Identification of candidate biomarkers and pathways associated with SCLC by bioinformatics analysis

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Abstract. Small cell lung cancer (SCLC) is one of the highly malignant tumors and a serious threat to human health. The aim of the present study was to explore the underlying molecular mechanisms of SCLC. mRNA microarray datasets GSE6044 and GSE11969 were downloaded from Gene Expression Omnibus database, and the differentially expressed genes (DEGs) between normal lung and SCLC samples were screened using GEO2R tool. Functional and pathway enrichment analyses were performed for common DEGs using the DAVID database, and the protein-protein interaction (PPI) network of common DEGs was constructed by the STRING database and visualized with Cytoscape software. In addition, the hub genes in the network and module analysis of the PPI network were performed using CentiScaPe and plugin Molecular Complex Detection. Finally, the mRNA expression levels of hub genes were validated in the Oncomine database. A total of 150 common DEGs with absolute fold-change >0.5, including 66 significantly downregulated DEGs and 84 upregulated DEGs were obtained. The Gene Ontology term enrichment analysis suggested that common upregulated DEGs were primarily enriched in biological processes (BPs), including 'cell cycle', 'cell cycle phase', 'M phase', 'cell cycle process' and 'DNA metabolic process'. The common downregulated genes were significantly enriched in BPs, including 'response to wounding', 'positive regulation of immune system process', 'immune response', 'acute inflammatory response' and 'inflammatory response'. Kyoto Encyclopedia of Genes and Genomes pathway analysis identified that the common downregulated DEGs were primarily enriched in the 'complement and coagulation cascades' signaling pathway; the common upregulated DEGs were mainly enriched in 'cell cycle', 'DNA replication', 'oocyte meiosis' and the 'mismatch repair' signaling pathways. From the PPI network, the top 10 hub genes in SCLC were selected, including topoisomerase IIα, proliferating cell nuclear antigen, replication factor C subunit 4, checkpoint kinase 1, thymidylate synthase, minichromosome maintenance protein (MCM) 2, cell division cycle (CDC) 20, cyclin dependent kinase inhibitor 3, MCM3 and CDC6, the mRNA levels of which are upregulated in Oncomine SCLC datasets with the exception of MCM2. Furthermore, the genes in the significant module were enriched in 'cell cycle', 'DNA replication' and 'oocyte meiosis' signaling pathways. Therefore, the present study can shed new light on the understanding of molecular mechanisms of SCLC and may provide molecular targets and diagnostic biomarkers for the treatment and early diagnosis of SCLC.

Introduction

Lung cancer is one of the highly malignant tumors and a serious threat to human health. The incidence and mortality rates of lung cancer are the highest of any type of cancer, particularly in China (1). Despite the advances and developments in the treatments for lung cancer, the 5-year survival rate of patients with lung cancer remains only 16%, and the 5-year recurrence rate is 50% (2). Based on differences in presentation and behavior, primary lung cancer is divided into two main histological subtypes: Small cell lung cancer (SCLC) and non-SCLC (NSCLC) (3). Although SCLC only accounts for 15% of lung cancers, it is an aggressive high-grade neuroendocrine tumor associated with early and widespread metastasis and development of resistance to chemotherapy, which contribute to the extremely poor prognosis of patients with the disease (4,5). Previously, several common genetic alterations in SCLC have been identified, including functional inactivation of the tumor-suppressor genes tumor protein p53 and RB transcriptional corepressor 1, as well as amplification of genes encoding Myc family members, enhancer of zeste homolog 2 (EZH2) involved in chromatin remodeling, epidermal growth factor receptor and B-cell lymphoma 2

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receptor tyrosine kinases, their downstream effectors, and Notch family proteins (4,6-10). These may provide opportunities for classification and therapeutic intervention, including poly (ADP-ribose) polymerase (PARP) inhibitors, EZH2 inhibition and Wee1 inhibitor (11-15). Therefore, more effort needs to be invested towards the investigation and understanding of molecular mechanisms in development and progression of SCLC, which are crucial for the development of more effective diagnostic and therapeutic strategies.

Recently, the gene expression profile chip, a high-throughput and effective technique, has been widely used in a variety of disease research fields to reveal the association between disease and genes, and provide the valuable clues for the pathogenesis of the diseases, including lung cancer (16-18). Kikuchi *et al* (19) identified several genes, which may be used for the prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. Yanaihara *et al* (20) identified that high hsa-mir-155 and low hsa-let-7a-2 expression levels correlated with poor survival, which indicated that micro (mi)RNA expression profiles are diagnostic and prognostic markers of lung cancer. Furthermore lung adenocarcinoma has been defined to represent distinct molecular subclasses according to the miRNA expression profiling data (21). Although the cellular and molecular genetic alterations underlying SCLC have become better understood, the molecular mechanisms of SCLC have yet to be fully elucidated.

In order to investigate the molecular mechanisms of SCLC, the present study re-analyzed the gene expression profiles of GSE6044 and GSE11969 (22,23) and identified the differentially expressed genes (DEGs) between normal lung tissue and SCLC. Subsequently, comprehensive bioinformatics analysis was used for biological process (BP) annotation and biological pathway enrichment analysis. The protein-protein interaction (PPI) network of common DEGs was constructed and analysis performed on the hub genes and modules of the PPI network. Therefore, the findings of the present study may provide further understanding of SCLC development and lead to an improved diagnosis of SCLC.

Materials and methods

Expression profile microarray. Data was downloaded from the Gene Expression Omnibus (GEO), a public repository for data storage (www.ncbi.nlm.nih.gov/geo) (24). A total of 2 mRNA expression datasets of SCLC, GSE6044 and GSE11969, were included in the present study (22,23). The dataset GSE6044 based on GPL201 (HG-Focus) Affymetrix Human HG-Focus Target Array platform (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA), included 5 normal lung and 9 SCLC samples; the dataset GSE11969 also included 5 normal lung and 9 SCLC samples based on the platform of GPL7015 Agilent *Homo sapiens* 21.6K custom array (Agilent Technologies, Inc., Santa Clara, CA, USA).

Identification of DEGs. The DEGs between normal lung and SCLC samples were screened by an interactive web tool, GEO2R (www.ncbi.nlm.nih.gov/geo/geo2r) (25). The adjusted P-value using the Benjamini and Hochberg false discovery rate (FDR) method was applied to correct for the occurrence of false positive results. The adjusted P-value <0.05 and |logFC| >0.5 were set as the cut-off criteria. The heat map of DEGs was generated using the gplots package for R (http://cran.r-project.org/web/packages/gplots/; version 3.4.3).

Gene ontology (GO) terms and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. The Database for Annotation, Visualization and Integrated Discovery (DAVID, david.abcc.ncifcrf.gov) is an online program that provides a comprehensive set of functional annotation tools for researchers to understand the biological meaning behind numerous genes (26). GO, including molecular function, biological processes (BP) and cellular components and KEGG pathway enrichment analyses were performed for identified DEGs using the DAVID database (version 6.7). FDR <0.05 was used as a cutoff for significance.

