Comprehensive analysis of differential expression profiles reveals potential biomarkers associated with the cell cycle and regulated by p53 in human small cell lung cancer

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Abstract. Small cell lung cancer (SCLC) is the subtype of lung cancer with the highest degree of malignancy and the lowest degree of differentiation. The purpose of this study was to investigate the molecular mechanisms of SCLC using bioinformatics analysis, and to provide new ideas for the early diagnosis and targeted therapy of SCLC. Microarray data were downloaded from Gene Expression Omnibus. Differentially expressed genes (DEGs) in SCLC were compared with the normal lung samples and identified. Gene Ontology (GO) function and pathway analysis of DEGs was performed through the DAVID database. Furthermore, microarray data was analyzed by using the clustering analysis tool GoMiner. Protein-protein interaction (PPI) networks of DEGs were constructed using the STRING online database. Protein expression was determined from the Human Protein Atlas, and SCLC gene expression was determined using Oncomine. In total, 153 DEGs were obtained. Functional enrichment analysis suggested that the majority of DEGs were associated with the cell cycle. *CCNB1, CCNB2, MAD2L1* and *CDK1* were identified to contribute to the progression of SCLC through combined use of GO, Kyoto Encyclopedia of Genes and Genomes enrichment analysis and a PPI network. mRNA and protein expression were also validated in an integrative database. The present study indicated that the formation of SCLC may be associated with cell cycle regulation. In addition, the four crucial genes *CCNB1*, *CCNB2*, *MAD2L1* and *CDK1*, which are downstream of p53, may have important roles in the occurrence and progression of SCLC, and thus may be promising potential biomarkers and therapeutic targets.

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Abbreviations: SCLC, small cell lung cancer; GEO, Gene Expression Omnibus; GO, Gene Ontology; PPI, protein-protein interaction; NCBI, National Center of Biotechnology Information; RMA, Robust Multiarray Average; FDR, false discovery rate; BP, biological process; CC, cellular component; MF, molecular function

Key words: small cell lung cancer, microarray analysis, function enrichment, DAVID, cell cycle

Introduction

Lung cancer (LC) is one of the most common malignancies, which causes great harm to human health and is currently the leading cause of cancer-associated death worldwide (1). According to the histological type classification, LC can be divided into two subtypes: Small cell LC (SCLC) and non-SCLC (NSCLC). SCLC is a special type of LC, featuring the highest degree of malignancy and the lowest degree of differentiation among all the subtypes of LC (2,3), and accounting for ~20% of the total reported LC cases (4-6). Patients suffering from SCLC may be discovered to have lymphatic metastasis, Similar to the viruses, cancer cells enter the circulation and are transferred to distant organs of the body. Among all types of lung tumors, SCLC has the worst prognosis. Due to its high sensitivity to radiotherapy and chemotherapy, SCLC is frequently treated with systemic chemotherapy, combined with radiotherapy and surgery (7). Such treatment can reduce the rate of local recurrence for patients with SCLC, and ~25% of patients are able to achieve long-term survival and an improved prognosis (8-10).

Despite the sensitivity of SCLC to chemotherapy, the 2-year survival rate for most patients only reaches 5%, due to the characteristic distant metastases that arise in the early stages. Overall, 90% of patients die within five years of the diagnosis (11,12). The treatment options for SCLC have not substantially improved over the last four decades, and no

targeted therapy has been approved, as compared with the treatments available for NSCLC (13). Therefore, ongoing progresses in elucidating the pathogenesis of SCLC may be a basis for the development of treatments for SCLC (14).

With the rapid development of molecular biology and molecular genetics, the molecular mechanisms of SCLC were determined to be partially the result of genetic abnormalities (14,15). Many carcinogenic factors can be induced by the activation of oncogenes and the inactivation of tumor suppressor genes, leading to cell transformation and carcinogenesis (16). Therefore, exploring the pathogenesis of related genes of key signaling pathways and the corresponding mediated signaling pathways is necessary to analyze the occurrence of SCLC; this may be of benefit for the discovery of targeted therapy molecular markers to assist early diagnosis, and may also provide a molecular basis for treatment.

In the cell cycle, a number of events occur in order to replicate all cellular components (CCs) and produce two daughter cells. The cell cycle is strictly controlled by a series of sophisticated signaling pathways guiding the growth, DNA replication and division of a normal cell (17). Moreover, it was widely considered that the G0/G1-S phase and the G2-M phase were the key periods of regulation in the cell cycle (18). Studies have shown that dysregulation of the cell cycle is one of the key characteristics of tumors. Tumor cells must break through the cell cycle arrest that controls cell division. High expression of cell cycle regulation-related genes is often observed in a variety of human tumors, indicating the important roles of the cell cycle during the occurrence and development of tumors.

In nonfunctional pituitary adenomas (NFPA), the overexpression of *CDK1* and *CDC25A* may have an important role in promoting pituitary tumors in the G2/M transition phase (19). It has been reported that cell proliferation in hepatocellular carcinoma, nasopharyngeal carcinoma, breast cancer and colorectal cancer can be inhibited by regulating the expression of genes that induce cell cycle arrest at the G1/S phase (20-22). Similarly, in LC, aberrant expression of the cell cycle regulation-related genes *Cyclin D1, Cyclin E, Cyclin A, CDC25A* and *CDK4* could facilitate the transcription and expression of genes that are associated with cell cycle progression (23). However, the functions of the cell cycle and its regulatory proteins in SCLCs have not been fully clarified.

