

Identification and validation of aberrant regulation of miR-445-3p /TTK , miR-140-5p/TTK and miR-133b/CDCA8 in small-cell lung cancer via bioinformatics analysis

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Abstract

Background: Small-cell lung cancer (SCLC) remains the leading form of malignant lung cancer, but little bioinformation on SCLC is available. This study explored the molecular targets of SCLC by evaluating differentially expressed genes (DEGs) and differentially expressed microRNAs (miRNAs) (DEMs).

Methods: Five mRNA expression profiles and two miRNAs expression profiles from Gene Expression Omnibus (GEO) were downloaded. R software was utilized to analyze the DEGs and DEMs between SCLC and normal samples. The DEGs were analyzed via functional enrichment analyses and were used to construct protein-protein interaction (PPI) networks. DEM targets were then predicted and intersected with the DEGs. Furthermore, the hub genes of SCLC in the overlapping DEGs were analyzed in Oncomine. Finally, the expression of DEM-hub gene pairs were verified in tissues by RT-qPCR and Western blotting.

Results: In total, 236 common DEGs and 104 common DEMs were identified. Functional enrichment analysis showed the DEGs were primarily enriched in 'cell cycle', 'DNA replication' and 'oocyte meiosis'. Twenty hub genes and five modules were identified from the PPI network. Furthermore, 6732 targeted genes of the DEMs were predicted. After intersecting with DEGs, 54 genes and 153 miRNA-mRNA pairs were eventually identified aberrant regulation in SCLC. MiR-445-3p/TTK, miR-140-5p/TTK and miR-133b/CDCA8 were identified as DEM-hub gene pairs. Oncomine analysis confirmed the overexpression of TTK and CDCA8 in SCLC. Further validation demonstrated that TTK and CDCA8 levels in SCLC tissue samples were markedly increased relative to normal controls, while miR-445-3p, miR-140-5p, and miR-133b levels were lower in SCLC samples than in controls.

Conclusions: Our results revealed key miRNA-mRNA pairs associated with SCLC, providing new insights into potential disease targets.

Background

Small-cell lung cancer (SCLC) remains the most prevalent malignant lung cancer subtype, accounting for approximately 20% of lung cancers [1]. While sensitive to chemotherapy and radiotherapy, SCLC often becomes resistant to these therapies and undergoes systemic metastasis [2]. In addition, there are few targeted drugs for SCLC in the clinic. Thus, the efficacy of SCLC treatment has not improved substantially over the past few decades [3]. Due to the unique carcinogenic mechanism of SCLC and the difficulty in obtaining samples, molecular mechanism studies as well as subsequent bioinformatics data of SCLC are also limited. Under these circumstances, further identification of the molecular mechanisms is essential to enhance SCLC patient treatment.

As next-generation sequencing technology advances rapidly, microarrays have been widely used in the study of tumor genes, molecular targets of antitumor drug therapy and prognosis monitoring [4]. The integrated Gene Expression Omnibus (GEO) database is an extensive public source of gene expression data, providing many cancer gene expression profile datasets, including SCLC. In addition, recent studies have demonstrated that aberrant microRNA (miRNA) and mRNA regulation may lead to tumorigenesis [5,

6]. MiRNAs, as noncoding endogenous regulatory RNAs, bind to target mRNA 3'-untranslated regions (UTRs) to inhibit translation [7]. MiRNAs play an indispensable role in regulating proliferation, survival, development, apoptosis, pathogenesis resistance, and tumorigenesis [8–10]. Several reports have found that miRNAs are related to the pathogenesis of SCLC. For instance, miR-25 has been revealed to be overexpressed as a carcinogenic regulator in SCLC by targeting cyclin E2 [11]. MiR-34b-3p and miR-27a-5p significantly inhibited the progression of SCLC by regulating their target genes [12]. Since the regulatory system between mRNAs and miRNAs plays a complex role in biological functions [13], comprehensively analyzing differentially expressed genes (DEGs) as well as miRNAs (DEMs) in multiple datasets from GEO will shed light on their potentially crucial molecular mechanisms.