Construction of PPI, hub gene identification and module analysis of the PPI network. The Search Tool for the Retrieval of Interacting Genes (STRING) database (version 10.5; http://string-db.org/), is an online tool designed to explore and analyze PPI information. To evaluate the interactive associations among common DEGs, the common DEGs were mapped using STRING, and interactions with a combined score >0.4 were selected. Then, the PPI network was constructed and visualized using Cytoscape software (version 3.5.1; www.cytoscape.org). In order to identify key elements in BP, the hub genes in the network defined as possessing a connective degree >10, were identified using CentiScaPe v2.0 plugin for Cytoscape (version 3.5.1; www.cytoscape.org). The topological properties of the PPI network, including average clustering coefficients, topological coefficients and shortest path lengths, were investigated using a Network Analyzer (version 2.7; med.bioinf.mpi-inf.mpg.de/netanalyzer/download.php) and Cytoscape (version 3.5.1; www.cytoscape.org) plugin app (27). Finally, module analysis was carried out by the plug-in Molecular Complex Detection (MCODE; version 1.5.1) with cut-off criterion: MCODE score >4 and number >5.

Validation of the expression of hub genes in oncomine database. Oncomine (www.oncomine.org; Ion Torrent; Thermo Fisher Scientific, Inc.) is an online cancer microarray database to facilitate the discovery of genome-wide expression analyses (28). To validate the expression level of hub genes in SCLC, Garber *et al* (29) and Bhattacharjee *et al* (21) lung cancer gene expression data in the Oncomine database were searched for expression levels of hub genes in the network with a P-value <0.05. Thresholds for fold-change and gene rank were set to 'all', whereas the data type was restricted to mRNA. Statistical significance was provided by Oncomine in the form of a Student's t-test.

Results

Identification of DEGs. Gene expression datasets GSE6044 and GSE11969 were downloaded from GEO datasets. GEO2R was applied to screen DEGs between normal lung tissue and SCLC samples. A total of 1,025 and 1,006 DEGs were identified from GSE6044 and GSE11969 datasets, respectively (Fig. 1A and B). Among them, 481 downregulated genes and 544 upregulated genes in the GSE6044 dataset, and

Table I. The 150 common DEGs in the GSE6044 dataset.

A, Downregulated DEGs

Gene symbol	Log fold-change	Adjusted P-value
CYP4B1	-4.26	$3.14x10^{-4}$
CX3CL1	-4.23	$7.58x10^{-4}$
FBLN5	-3.26	$1.22x10^{-3}$
SCGB1A1	-3.16	$5.10x10^{-3}$
AQP3	-2.76	$3.70x10^{-2}$
ADH1C	-2.75	$9.50x10^{-4}$
CSTA	-2.60	$1.87x10^{-2}$
ALDH1A1	-2.49	$2.66x10^{-3}$
CFH	-2.32	$1.49x10^{-3}$
CLU	-2.08	$1.28x10^{-3}$
ADH1B	-2.07	$3.39x10^{-3}$
PTGDS	-1.94	$5.01x10^{-3}$
PROS1	-1.87	$1.93x10^{-3}$
TGFBR3	-1.80	$7.75x10^{-3}$
ANXA11	-1.73	$4.77x10^{-3}$
LAMB3	-1.73	$4.78x10^{-3}$
DMBT1	-1.72	$4.59x10^{-2}$
F13A1	-1.72	$2.19x10^{-2}$
FLRT3	-1.72	$4.58x10^{-2}$
RRAD	-1.70	$1.88x10^{-3}$
TACSTD2	-1.67	$2.54x10^{-2}$
C3	-1.66	$1.03x10^{-2}$
PLK2	-1.66	$5.62x10^{-3}$
EPAS1	-1.65	$6.31x10^{-3}$
PZP	-1.57	$2.83x10^{-3}$
CXCL1	-1.55	$4.10x10^{-3}$
CAST	-1.49	$3.13x10^{-3}$
ANXA1	-1.42	$3.49x10^{-2}$
RNASE4	-1.39	$7.39x10^{-4}$
CTSH	-1.33	$7.49x10^{-4}$
CD9	-1.31	$2.85x10^{-2}$
ADRB2	-1.30	$2.62x10^{-2}$
PTGER4	-1.26	$1.13x10^{-2}$
FOLR1	-1.22	$5.56x10^{-3}$
BAG3	-1.21	$1.50x10^{-2}$
CAPN2	-1.21	$5.21x10^{-3}$
CD81	-1.21	$2.21x10^{-2}$
SERPINA1	-1.21	$2.26x10^{-2}$
VAMP8	-1.21	$1.51x10^{-2}$
GPX3	-1.19	$1.45x10^{-2}$
MYO5C	-1.19	$1.46x10^{-2}$
PCSK5	-1.19	$2.10x10^{-3}$
HLA-E	-1.18	$5.52x10^{-3}$
FBLN1	-1.12	$1.24x10^{-2}$
A2M	-1.11	$1.67x10^{-2}$
TGM2	-1.07	$7.86x10^{-3}$
TGFBR2	-1.06	$1.98x10^{-2}$
PXMP2	0.50	$4.38x10^{-2}$
NOL4	0.51	$4.98x10^{-3}$
MKI67	0.52	$4.11x10^{-2}$

Table I. Continued.

A, Downregulated DEGs

Gene symbol	Log fold-change	Adjusted P-value
LYN	-1.04	$3.06x10^{-2}$
C6	-1.02	$4.37x10^{-3}$
HNMT	-1.01	$4.43x10^{-3}$
PRNP	-1.01	$2.72x10^{-2}$
CCND1	-0.98	$1.31x10^{-2}$
TCF21	-0.96	$6.18x10^{-3}$
CST3	-0.95	$2.20x10^{-3}$
CNN2	-0.95	$1.07x10^{-2}$
NEDD9	-0.91	$1.29x10^{-2}$
IL4R	-0.91	$3.37x10^{-2}$
THBD	-0.90	$4.91x10^{-2}$
EPHA2	-0.87	$2.92x10^{-2}$
ZFP36L2	-0.86	$4.88x10^{-2}$
SLC16A5	-0.83	$2.59x10^{-3}$
STAT6	-0.83	$2.09x10^{-2}$
SP110	-0.69	$3.10x10^{-2}$
TLR2	-0.63	$4.12x10^{-2}$
CFTR	-0.61	$2.77x10^{-2}$
VAV1	-0.53	$3.77x10^{-3}$

B, Upregulated DEGs

Gene symbol	Log fold-change	Adjusted P-value
KCNH2	0.52	$2.49x10^{-2}$
RAD54L	0.52	$2.49x10^{-2}$
CBX5	0.59	$1.01x10^{-2}$
DDC	0.61	$3.27x10^{-2}$
RECQL4	0.62	$5.10x10^{-3}$
CHEK1	0.64	$4.79x10^{-2}$
ENC1	0.64	$2.00x10^{-2}$
SOX11	0.67	$1.92x10^{-2}$
BIRC5	0.69	$5.60x10^{-3}$
CKS1B	0.69	$1.87x10^{-2}$
GNG4	0.70	$2.24x10^{-2}$
EZH2	0.71	$9.45x10^{-4}$
FANCA	0.71	$9.45x10^{-4}$
STMN1	0.71	$9.45x10^{-4}$
EXO1	0.71	$7.39x10^{-3}$
GRP	0.71	$7.39x10^{-3}$
CDKN3	0.73	$7.18x10^{-3}$
FKBP3	0.78	$7.93x10^{-3}$
NRTN	0.79	$2.86x10^{-2}$
ASCL1	0.81	$8.66x10^{-3}$
CENPF	0.81	$5.56x10^{-3}$
PCSK1	0.83	$5.62x10^{-3}$
MYBL2	0.86	$2.27x10^{-2}$
TRIM36	0.86	$2.26x10^{-2}$
MSH6	0.86	$5.21x10^{-3}$
TPD52	0.89	$1.64x10^{-2}$

Table I. Continued.