In this study, we analyzed microarray data from SCLC using a series of bioinformatics tools in an integrated manner. Aiming to elucidate the molecular mechanism of SCLC, crucial genes associated with the cell cycle were identified, which may be helpful for SCLC diagnosis and targeted therapy.

Materials and methods

Data source of gene expression. The gene expression profiles of GSE43346 were obtained from the Gene Expression Omnibus (GEO) database of the National Center of Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/geo). The platform of the GPL570 [(HGU133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array] was applied in the expression array (24). There were 24 samples contained in the datasets used for the analysis, including 23 sample groups of SCLC and 1 control group. The data (CEL form) and annotation files were downloaded for further an alysis.

Data pre-processing. The original expression datasets under all conditions were normalized by the Robust Multiarray Average (RMA) method (25) in the clusterProfiler package of R software (26) to obtain the gene expression matrix. The t-test method in the Limma package in R software was used to identify DEGs between SCLC and normal lung samples. Values of |log fold-change (FC)| >2.0 and P-value <0.05 were selected as the cut-off criteria.

Functional enrichment analysis. In order to better understand the functions of the DEGs, R software was used to perform enrichment analysis on the Gene Ontology (GO) (27) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (28). In this study, the KEGG database was applied to perform enrichment analysis of the DEGs, in order to identify signaling pathways that may be involved in the occurrence and development of SCLC. We relied on the web-based search engine DAVID (https://david.ncifcrf.gov) to count GO terms and KEGG pathways (29) with a P-value of <0.01 and a gene number of >2. Based on the standard clustering analysis method and novel gene clustering scheme, GoMiner (https://discover.nci.nih.gov) (30) was adopted to study the microarray data of SCLC for the purposes of further evaluating the biological relevance, functional characteristics and false discovery rate (FDR) <0.05 and P-value <0.05.

Protein-protein interaction (PPI) network construction. The tool used to retrieve interactions between the genes and proteins, STRING (31), is a database that can provide comprehensive information regarding the interactions between proteins, including the prediction of interactions and experimental studies data. Cytoscape is a conventional bioinformatics software program used for visual biological networks and data integration. In this study, the STRING online tool was applied to analyze the PPIs with interactions of combined score >0.4 among the DEGs. Then, the network was constructed with Cytoscape (32).

Analysis of mRNA expression in human SCLC. Protein expression in SCLC and normal tissues was determined using the Human Protein Atlas (www.proteinatlas.org). SCLC gene expression was identified by analyzing the Roessler and TCGA databases available in Oncomine (Compendia Biosciences; www.oncomine.org). High and low values were defined as above and below the mean value, respectively.

Results

Identification of DEGs. In our study, gene expression profiles from GSE43346 were utilized in order to compare the expression of specific genes between SCLC and normal lung samples. Genes with corrected P-values of <0.05 and an absolute FC of >2 were considered to be DEGs. As shown in Fig. 1, blue represents the data prior to correction and the middle line represents the median (Fig. 1A). The Limma data package was used to correct the original data (Fig. 1B). The results showed that 153 genes between the SCLC and the normal

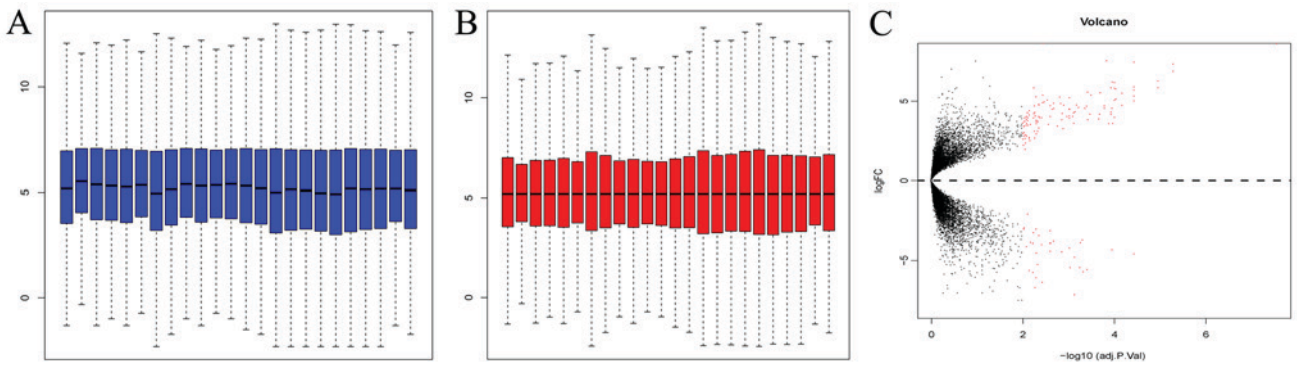

Figure 1. Identification of expression differences between normal and SCLC. data normalization boxplot. (A) The original data of the boxplot. (B) the normalized boxplot. The original data were normalized by R package limma. (C) Volcano plot of the differential mRNA expression analysis. X-axis: -log10 (FDR P-value); Y-axis: log2 fold-change for each probes; Vertical dotted lines: Fold-change ≥2 or ≤2; Horizontal dotted line: the significance cutoff (FDR P-value=0.01). the Valcano figure compiled from data of GSE43346, which demonstrated 21,653 probe sets representing 153 DEGs between the two groups of SCLC vs. normal cell in tumor patients, the red indicate the DEGs. SCLC, small cell lung cancer; FDR, false discovery rate; DEG, differentially expressed genes.