Though a previous study has explored DEGs and DEMs in SCLC [14], the sample size of the study was small, and only one dataset each for DEGs and DEMs was analyzed, which might have led to false positive results. In addition, to our knowledge, no bioinformatics studies of SCLC have validated the results in tissue. In the present study, with the larger sample size, the RobustRankAggreg package was used to eliminate batch differences as a means of improving precision. Furthermore, the overlapping genes between DEM-target genes and DEGs were extracted, and the most critical miRNA-mRNA pairs were selected for validation in tissue.

Methods

Expression prolife data

The GEO (<http://www.ncbi.nlm.nih.gov/geo>) database was used to collect expression data of genes and miRNAs. A total of five transcription profiles, GSE1037, GSE6044, GSE11969, GSE43346, and GSE108055, and two noncoding profiles, GSE19945 and GSE74190, were downloaded. The characteristics of all the mRNA and miRNA datasets are presented in Table 1.

Screening DEGs and DEMs

R (v 3.5.1; <https://www.r-project.org/>) with the limma package was used to screen relevant DEGs and DEMs. Original data were downloaded if the standardized data were not available. Log₂ conversion was performed for values that were not described as logarithms. The affy package was utilized to read CEL file expression data, whereas limma was employed to obtain the DEGs and DEMs from each dataset. The common DEGs were identified by integrating each DEG in the five mRNA datasets using the RobustRankAggreg package. The common DEMs were obtained by intersecting the two miRNA datasets. The included DEGs and DEMs met the criteria of adjusted $P < 0.05$ and $|\log_2 \text{fold change}| > 1.0$.

Enrichment analysis

GO and KEGG enrichment analyses were performed with the ClusterProfiler package of R software. GO enrichment analysis is widely used to investigate gene function, with GO covering three main ontologies: molecular function (MF), cellular component (CC), and biological process (BP). KEGG is a database

widely used for systematically analyzing high-level gene functions. The cutoffs for significance were both adjusted to $p < 0.05$ and $q < 0.05$.

Protein-protein interaction (PPI) network construction

The STRING tool (<https://string-db.org/>) was utilized to assess interactions among the DEGs and identify hub genes. Interactions with an interaction score > 0.9 were selected. We excluded disconnected nodes in the network. Significant network modules were screened with Cytoscape (v3.6.1; <http://www.cytoscape.org/>) software with the MCODE plug-in (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. depth = 100). Functional enrichment analysis was also performed for the genes in the significant modules.

DEM-DEG pair identification

FunRich software (version 3.1.3; www.cytoscape.org) was used to predict DEM target genes [15]. The overlapping genes between DEGs and the predicted genes of the counter-regulated DEMs with the same regulatory alterations were extracted using the Venny online Tool (version 2.1; <http://bioinfogp.cnb.csic.es>). Cytoscape software was utilized to illustrate and visualize the miRNA-mRNA regulatory network.

Cross-database validation via Oncomine analysis

Oncomine (oncomine.org) is considered to be the world's largest database of oncogene chips and comprehensive data mining platforms [16]. The hub genes among the overlapping genes between the DEGs and DEM-target genes were selected for verification in the Oncomine database. Oncomine data corresponding to expression value data (the preprocessed expression level was \log_2 normalized and median-centered) of normal and SCLC tissues were obtained. GraphPad Prism software (version 8.2; GraphPad, Inc.) was used to create box diagrams, and data were compared by Student's t-tests with $P < 0.05$ as the significance threshold.

SCLC patient samples

The Ethics Committee of The First Hospital of Jilin University (Changchun, China) approved the present study with the approval document number 2019-279. In total, 5 pairs of tumor and paracancerous tissues were obtained from SCLC patients at The First Hospital of Jilin University between January 2019 and January 2020. All patients provided informed consent before sample collection.