B, Upregulated DEGs		
Gene symbol	Log fold-change	Adjusted P-value
CDC7	0.90	$1.98x10^{-3}$
PSIP1	0.90	$1.98x10^{-3}$
PRDX2	0.91	$2.19x10^{-2}$
FZD3	0.91	$4.66x10^{-3}$
HDAC2	0.94	$3.14x10^{-4}$
MCM6	0.94	$3.14x10^{-4}$
MEST	0.94	$3.14x10^{-4}$
SOX4	0.94	$3.14x10^{-4}$
TOP2A	0.94	$3.14x10^{-4}$
TYMS	0.94	$3.14x10^{-4}$
CDC20	0.95	$1.88x10^{-3}$
LHX2	0.97	$2.16x10^{-2}$
HPRT1	0.99	$9.71x10^{-3}$
PARP1	0.99	$9.71x10^{-3}$
CDC6	1.02	$2.97x10^{-3}$
PCNA	1.08	$1.84x10^{-3}$
NELL1	1.09	$1.92x10^{-2}$
SHMT2	1.11	$9.89x10^{-3}$
FANCG	1.19	$1.62x10^{-3}$
TTK	1.19	$1.62x10^{-3}$
BUB1	1.20	$2.72x10^{-3}$
PAFAH1B3	1.23	$2.62x10^{-3}$
SPAG5	1.25	$4.19x10^{-3}$
CELSR3	1.26	$2.51x10^{-3}$
ITGB3BP	1.27	$1.56x10^{-2}$
DTYMK	1.29	$1.77x10^{-3}$
DLK1	1.31	$3.32x10^{-3}$
DEK	1.33	$6.17x10^{-4}$
RFC5	1.39	$1.21x10^{-3}$
KIF11	1.41	$3.53x10^{-3}$
NEK2	1.41	$3.09x10^{-3}$
UNG	1.48	$1.28x10^{-3}$
MCM3	1.52	$1.39x10^{-3}$
CAMK2B	1.53	$2.43x10^{-2}$
TIMELESS	1.60	$2.07x10^{-3}$
USP1	1.60	$6.35x10^{-4}$
CCNB2	1.61	$3.13x10^{-3}$
FBXO5	1.62	$1.46x10^{-3}$
ZWINT	1.67	$7.39x10^{-4}$
GMNN	1.79	$1.52x10^{-3}$
COCH	1.83	$1.45x10^{-3}$
PTTG1	1.85	$7.46x10^{-3}$
MCM2	1.90	$4.17x10^{-3}$
MAD2L1	1.98	$2.59x10^{-3}$
CCNE2	1.99	$7.62x10^{-3}$
RACGAP1	2.00	$1.77x10^{-3}$
CHGB	2.04	$3.42x10^{-3}$
ASNS	2.16	$2.61x10^{-3}$
RRM2	2.40	$2.24x10^{-3}$
RFC4	2.52	$3.41x10^{-4}$
CKS2	2.60	$1.09x10^{-3}$
RBP1	2.75	$5.41x10^{-3}$
UCHL1	3.08	$3.37x10^{-4}$
ISL1	3.17	$4.91x10^{-3}$
INSM1	3.65	$5.28x10^{-3}$

DEGs, differentially expressed genes.

706 downregulated genes and 300 upregulated genes in the GSE11969 dataset, were identified. In addition, 150 common DEGs were obtained (Tables I and II), comprising 66 co-downregulated genes and 84 co-upregulated genes (Fig. 1C and D).

Biological classification and pathway enrichment analysis of common DEGs. To gain an understanding of the GO categories of common DEGs, all common DEGs were uploaded to the DAVID database. The downregulated DEGs were significantly enriched in BPs, including 'response to wounding', 'positive regulation of immune system process', 'immune response', 'acute inflammatory response' and 'inflammatory response'; the upregulated genes were significantly enriched in 'cell cycle', 'cell cycle phase', 'M phase', 'cell cycle process' and 'DNA metabolic process'. For cellular component, the downregulated DEGs were significantly enriched in the 'extracellular region', 'extracellular region part', 'extracellular space', 'platelet α-granule', and 'cytoplasmic vesicle part'; and the upregulated DEGs were significantly enriched in 'chromosome', 'chromosomal part', 'nuclear lumen', 'spindle' and 'intracellular organelle lumen'. In addition, MF analysis also indicated that the downregulated DEGs were significantly enriched in 'enzyme inhibitor activity', 'endopeptidase inhibitor activity' and 'peptidase inhibitor activity' (Fig. 2A and B).

Following KEGG pathway enrichment analysis, the common downregulated DEGs were identified to be primarily enriched in the 'complement and coagulation cascades' signaling pathways; the common upregulated DEGs were mainly enriched in 'cell cycle', 'DNA replication', 'oocyte meiosis' and 'mismatch repair' signaling pathways (Table III). Therefore, these significantly enriched GO terms and pathways could aid further understanding of the roles of these DEGs, involved in the occurrence and development of SCLC.

Construction of PPI network and module analysis. PPI network of common DEGs was constructed using the STRING online database and Cytoscape software (Fig. 3). A total of 123 DEGs (50 downregulated and 73 upregulated) of the 150 commonly altered DEGs were filtered into the DEGs PPI network complex, containing 123 nodes and 869 edges, and 27 of the 150 DEGs fell outside the DEGs PPI network (Fig. 3A). Then, the hub genes in the networks with a connectivity degree

Table II. The 150 common DEGs in the GSE11969 dataset.