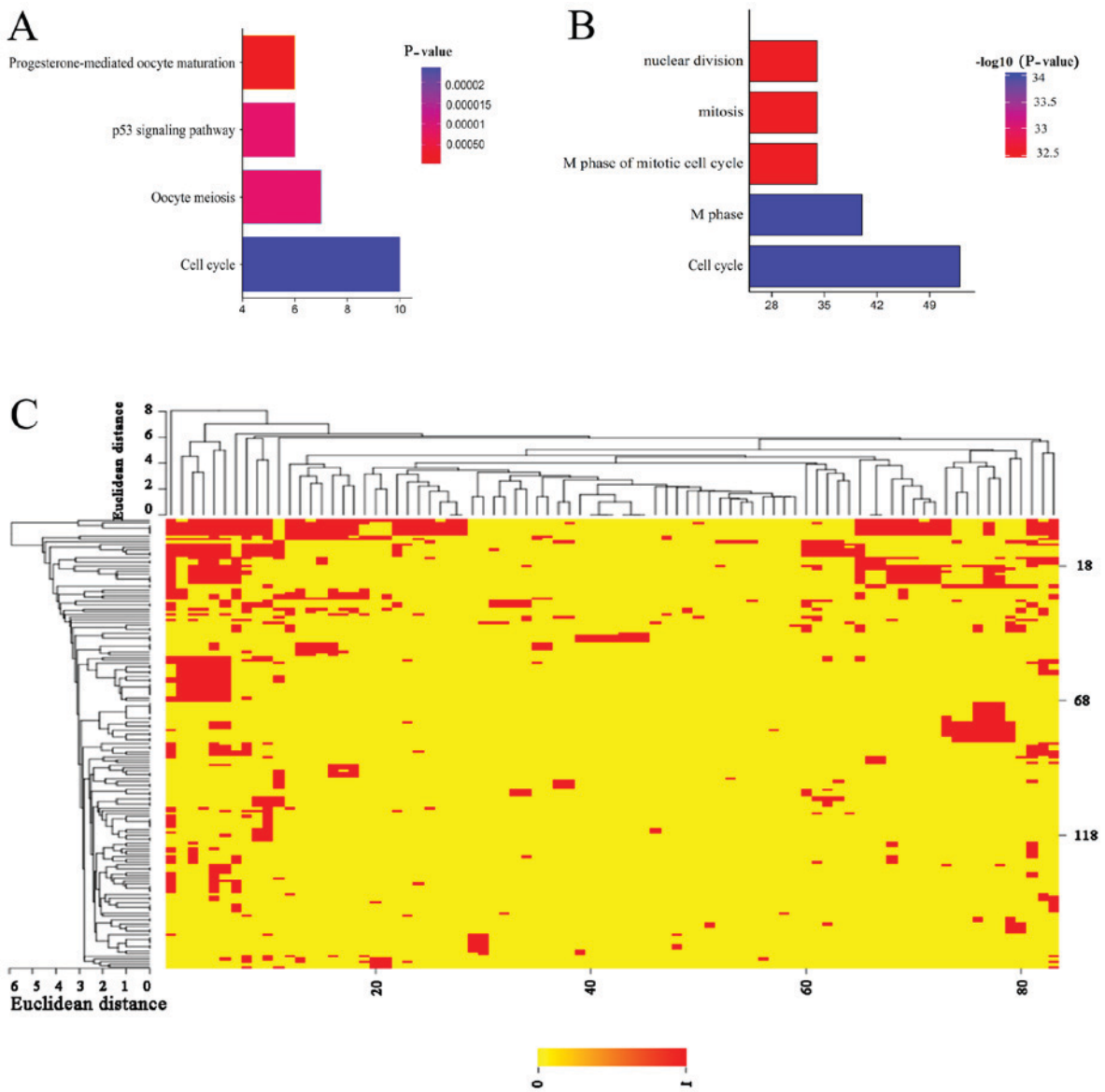

Figure 2. KEGG pathway and enriched GO terms of functional enrichment analysis. (A) four pathways enriched KEGG pathway. gene count of the cell cycle, oocyte meiosis, p53 signaling pathway, progesterone-mediated oocyt. (B) Top five enriched GO for differentially expressed genes. Gene count of M phase, cell cycle, nuclear division, mitosis, M phase of mitotic cell cycle. (C) CIM cluster with functional categories related to cell cycle, FDR <0.05. The cluster is enriched for cell division and cell cycle regulation. Red, genes are mapped to GO categories; yellow, no association; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; FDR, false discovery rate

Table I. The four enriched KEGG pathways for differentially expressed genes.