RT-qPCR

TRIzol (Invitrogen; Thermo Fisher Scientific, Inc. Waltham, MA, USA) was used for extracting sample RNA based on manufacturer's instruction. Reverse transcription (RT) of miRNA was conducted using a PrimeScript RT Kit (Thermo Fisher Scientific, Inc.), and a SuperScript II cDNA Conversion Kit (Thermo Fisher Scientific, Inc.) was used to prepare cDNA. RT-qPCR amplification of the cDNA was performed subsequently. GAPDH and U6 snRNA were used to normalize mRNA and miRNA expression, respectively.

SYBR Green Realtime PCR Master Mix was used for RT-qPCR with appropriate primers (Table 2) using an ABI 7500 Fast Real-Time PCR System. The $2^{\Delta\Delta Ct}$ approach was used for assessing relative gene expression.

Western blotting

RIPA buffer (Beyotime, Shanghai, China) was used for extracting protein prior to the use of a BCA kit for measuring protein concentrations (Beyotime). Protein samples of the same quantity were separated using SDS-PAGE prior to transfer onto PVDF membranes. The membranes were then blocked with 5% nonfat dry milk and incubated with anti-TTK primary (ab187520; 1:2,000; Abcam, Cambridge, UK) and anti-CDCA8 (ab74473; 1:1,000; Abcam) at 4 °C overnight. Appropriate HRP-conjugated secondary antibodies were then used to probe blots. Enhanced chemiluminescence (ECL; Thermo Fisher Scientific, Inc.) was then employed to detect proteins. The relative intensity was calculated by correcting for GAPDH (5174; 1:1000; CST, Boston, Massachusetts, USA) with ImageJ software.

Results

DEG and DEM identification

Compared with that in normal tissue samples, 910 DEGs, 442 DEGs, 1704 DEGs, 522 DEGs and 1793 DEGs were identified in SCLC samples in the GSE1037, GSE6044, GSE11969, GSE43346, and GSE108055 datasets, respectively. The volcano maps are shown in Fig. 1a-e. After integration, 236 DEGs, including 117 downregulated DEGs and 119 upregulated DEGs, were identified. The top 20 most significant up- and downregulated common DEGs are shown in Fig. 1f. By comparing the SCLC samples and normal tissue samples, 142 DEMs and 159 DEMs were detected in the GSE19945 and GSE74190 datasets, respectively (Fig. 2a and b). After intersection, 104 DEMs, including 60 downregulated DEMs and 44 upregulated DEMs (Fig. 2c and d), were identified.

DEG functional enrichment analysis

GO analysis of the DEGs demonstrated that the BPs were primarily enriched in 'nuclear division', 'mitotic nuclear division', 'organelle fission', 'regulation of mitotic cell cycle phase transition', and 'regulation of cell cycle phase transition'. Variations in CCs were enriched in 'spindle', 'chromosomal region', 'spindle pole', 'condensed chromosome', and 'cyclin-dependent protein kinase holoenzyme complex'. Regarding the MFs, the DEGs were enriched in 'cyclin-dependent protein serine/threonine kinase regulator activity', 'long-chain fatty acid binding', 'tubulin binding', 'water channel activity' and 'water transmembrane transporter activity'. The top 10 significant functions of the DEGs in the BP, CC, and MF categories were determined (Fig. 3a-c).

In KEGG analysis, 48 DEGs were mapped into 11 enriched pathways, including 'cell cycle', 'oocyte meiosis', 'complement and coagulation cascades', 'human T-cell leukemia virus 1 infection', 'DNA

replication', 'p53 signaling pathway', 'cellular senescence', 'tyrosine metabolism', 'mismatch repair', 'drug metabolism - cytochrome P450', and 'progesterone-mediated oocyte maturation' (Fig. 3d).