A, Downregulated DEGs

Gene symbol	Log fold-change	Adjusted P-value
TGFBR2	-1.19	3.30×10^{-4}
CFTR	-1.16	1.03×10^{-3}
CXCL1	-1.14	5.16×10^{-4}
TGM2	-1.11	4.73×10^{-5}
THBD	-1.11	4.73×10^{-5}
EPAS1	-1.10	4.13×10^{-4}
AQP3	-1.10	1.00×10^{-5}
CD9	-1.07	3.03×10^{-3}
ALDH1A1	-1.03	4.22×10^{-3}
RRAD	-1.01	1.52×10^{-4}
NEDD9	-0.99	3.32×10^{-4}
CX3CL1	-0.98	6.32×10^{-5}
ANXA1	-0.97	4.15×10^{-4}
CNN2	-0.95	3.65×10^{-4}
SLC16A5	-0.95	1.53×10^{-4}
LAMB3	-0.94	2.82×10^{-4}
PROS1	-0.93	1.49×10^{-3}
FBLN1	-0.92	8.81×10^{-4}
SP110	-0.89	1.36×10^{-2}
TGFBR3	-0.88	7.00×10^{-5}
RNASE4	-0.87	1.76×10^{-4}
DMBT1	-0.86	6.02×10^{-3}
CSTA	-0.85	5.07×10^{-3}
GPX3	-0.85	1.10×10^{-3}
CTSH	-0.85	3.15×10^{-3}
PTGER4	-0.85	3.15×10^{-3}
VAV1	-0.84	8.80×10^{-3}
TCF21	-0.83	4.99×10^{-4}
F13A1	-0.83	1.22×10^{-3}
TACSTD2	-0.83	5.82×10^{-4}
CST3	-0.83	2.69×10^{-2}
STAT6	-0.83	1.02×10^{-2}
ADH1B	-0.81	3.64×10^{-3}
ZFP36L2	-0.81	6.86×10^{-4}
PZP	-0.81	5.75×10^{-3}
C3	-0.80	2.70×10^{-2}
CLU	-0.76	4.60×10^{-2}
CYP4B1	-0.75	3.74×10^{-3}
SCGB1A1	-0.75	5.88×10^{-3}
FLRT3	-0.74	3.15×10^{-2}
PCSK5	-0.74	2.91×10^{-3}
CFH	-0.72	2.45×10^{-2}
LYN	-0.72	1.19×10^{-2}
ADRB2	-0.71	6.46×10^{-5}
HLA-E	-0.71	9.33×10^{-3}
CAPN2	-0.70	8.30×10^{-3}
BAG3	-0.69	1.44×10^{-3}
TLR2	-0.69	1.15×10^{-3}
FBLN5	-0.69	3.74×10^{-4}
A2M	-0.66	2.23×10^{-2}
C6	-0.66	1.32×10^{-4}
FOLR1	-0.65	9.53×10^{-4}
PLK2	-0.65	1.92×10^{-4}
HNMT	-0.63	1.23×10^{-2}
MYO5C	-0.61	1.28×10^{-2}
CAST	-0.61	1.05×10^{-2}
PTGDS	-0.61	4.53×10^{-3}
ANXA11	-0.59	2.59×10^{-2}
CCND1	-0.58	3.88×10^{-5}
EPHA2	-0.58	3.88×10^{-5}
SERPINA1	-0.58	5.84×10^{-4}
PRNP	-0.57	4.65×10^{-2}
VAMP8	-0.56	5.03×10^{-3}
ADH1C	-0.54	1.74×10^{-3}
CD81	-0.52	3.13×10^{-3}
IL4R	-0.51	3.98×10^{-4}

B, Upregulated DEGs

Gene symbol	Log fold-change	Adjusted P-value
CCNB2	0.50	1.59×10^{-4}
DDC	0.50	1.59×10^{-4}
HPRT1	0.50	1.59×10^{-4}
CDC20	0.50	3.39×10^{-4}
PARP1	0.51	3.92×10^{-3}
FANCG	0.51	3.74×10^{-4}
PSIP1	0.51	3.74×10^{-4}
ENC1	0.51	4.43×10^{-2}
DEK	0.52	2.91×10^{-3}
FZD3	0.52	6.44×10^{-3}
ZWINT	0.52	2.34×10^{-4}
UNG	0.52	3.02×10^{-4}
CDC7	0.53	3.98×10^{-5}
MCM3	0.53	8.82×10^{-3}
CHEK1	0.53	5.88×10^{-3}
NRTN	0.53	1.43×10^{-3}
RBP1	0.53	5.99×10^{-4}
MSH6	0.53	2.16×10^{-4}
DTYMK	0.54	6.60×10^{-5}
MCM2	0.55	4.74×10^{-4}
CHGB	0.56	3.30×10^{-4}
EXO1	0.56	3.30×10^{-4}
CELSR3	0.56	8.00×10^{-4}
CDKN3	0.56	7.01×10^{-3}
SHMT2	0.56	1.85×10^{-4}
TRIM36	0.56	1.58×10^{-3}
BUB1	0.56	1.23×10^{-3}
CDC6	0.56	4.94×10^{-5}
USP1	0.57	5.25×10^{-4}

Table II. Continued.

B, Upregulated DEGs		
Gene symbol	Log fold-change	Adjusted P-value
PXMP2	0.57	$3.87x10^{-5}$
RACGAP1	0.57	$3.87x10^{-5}$
FKBP3	0.57	$1.01x10^{-3}$
MAD2L1	0.57	$1.01x10^{-3}$
DLK1	0.58	$1.17x10^{-2}$
HDAC2	0.58	$9.39x10^{-5}$
ASCL1	0.60	$3.29x10^{-3}$
SOX4	0.60	$1.89x10^{-2}$
COCH	0.60	$4.20x10^{-4}$
PRDX2	0.61	$7.76x10^{-5}$
FANCA	0.62	$4.21x10^{-4}$
RFC5	0.62	$1.00x10^{-5}$
ITGB3BP	0.63	$1.14x10^{-3}$
LHX2	0.64	$8.68x10^{-4}$
MCM6	0.64	$5.32x10^{-5}$
CKS2	0.65	$1.92x10^{-3}$
CCNE2	0.66	$2.14x10^{-4}$
ASNS	0.68	$5.25x10^{-5}$
CKS1B	0.69	$1.55x10^{-3}$
FBXO5	0.69	$9.25x10^{-5}$
KCNH2	0.71	$2.95x10^{-3}$
TPD52	0.75	$1.99x10^{-4}$
STMN1	0.75	$2.13x10^{-5}$
TYMS	0.75	$2.13x10^{-5}$
RRM2	0.76	$6.99x10^{-5}$
CBX5	0.76	$2.44x10^{-3}$
KIF11	0.76	$1.09x10^{-3}$
SOX11	0.77	$1.42x10^{-2}$
RECQL4	0.79	$3.77x10^{-4}$
PCNA	0.79	$4.39x10^{-4}$
GMNN	0.80	$1.27x10^{-5}$
MYBL2	0.81	$7.84x10^{-4}$
TTK	0.83	$5.23x10^{-5}$
TOP2A	0.84	$1.95x10^{-4}$
EZH2	0.85	$7.70x10^{-6}$
PAFAH1B3	0.85	$7.70x10^{-6}$
RAD54L	0.85	$7.70x10^{-6}$
TIMELESS	0.85	$2.82x10^{-5}$
GNG4	0.87	$1.04x10^{-5}$
SPAG5	0.87	$1.04x10^{-5}$
PTTG1	0.88	$3.01x10^{-5}$
NELL1	0.92	$3.40x10^{-3}$
MEST	0.93	$1.25x10^{-3}$
NOL4	0.93	$2.14x10^{-3}$
UCHL1	0.94	$3.18x10^{-3}$
INSM1	0.97	$2.25x10^{-4}$
MKI67	0.98	$1.13x10^{-3}$
PCSK1	0.98	$1.20x10^{-2}$
BIRC5	1.00	$3.28x10^{-5}$
RFC4	1.01	$9.53x10^{-6}$

Table II. Continued.

B, Upregulated DEGs		
Gene symbol	Log fold-change	Adjusted P-value
NEK2	1.01	$9.94x10^{-4}$
CENPF	1.08	$1.84x10^{-4}$
CAMK2B	1.10	$1.89x10^{-4}$
ISL1	1.30	$7.31x10^{-3}$
GRP	1.32	$1.16x10^{-2}$

DEG, differentially expressed genes.