KEGG pathway	Gene counts	P-value	Genes
hsa04110:Cell cycle	10	7.67E-08	*CCNB1, CDK1, MAD2L1, CCNB2, BUB1, TTK, CHEK1, PTTG1, GADD45B, CCNA2*
hsa04114:Oocyte meiosis	7	7.34E-05	*CCNB1, CDK1, MAD2L1, CCNB2, BUB1, AURKA, PTTG1*
hsa04115:p53 signaling pathway	6	7.59E-05	*CCNB1, CDK1, CCNB2, RRM2, CHEK1, GADD45B*
hsa04914:Progesterone-mediated oocyte maturation	6	2.33E-04	*CCNB1, CDK1, MAD2L1, CCNB2, BUB1, CCNA2*

KEGG, Kyoto Encyclopedia of Genes and Genomes

group (119 upregulated and 34 downregulated genes) differed greatly in expression. Additionally, the number of upregulated genes was markedly increased compared with the downregulated genes. Red dots above and below the imaginary line in the volcano plot represent the upregulated and downregulated genes, respectively (Fig. 1C).

KEGG and GO enrichment. To improve our understanding of the function of these DEGs, we carried out GO enrichment and KEGG pathway analysis on the aforementioned 153 DEGs using the DAVID database, with P<0.05. KEGG pathway enrichment showed that four pathways were enriched: Cell cycle, p53 signaling pathway, oocyte meiosis, and progesterone-mediated oocyte maturation (Fig. 2A). DEGs, including cyclin B1 (*CCNB1*), cyclin-dependent kinase 1 (*CDK1*), mitotic spindle assembly checkpoint protein (*MAD2L1*) and checkpoint kinase 1 (*CHEK1*) were identified in the cell cycle and p53 signaling pathways in this study, and are listed in Table I.

GO enrichment results showed that a total of 50 terms containing upregulated and downregulated DEGs were obtained (Fig. 2B). With GoMiner, clustering was carried out in public databases to discover some information associated with cell development and function. Relying on GoMiner, we identified that the enrichment results were associated with cell division and cell cycle regulation. From the Fig. 2C, it can be concluded that 80 genes were enriched in 167 GO function terms, and were closely correlated with cell cycle development. Among the clustering results obtained, the top ten genes were *CCNB1*, *TTK, BUB1*, *MAD2L1*, *CENPE*, *NDC80*, *BIRC5*, *CCNA2*, *CDK1* and *BRCA1*. Meanwhile, cell division, M phase, mitosis, organelle fission and mitotic cell cycle in the M phase ranked as the top five, respectively, among the enriched GO terms.

The upregulated genes were mainly enriched in GO biological process (BP) terms. The GO CC terms comprised the spindle, condensed chromosome and microtubule cytoskeleton. The GO terms gathering the largest number of downregulated genes included conditions of response to chromosome organization, regulation of response to external stimuli, and positive regulation of cellular protein metabolic processes. Furthermore, downregulated genes were primarily correlated with the compound terms of spindle microtubule, nucleoplasm and microtubule in CC of the GO results, and associated with terms of polysaccharide binding, pattern binding and ribonucleotide binding in molecular function (MF) of the GO results. The top three terms were M phase (*KIF23*, *KIFC1*, *PRC1* and *TTK*) (P=1.66E-34), cell cycle (*KIF23*, *KIFC1*, *CLSPN* and *PRC1*) (P=3.02E-34) and nuclear division (*KIF23*, *KIFC1*, *ANLN*, *AURKA* and *PTTG1*) (P=1.74E-32), respectively, as shown in Table II.

PPI network construction and analysis. The PPI networks of upregulated and downregulated genes are shown in Fig. 3A. The PPI relationships between the 90 DEGs were determined using the STRING tool. Then, a PPI network was constructed using Cytoscape; we built the network with 97 nodes and 609 edges. The top 15 degree hub nodes in the PPI network were as follows: *TOP2A, PRC1, CDK1, NUSAP1, BUB1, SPC24, CENPF, CCNB2, MAD2L1, KIFC1, BRCA1, BIRC5, CHEK1, KIFC1* and *TTK*. These genes (proteins) could have an important role in the progression of SCLC.

The original PPI data was statistically analyzed. The abscissa represents the number of nodes, each of which has five gradients. The ordinate represents the number of genes. As shown in the figure, nodes for genes 0-5 achieved the largest number of genes and nodes for genes 21-25 reached the minimum number of genes (Fig. 3B). Meanwhile, the Cytoscape Networker Analyzer was adopted to analyze the network and identify the shortest path between two nodes, the results of which are presented as a histogram (Fig. 3C). The shortest path length distribution may indicate the inherent property of the analyzed network. The predictive ability of the biological network can be enhanced by decreasing the complexity of the data.

Key genes cross analysis and validation. We analyzed the enrichment analysis data of DEGs of the KEGG pathway and the PPI network. The four significantly enriched KEGG pathways in the module included cell cycle, oocyte meiosis, p53 signaling pathway and progesterone-mediated oocyte maturation. It is worth noting that *CCNB1*, *CDK1*, *MAD2L1* and *CCNB2* were significantly enriched in these four pathways. Specifically, it was found that the four genes in the SCLC specimens exhibited strong expression in compared with those in normal lung sample (Fig. 4). The immunohistochemical schematic diagrams for the *CCNB1*, *CDK1*, *MAD2L1* and *CCNB2* genes from SCLCs and

Table II. Top ten enriched GO for differentially expressed genes.