>10 were identified. The most significant 10 node degree genes were topoisomerase IIα (TOP2A), proliferating cell nuclear antigen (PCNA), replication factor C subunit 4 (RFC4), checkpoint kinase 1 (CHEK1), thymidylate synthase (TYMS), minichromosome maintenance protein (MCM) 2, cell division cycle (CDC) 20, cyclin dependent kinase inhibitor 3 (CDKN3), MCM3 and CDC6. The heat map of the most significant hub genes expression in GSE11969 is shown in Fig. 3B. To assess the basic properties of the PPI network, the Network Analyzer was used to compute several indices, including average clustering coefficient distribution, closeness centrality, average neighborhood connectivity, node degree distribution, shortest path length distribution and topological coefficients. In scale-free networks, the majority of nodes have a low degree, increasing the likely accuracy of the network (30). The computed parameters revealed that the constructed network was scale-free and stable (Fig. 4). In addition, one significant module was obtained from the PPI network of DEGs using MCODE, consisting of 35 nodes and 550 edges (Fig. 3C). Functional and KEGG pathway enrichment analysis revealed that genes in this module were primarily associated with 'cell cycle', 'DNA replication' and 'oocyte meiosis' signaling pathways (Table IV).

Validation of hub genes mRNA level in the oncomine database. Based on the Oncomine database, it was identified that the mRNA expression levels of TOP2A, PCNA, RFC4, CHEK1, TYMS, CDC20, CDKN3, MCM3 and CDC6 were significantly increased in SCLC samples compared with normal lung samples, while MCM2 was not significantly differentially expressed, which was inconsistent with the bioinformatics investigation (Fig. 5).

Discussion

Although research on SCLC has made great progress in the past decade (31,32), the pathogenesis of SCLC has yet to be fully elucidated due to its complexity of biological traits and high heterogeneity. As a result, the early diagnosis and treatment of SCLC remains a problem. Therefore, understanding of molecular mechanisms of SCLC based on microarray technology, which has developed rapidly and has been widely used to reveal the general genetic alteration in progression of diseases (16-18), may aid the identification of the key gene

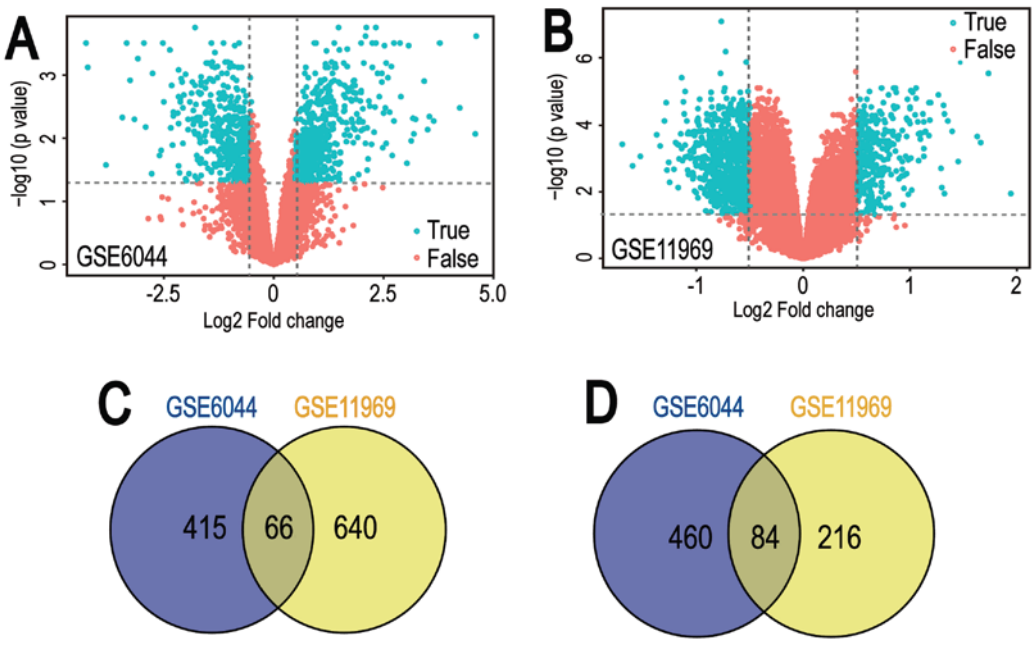

Figure 1. Volcano plot and Venn diagram of DEGs in mRNA expression profiling datasets. Volcano plots of DEGs in normal lung and small cell lung cancer samples in (A) GSE6044 and (B) GSE11969 datasets. DEGs were selected by P<0.05 and $|\log_2$ (fold-change)$|$ >0.5. The x-axis shows the fold-change in gene expression between normal lung and small cell lung cancer samples, and the y-axis shows the statistical significance of the differences. Colors represent different genes: Red for genes without significantly different expression and blue for significantly differentially expressed genes. Venn diagrams illustrating the number of (C) downregulated and (D) upregulated genes in the two datasets, respectively. The intersection in grey represents the DEGs common between the two datasets. DEG, differentially expressed genes.

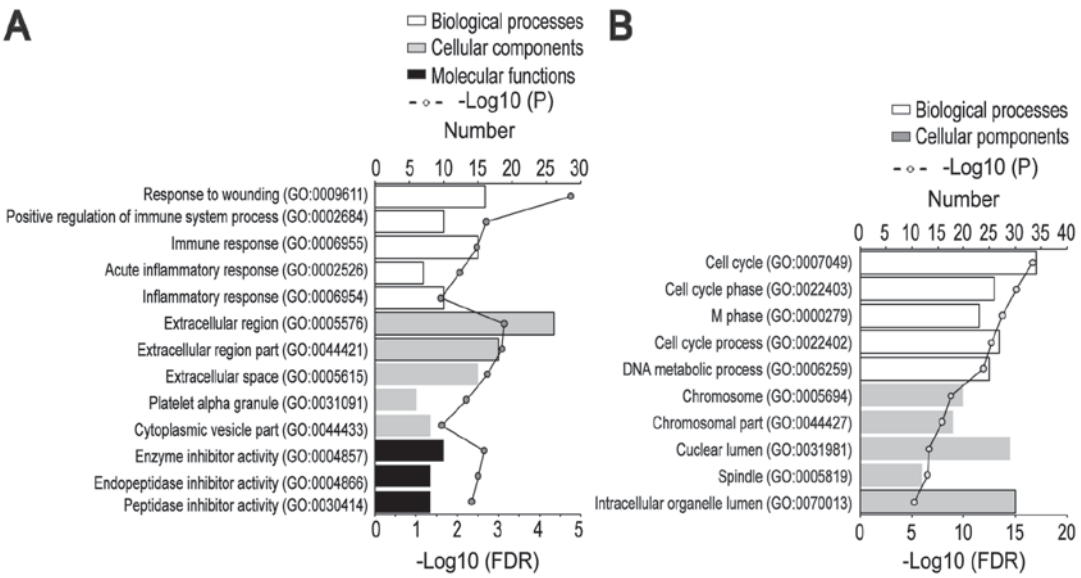

Figure 2. GO classification of common DEGs in the listed categories (top 5 biological processes and cellular components, and top 3 molecular functions). GO enrichment analysis results of (A) downregulated and (B) upregulated common DEGs with P<0.05 and absolute fold-changes >0.5. The top x axis represents the number of genes in the marked category; the bottom x axis indicates the minus Log_{10} (FDR) of categories. Only functional categories with P-value <0.05 are shown. GO, Gene Ontology; DEG, differentially expressed genes; FDR, false discovery rate.

targets or signaling pathways for diagnosis, treatment, and prognosis of SCLC.