GO ID	Term	Count	P-value	Genes
GO:0000279	M phase	40	1.66E-34	*KIF23*, *KIFC1*, *PRC1*, *TTK*, *CHEK1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *SGOL2*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *MND1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *RAD54L*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0007049	Cell cycle	53	3.02E-34	*KIF23*, *KIFC1*, *CLSPN*, *PRC1*, *DTYMK*, *E2F8*, *TTK*, *AURKA*, *PTTG1*, *CDCA2*, *CDCA5*, *CCNA2*, *ASPM*, *CDCA3*, *CDK1*, KIF11, SGOL2, KIF15, MND1, NUSAP1, PBK, UHRF1, *MAD2L1*, *ZWINT*, *G0S2*, *HAUS8*, *SPAST*, *FOXM1*, *CHEK1*, *ANLN*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *HJURP*, *BUB1*, *HELLS*, *CKAP2*, *DLGAP5*, *GMNN*, *NUF2*, *CENPF*, *BIRC5*, *NDC80*, *CENPE*, *RACGAP1*, *RAD54L*, *BRCA1*, *CCNB1*, *NEDD1*, *CCNB2*, *CHAF1B*
GO:0000280	Nuclear division	34	1.74E-32	*KIF23*, *KIFC1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0007067	Mitosis	34	1.74E-32	*KIF23*, *KIFC1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0000087	M phase of mitotic cell cycle	34	3.23E-32	*KIF23*, *KIFC1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0048285	Organelle fission	34	6.86E-32	*KIF23*, *KIFC1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0022403	Cell cycle phase	40	1.30E-30	*KIF23*, *KIFC1*, *PRC1*, *TTK*, *CHEK1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *SGOL2*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *MND1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *RAD54L*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0000278	Mitotic cell cycle	37	1.17E-28	*KIF23*, *KIFC1*, *PRC1*, *TTK*, *CHEK1*, *ANLN*, *AURKA*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *BUB1*, *CDCA2*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *DLGAP5*, *KIF15*, *NUF2*, *CENPF*, *NUSAP1*, *BIRC5*, *CENPE*, *NDC80*, *PBK*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*
GO:0051301	Cell division	34	3.41E-28	*KIF23*, *KIFC1*, *PRC1*, *ANLN*, *PTTG1*, *SPC24*, *SPC25*, *NCAPH*, *SAC3D1*, *NCAPG*, *CDCA2*, *BUB1*, *CCNA2*, *CDCA5*, *ASPM*, *HELLS*, *CDCA3*, *CDK1*, *KIF11*, *SGOL2*, *NUF2*, *CENPF*, *NUSAP1*, *BIRC5*, *CENPE*, *NDC80*, *RACGAP1*, *CCNB1*, *NEDD1*, *MAD2L1*, *CCNB2*, *ZWINT*, *HAUS8*, *SPAST*

GO, gene ontology.