In the present study, two microarray datasets were obtained to identify the DEGs common to normal lung tissues and SCLC samples. A total of 150 common DEGs, including 66 significantly downregulated DEGs and 84 upregulated DEGs were identified and used for further analysis. To interpret the biological functions of these common DEGs, GO and pathway analysis based on the DAVID tool was performed. GO and pathway analysis for the common DEGs indicated that the common upregulated DEGs were mainly enriched in cell cycle, cell cycle phase, M phase, cell cycle process and DNA metabolic process, and the common downregulated genes were significantly enriched in response to wounding, positive regulation of immune system process, immune response, acute inflammatory response and inflammatory response. These

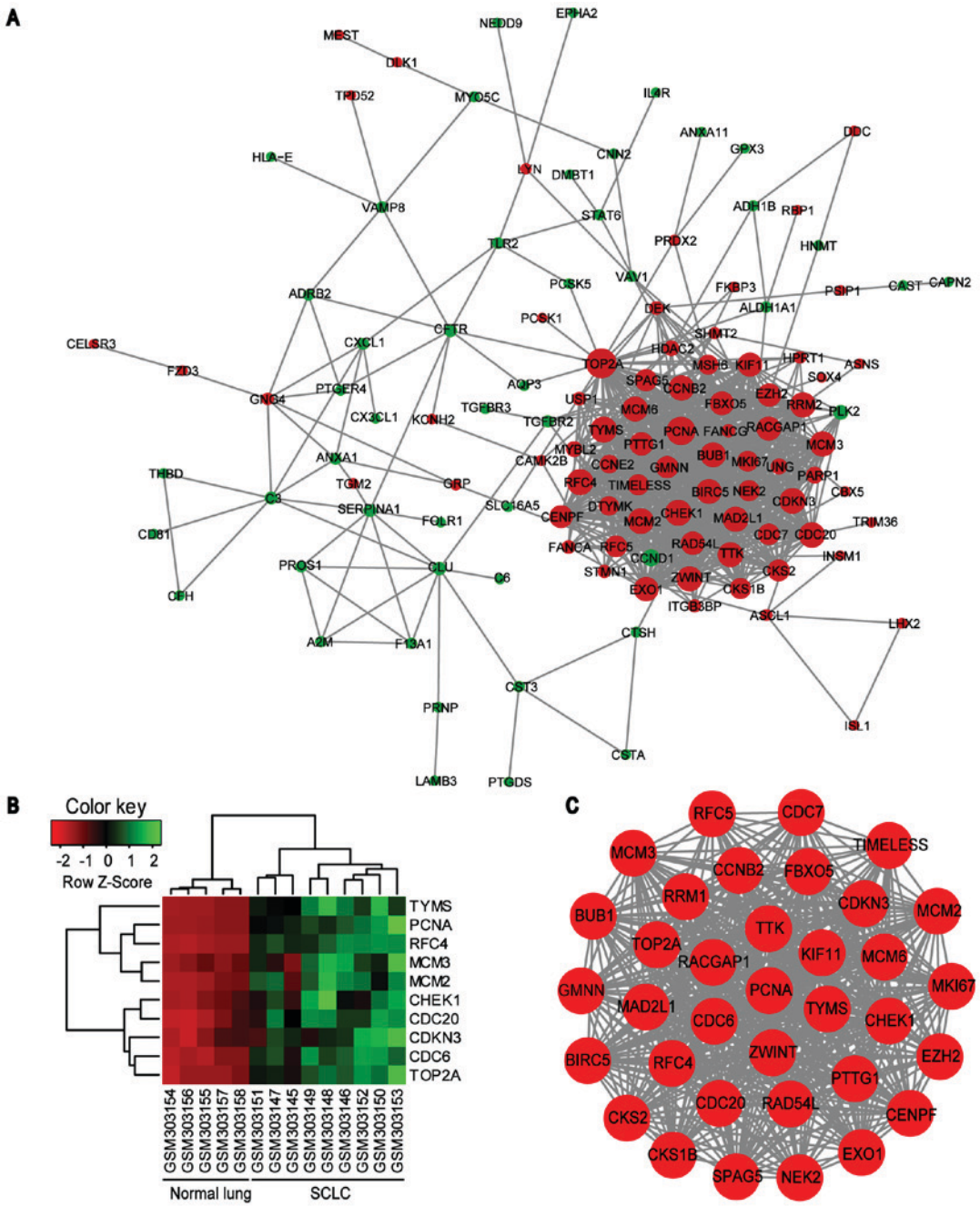

Figure 3. PPI network constructed from the common DEGs, module analysis and hub genes. (A) Using the STRING online database, a total of 123 DEGs were filtered into the DEGs PPI network complex. The nodes represent proteins, the edges represent the interaction of proteins and green circles and red circles indicate downregulated and upregulated DEGs, respectively. (B) Expression heat map of the top 10 hub genes in GSE11969. (C) The most significant module in the PPI network with MCODE score ≥4 and node >5. PPI, protein-protein interaction; DEG, differentially expressed genes; MCODE, Molecular Complex Detection plug in; SCLC, small cell lung cancer; TYMS, thymidylate synthase; PCNA, proliferating cell nuclear antigen; RFC4, replication factor C subunit 4; MCM, minichromosome maintenance protein; CHEK1, checkpoint kinase 1; CDC, cell division cycle; CDKN3, cyclin dependent kinase inhibitor 3; TOP2A, topoisomerase IIα.

results are consistent with the evidence that disorders in cell cycle regulation and alterations of immune response contribute to carcinogenesis and development of tumor (33-35). KEGG pathway analysis indicated that the common downregulated DEGs were mainly enriched in the complement and coagulation cascades signaling pathways. Previous studies have shown that the tissue factor-activated coagulation cascade in the tumor microenvironment, in addition to coagulation, can facilitate the spreading of tumor cell in the pulmonary vasculature during early metastatic colony formation (36,37). Conversely, the common upregulated DEGs were mainly enriched in cell cycle, DNA replication, oocyte meiosis and mismatch repair signaling pathway, consistent with the results from GO and pathway analysis.

To predict the associations of protein functions of the identified 110 common interacting genes, a PPI network was constructed in which the top 10 hub genes with the highest connective degree were selected, including TOP2A, PCNA, RFC4, CHEK1, TYMS, MCM2, CDC20, CDKN3, MCM3 and CDC6, which were also primarily associated with 'cell cycle',

Table III. Signaling pathway enrichment analysis of common DEGs in normal lung and small cell lung cancer.

Pathway	Name	Gene count	Genes	FDR
Common downregulated DEGs				
KEGG_PATHWAY: hsa04610	Complement and coagulation cascades	8	A2M, THBD, C3, C6, F13A1, CFH, SERPINA1, PROS1	$3.17x10^{-4}$
Common upregulated DEGs				
KEGG_PATHWAY: hsa04110	Cell cycle	15	CDC7, CDC6, TTK, CHEK1, CDC20, PTTG1, MCM2, MCM3, MCM6, CCNE2, CCNB2, HDAC2, MAD2L1, PCNA, BUB1	$5.06x10^{-11}$
KEGG_PATHWAY: hsa03030	DNA replication	6	RFC5, RFC4, PCNA, MCM2, MCM3, MCM6	$4.67x10^{-3}$
KEGG_PATHWAY: hsa04114	Oocyte meiosis	8	CCNE2, MAD2L1, CCNB2, BUB1, FBXO5, CDC20, CAMK2B, PTTG1	$1.04x10^{-4}$
KEGG_PATHWAY: hsa03430	Mismatch repair	5	RFC5, EXO1, MSH6, RFC4, PCNA	$1.80x10^{-2}$

DEG, differentially expressed genes; FDR, false discovery rate; KEGG, kyoto encyclopedia of genes and genomes.

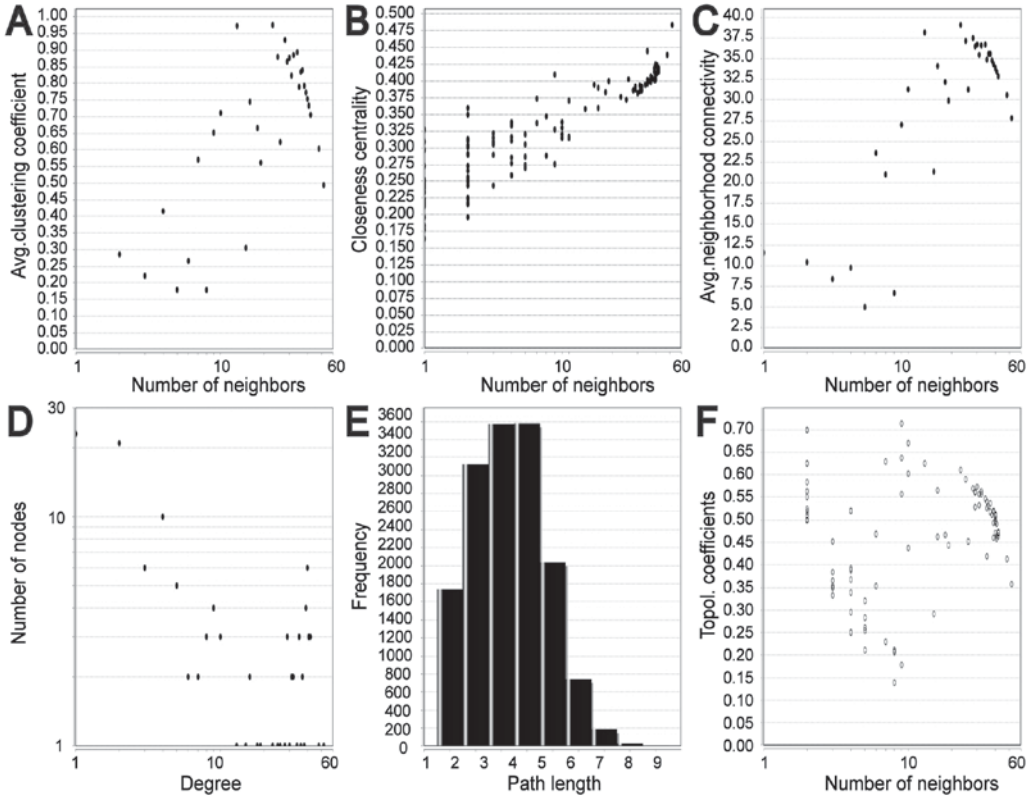

Figure 4. Topological parameters of the protein-protein interaction network. (A) Average clustering coefficient distribution. (B) Closeness centrality. (C) Average neighborhood connectivity distribution. (D) Node-degree distribution. (E) Shortest path length distribution. (F) Topological coefficients. Avg, average; topol, topological.

'DNA replication' and 'oocyte meiosis' signaling pathways. In addition, to validate the expression levels of these hub genes, the mRNA expression level of hub genes was searched for by mining the Oncomine database, which further supported the bioinformatics data. Although previous research has suggested that the majority of these deregulated hub genes correlated with diagnosis, treatment and prognosis of the various malignancies, the precise roles and molecular mechanism of them in the occurrence and development of SCLC have not yet been fully elucidated.

The TOP2A gene encodes a 170 kDa nuclear enzyme that catalyzes the ATP-dependent transport of one intact DNA double helix through another, by which TOP2A is involved in the chromosome segregation and cell cycle progression (38), and numerous studies indicated that the expression, genetic alteration and enzyme activity of TOP2A have been identified

Table IV. GO function enrichment analysis of gene in module.

Category	Term	Description	Count	Genes	FDR
KEGG	hsa04110	Cell cycle	13	CDC7, CDC6, TTK, CDC20, CHEK1, PTTG1, MCM2, MCM3, MCM6, CCNB2, MAD2L1, PCNA, BUB1	$5.06x10^{-12}$
KEGG	hsa03030	DNA replication	6	RFC5, RFC4, PCNA, MCM2, MCM3, MCM6	$1.81x10^{-4}$
KEGG	hsa04114	Oocyte meiosis	6	CCNB2, MAD2L1, BUB1, FBXO5, CDC20, PTTG1	$4.83x10^{-2}$

GO, Gene Ontology; FDR, false discovery rate; KEGG, kyoto encyclopedia of genes and genomes; Count, the number of enriched genes in each term.