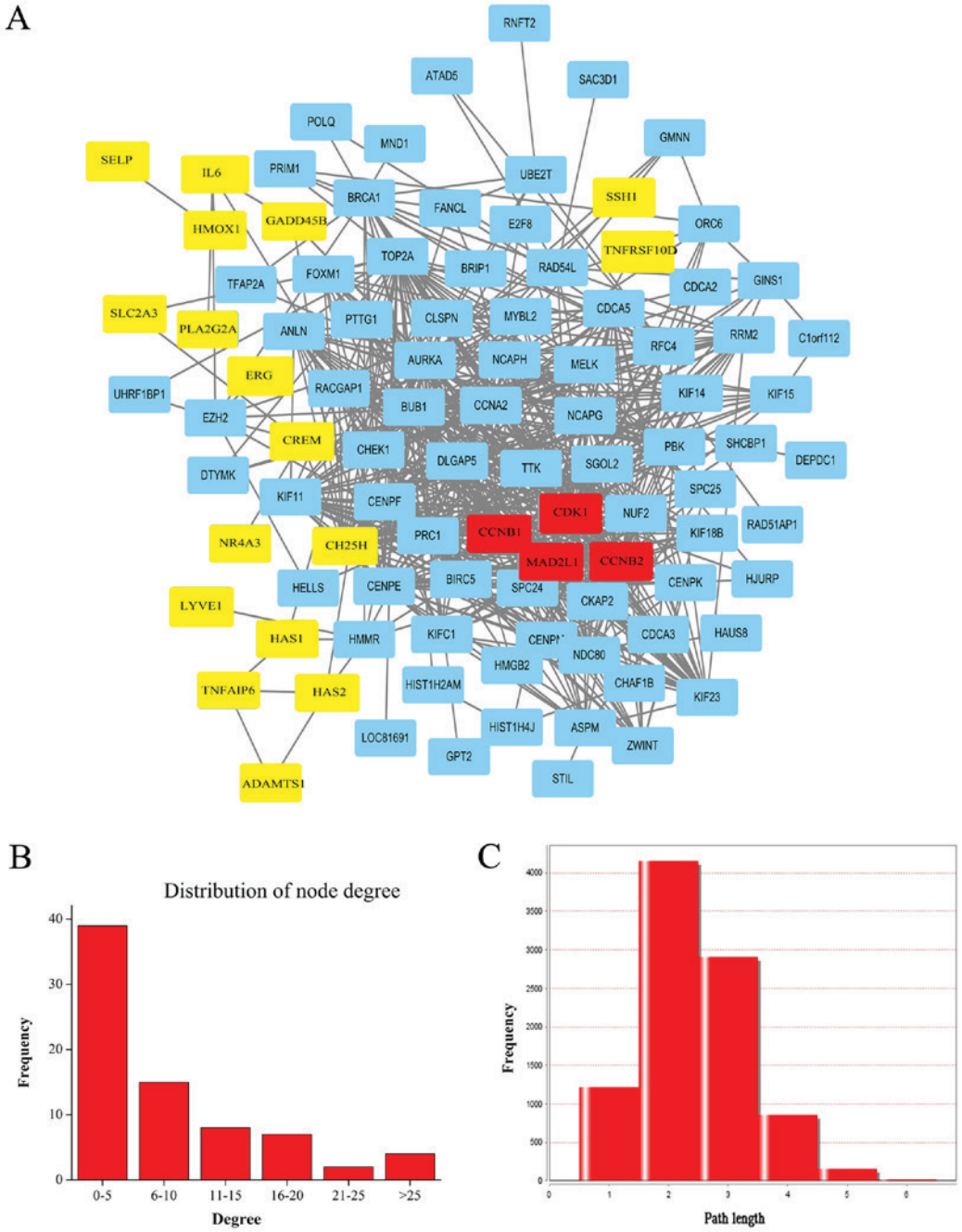

Figure 3. PPI networks of DEGs. (A) Nodes stand for proteins and edges represent interactions between two proteins. Yellow, downregulated genes; blue, upregulated genes; red, key genes. (B) Histogram statistics of node distribution in PPI. (C) distribution of shortest path. PPI, protein-protein interaction; DEGs, differentially expressed genes.

normal lung tissues were obtained through the Human Protein Atlas. Additionally, the mRNA expression levels of these genes in SCLC tissues were analyzed by Oncomine, the results of which were consistent with our analysis.

Discussion

In this study, we analyzed GSE43346 to obtain 153 differentially-expressed genes, including 118 upregulated and 34 downregulated genes. We found that these genes were significantly enriched in four pathways, including cell cycle, oocyte meiosis, p53 signaling pathway, and progesterone-mediated oocyte maturation. Certain genes, including *CCNB1*, *CCNB2*, *CDK1* and *MAD2L1*, were identified to be significantly upregulated in these pathways. In our further study, we will collaborate with clinical research teams to verify the roles of candidate targets in tumorigenesis, cancer progression and evolvement of resistance to therapy.

Tumor suppressor p53 is encoded by the homologous *TP53* genes, which can slow down or monitor cell division (33). In total, >50% of all malignant tumors have been found to exhibit mutations in this gene (34). Mutant p53, as a result of a *TP53* gene mutation, is a tumor-promoting factor, which can lead to tumor formation or cell transformation and eliminate the functions of normal *TP53* genes (35,36). The *TP53* gene is ranked first among all the genes discovered in terms of its correlation

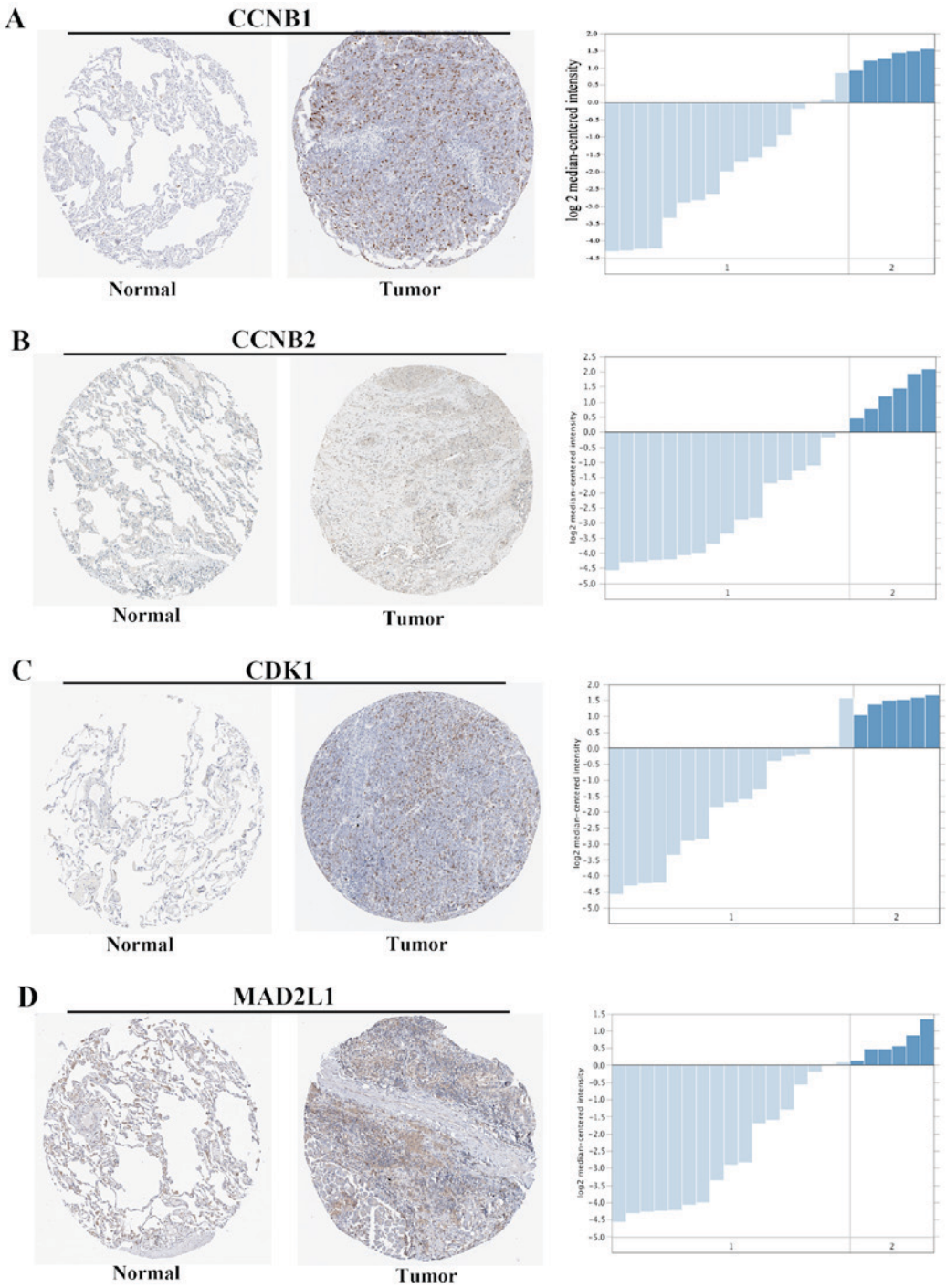

Figure 4. Gene expression in human small cell lung cancer specimens. (A) *CCNB1*, (B) *CCNB2*, (C) *CDK1*, (D) *MAD2L1*. Genes expression of four genes in normal lung tissue and small cell lung cancer specimens were indicated. Images were taken from the Human Protein Atlas (http://www.proteinatlas.org) online database (left). Oncomine data showing gene expression in normal vs. tumor of lung (n=22) (right). CCNB1, cyclin B1; CDK1, cyclin-dependent kinase 1; MAD2L1, mitotic spindle assembly checkpoint protein.

with human cancer cases (37,38). Mutations in this gene are likely to be an important factor underlying tumorigenesis in humans. In the cell cycle, the G2/M phase DNA damage checkpoint is the last chance for cells to repair DNA damage prior to mitosis. The regulatory function of p53 in monitoring the cell cycle is observed at the G1 and G2/M stage (39,40); *CCNB1* and *CDK1* also act on these two monitoring points.

CCNB1 is a cell cycle protein that serves an essential role in the G2/M phase. Studies on NSCLC have shown that high expression of CCNB1 is associated with poor prognosis (41). The expression level of CCNB1 can also be used as a marker to determine the prognosis of patients with breast cancer (42). The expression level of CCNB1 is associated with the tumor grade; a higher level of CCNB1 indicates a larger tumor size and a higher probability of metastasis. Therefore, the expression of CCNB1 can act as a prognostic predictor (43,44). *CDK1* is the cell cycle regulatory protein with the greatest pleiotropy (45). CDK1 interacts with nine different cyclins throughout the cell cycle. These interactions with cyclins are important for activating its kinase activity, and also for the recruitment and selection

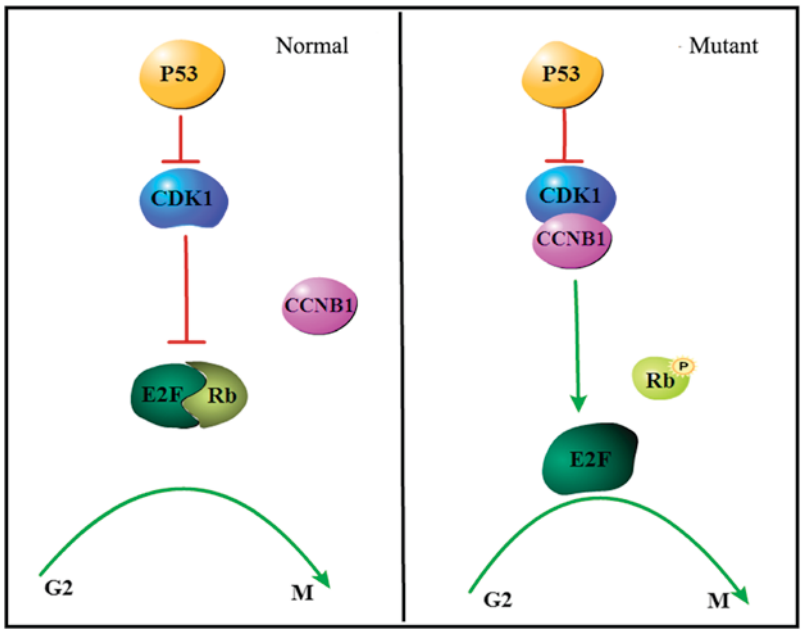

Figure 5. A schematic representation of CDK1/CCNB1 in regulation of small cell lung cancer cell cycle. In normal cells, p53 protein activates and inhibiting the binding of CDK1 and CCNB1, resulting in Rb non-phosphorylation possibly, then, cell cycle arrest (left). In the mutant, p53 expression is abnormal, cdk inhibitory activity is not activated, After binding to CCNB1, the CDK1 protein acted on the E2F-Rb complex, which could lead to the phosphorylation of Rb and promote the transition of the cell cycle from the G2 phase to the M phase (right). CDK1 cyclin-dependent kinase 1; CCNB1, cyclin B1.