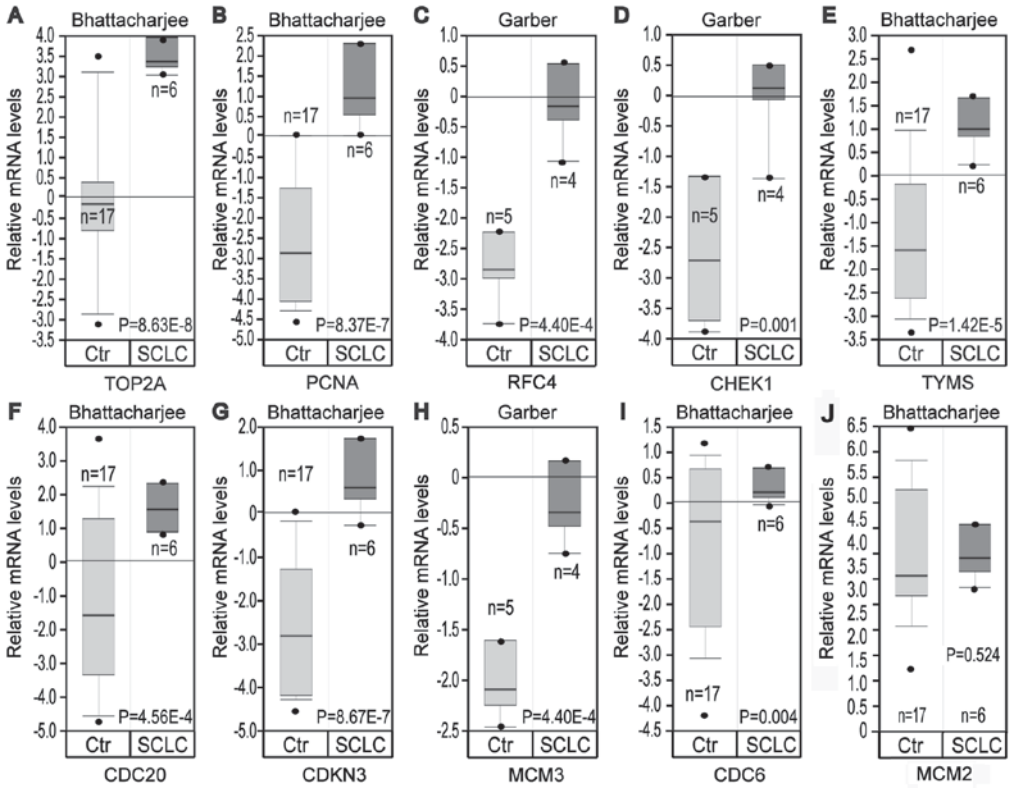

Figure 5. Analysis of expression of hub genes in the Oncomine database. Gene expression data was obtained from Garber and Bhattacharjee lung datasets and analyzed with Oncomine. mRNA expression levels of (A) TOP2A, (B) PCNA, (C) RFC4, (D) CHEK1, (E) TYMS, (F) CDC20, (G) CDKN3, (H) MCM3, (I) CDC6 and (J) MCM2 in normal lung vs. SCLC were compared. Pre-processed expression levels are Log_2 normalized and median centered. Data are presented as box plot with minimum (from bottom to top), 10th percentile, 25th percentile, median, 75th percentile, 90th percentile and maximum. Ctr, control; TOP2A, topoisomerase IIα; PCNA, proliferating cell nuclear antigen; RFC4, replication factor C subunit 4; CHEK1, checkpoint kinase 1; TYMS, thymidylate synthase; CDC, cell division cycle; CDKN3, cyclin dependent kinase inhibitor 3; MCM, minichromosome maintenance protein; SCLC, small cell lung cancer.

in several types of malignancies; therefore, it *TOP2A* should be investigated to determine whether it represents an effective therapeutic target for a wide variety of malignancies, such as SCLC, testicular cancer, neuroblastoma, leukemia and lymphoma (39-41).

PCNA encodes a nuclear protein acting as a subunit of DNA polymerase δ, which is essential for DNA replication (42). Although several studies have been performed to investigate the association between PCNA expression and clinical properties of NSCLC, the data is controversial; certain studies claimed that patients with increased expression of PCNA had a worse outcome compared with patients with a lower expression of PCNA (43-45), however, a subsequent study indicated that PCNA cannot predict disease-free survival in patients with lung adenocarcinoma (46). Furthermore, no correlation has been observed between PCNA expression in biopsy specimens and tumor responsiveness to chemotherapy (47).

RFC4 encodes the fourth largest subunit of the RFC complex, which helps PCNA load onto DNA in an ATP-dependent process during DNA synthesis and serves an important role in DNA repair activities following DNA damage. It has been reported that the expression level of RFC4 is upregulated in colorectal cancer, correlates with tumor progression and can predict the prognosis for colorectal cancer (48).

CHEK1 is an evolutionarily conserved Ser/Thr kinase, which mediates cell-cycle arrest following DNA damage (49). Previous results demonstrated that upregulated CHEK1 has been considered a potential target for cancer therapy (50,51).

Therefore, CHEK1 inhibitors (including LY2606368) have been tested as treatment for several types of cancer including lung cancer, and the inhibitors may affect the sensitivity of radiotherapy and chemotherapy, including cisplatin or the PARP inhibitor olaparib (52-55).

TYMS is a key enzyme in the *de novo* synthesis of thymidine and is upregulated in different histological types of lung cancer, particularly in SCLC (56). In addition, the expression level of TYMS is considered to be associated with the prognosis and treatment efficacy of chemotherapy (57,58).

MCM2 is a component of the prereplicative complex, which is essential for eukaryotic DNA replication and is only expressed in proliferating cells. Several studies indicated that the expression of MCM2 is also upregulated in NSCLC and is a predictor of survival in patients with NSCLC (59). Recently, Cheung *et al* (60) performed a multi-dimensional proteomic analysis to investigate the biological networks of MCM2 in the lung cancer and the results indicated that the deregulation of MCM2 is involved in lung cancer cell proliferation, the cell cycle and migration.

MCM3, another family member of MCMs, has been proved to be overexpressed in various human cancers, including leukemia, malignant melanoma, lymphoma, and carcinomas of the uterine cervix, colon, lung, stomach, kidney and breast (61).

CDC20, a homolog of *Saccharomyces cerevisiae* cell division cycle 20 protein, is an activator for the anaphase-promoting complex. Evidence has demonstrated that CDC20 is essential to govern cell cycle progression for cell division by targeting several key substrates including securin, cyclin B1, cyclin A, Nek2A, p21 and myeloid cell leukemia-1 for degradation (62,63). Subsequent studies have indicated that CDC20 is frequently upregulated in numerous types of malignancies, including NSCLC, and is associated with the prognosis of patients with tumors (64,65).

CDKN3 is a negative regulator of CDK1 and CDK2 (66). Since CDK-driven cell cycle is essential for proliferation of cancer cells and CDKN3 inhibits CDK activities, CDKN3 was initially perceived as a tumor suppressor (66). However, the overexpression of CNKN3 in a number of types of cancers has recently demonstrated that CDKN3 mRNA overexpression in cancer is due to the presence of dominant-negative CDKN3 mutations (67,68). Although upregulated CDKN3 may be a prognostic marker in lung adenocarcinoma and serve functional roles in the pathogenesis and diagnosis of SCLC, it has not been investigated other aggressive forms of lung tumors (69).

CDC6, initially identified to participate in the assembly of pre-replication complexes, is essential for DNA replication in mammalian cells (70). CDC6 expression represses E-cadherin transcription, and loss of this gene occurs frequently in carcinogenesis, contributing to invasion and metastasis (71). In addition, previous studies have confirmed the association between CDC6 and prognosis and the treatment sensitivity of patients with tumors (72,73).

Therefore, given the key roles, associated signaling pathways and results of the present study on the hub genes mentioned above, future studies may focus on these to explore their roles in the pathogenesis and diagnosis of SCLC. However, the present study has certain limitations: One is that the microarray data were obtained from GEO database, not generated by the authors. Another limitation of the study is the relatively small sample size.

In summary, based on the gene expression profile analysis of microarray datasets, the present study identified the common deregulated DEGs between normal lung tissues and SCLC tissues, associated signaling pathways and hub genes in the network in different datasets, which may possess important roles in the carcinogenesis and development of SCLC. These findings may provide new clues for the investigation of the potential biomarkers and biological mechanisms of SCLC, further developing the potential diagnosis and therapeutic intervention methods of SCLC.

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Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding authors on reasonable request.

Authors' contributions

YL and JG designed the study. PW, TC, ZS and TW analyzed the microarray datasets and interpreted the results. HG and NW downloaded the gene expression profile from the Gene Expression Omnibus. TC and TW wrote and edited the manuscript. All authors critically reviewed the content and approved the final version for publication.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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