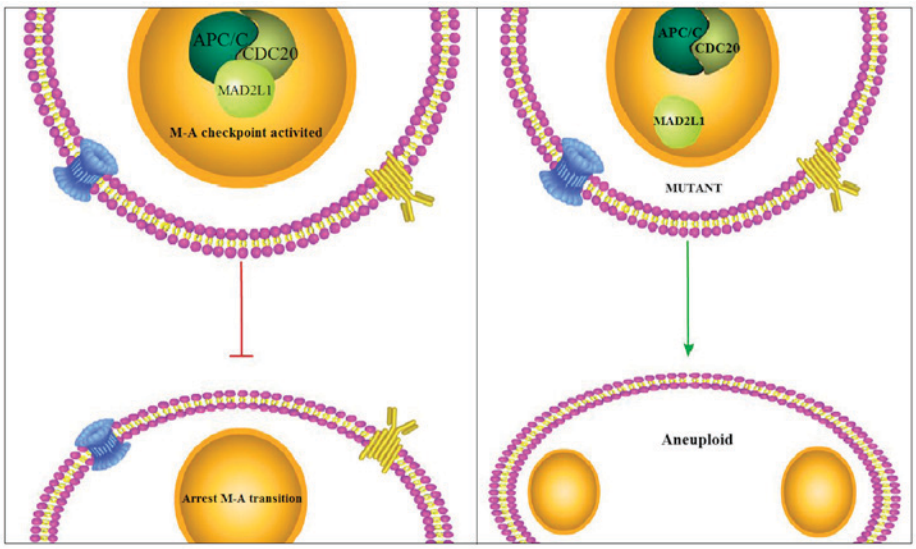

Figure 6. A schematic representation of MAD2L1 in regulation of small cell lung cancer mitotic arrest. Cell cycle abnormalities, mitotic monitoring points are activited, MAD2L1 binding to the APC/C-CDC20 complex, inhibit the ubiquitination function of complex, making mitotic arrest (left). In tumor cells, MAD2L1 is highly expressed in small cell lung cancer, which may affect the function of APC/C-CDC20 complex, then separation of chromosomal abnormalities, resulting in polyploid generation (right). MAD2L1, mitotic spindle assembly checkpoint protein.

of downstream proteins (46). For example, CDK1-CCNB1 complexes can induce abnormal regulation of downstream protein phosphorylation, leading to uncontrolled cell proliferation (47). Therefore, selective inhibition of CCNB1 or CDKs can limit the progress of the cell cycle and/or induce cellular apoptosis. To date, there have been many studies showing that inhibiting the expression of CCNB1 or CDK1 could suppress the occurrence and development of tumors, via the p53/Bax apoptosis pathway in tumor cells. Therefore, CCNB1, CDK1 and their downstream effector CDC25C could be therapeutic targets for antineoplastic treatment (48).

In mitosis, the segregation of chromosomes is controlled by the cyclin checkpoint. If the chromosomes are not properly attached to the mitotic spindle, the cell cycle may be arrested (49). The spindle monitoring point is the key time at which to correct the separation of the sister chromatid. The spindle checkpoint regulator MAD2L1 may protect cells from abnormal chromosome segregation (50). However, defects in the spindle monitoring point may lead to aneuploidy. The mitotic arrest defective protein MAD2L1 is an important component of the anaphase-promoting complex (APC/C). By directly binding to CDC20 and suppressing the activity of APC/CCDC20, cell

arrest may be inhibited during anaphase (51). High expression of MAD2L1 is a common phenomenon in a variety of tumors, and is associated with poor prognosis in breast, gastric and colorectal cancer, as well as in other tumor types (52-55).

Abnormal regulation of the cell cycle will lead to abnormal chromosomes, uncontrolled cell proliferation and apoptosis, and eventually the formation of malignant tumors. Based on the results of this study, it can be speculated that the DNA in SCLC cells suffers damage. As shown in Fig. 5, in normal cells, if the DNA is damaged, p53 inhibits the binding of CDK1 and CCNB1, and thus CDK1 is disable to induce dissociation of the E2F-Rb complex, leading to further cell cycle arrest at the G2-M phase, where apoptosis may be induced. By comparison, the dysfunctional p53 fails to suppress formation of the CDK1-CCNB1 complex, and this newly formed CCK1-CCNB1 complex promotes the dissociation of the E2F-Rb complex via phosphorylation of Rb in the G1 phase. Subsequently, the E2F obtained may accelerate cell cycle progression from the G2 to the M phase (56,57).

It was widely accepted that MAD2L1 binds with the APC/C and CDC20 to form a ternary complex that can activate the M-A checkpoint to arrest the M-A transition of a normal cell, if there exists abnormal segregation of chromatin. However, for atypical cells, the overproduction of E2F1, which is the transcription factor of *MAD2L1*, might influence the formation of the MAD2L1-APC/C-CDC20 ternary complex and, thus, fail to activate the M-A checkpoint of the cell, ultimately resulting in the production of atypical cells with aneuploidy (Fig. 6) (58).

In conclusion, the aforementioned speculations require further experimental validation. The p53 signaling pathway warrants further investigation, with the aim of finding novel and targeted therapies for SCLC, as well as tumor markers, and ultimately to promote a breakthrough in the treatment of LC.

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