PPI network analysis

STRING was employed to prepare the PPI network containing 154 nodes and 695 edges (Fig. 4a). Based on the analysis results of the Cytoscape analysis, twenty DEGs with the highest degree of nodes were identified as hub genes (Fig. 4b). The MCODE plug-in was used to calculate five modules, among which the most significant module comprised 46 nodes (Fig. 4c) and was significantly enriched in pathways including 'oocyte meiosis', 'progesterone-mediated oocyte maturation', 'cell cycle', and 'p53 signaling pathway' (Fig. 4d).

MiRNA-mRNA regulatory network

To further explore the miRNA-mRNA regulatory network in SCLC, a total of 6732 DEM-target genes were predicted by FunRich software. After intersecting with common DEGs, there were 54 genes and 153 miRNA-mRNA pairs finally identified aberrant regulation in SCLC (Fig. 5a). Importantly, two hub genes (TTK and CDCA8) of SCLC were found among the intersecting genes, and miR-445-3p/TTK, miR-140-5p/TTK and miR-133b/CDCA8 were identified as DEM-hub gene pairs. The predicted binding sites of the three DEM-hub gene pairs are shown in Fig. 5b.

Validation of expression on the Oncomine database

TTK and CDCA8 were selected as hub genes among the overlapping genes for cross-validation in the Oncomine database. The Oncomine analysis indicated that TTK and CDCA8 were overexpressed in multiple cancers, with significantly increased expression ($P < 0.05$) in lung cancer samples in six and ten datasets, respectively (Fig. 6a). We further queried and downloaded the SCLC datasets containing TTK or CDCA8 in the Oncomine database. The analysis indicated that TTK and CDCA8 levels were elevated in SCLC tissues relative to normal tissues ($P < 0.001$; Fig. 6b and c).

RT-qPCR and Western blotting verification results

Two mRNAs (TTK and CDCA8) and three miRNAs (miR-455-3p, miR-140-5p and miR-133b) were verified in tissue samples. TTK and CDCA8 were markedly upregulated in SCLC tissues versus controls, while miR-455-3p, miR-140-5p, and miR-133b were markedly reduced in SCLC tissues relative to controls (Fig. 7a). The validation results of Western blotting were consistent with those of RT-qPCR, confirming that TTK and CDCA8 levels in SCLC tissues were increased relative to those in normal tissues at the protein level (Fig. 7b).

Discussion

SCLC is a rather malignant lung cancer that exhibits a low degree of differentiation, grows rapidly, has high vascularity, and undergoes early extensive dissemination, with extremely poor associated survival

[17]. Those with extensive disease have a survival of only eight to thirteen months, with a two-year survival rate of approximately 5% [18]. The first-line therapy of SCLC has not changed for decades in the clinic, and an effective therapeutic option for such recurrent-prone disease is still lacking [18, 19]. Although numerous studies have indicated that several molecules regulate the progression of SCLC, the underlying carcinogenesis mechanism remains unclear.

Herein, through the integrated analysis of SCLC, we detected 236 common DEGs and 104 common DEMs and conducted functional enrichment analysis of the former, revealing these DEGs to be significantly enriched in multiple signaling pathways, such as 'cell cycle', 'DNA replication', 'human T-cell leukemia virus 1 infection', 'oocyte meiosis', and 'p53 signaling pathway'. The PPI network filtered five molecules and twenty hub genes, which were considered to be the key genes for the development of SCLC. Subsequent integrated analysis of the DEG regulatory pairs revealed a total of 54 overlapping genes between the DEM-target genes and the DEGs. Particularly, TTK and CDCA8 were identified as the hub genes among the 54 overlapping genes and were targeted by miR-455-3p, miR-140-5p, and miR-133b.

Thr/Tyr kinase (TTK) phosphorylates serine, tyrosine, and threonine residues [20, 21]. TTK is important for mitosis as it influences the precise segregation of chromosomes and the duplication of centrosomes [22, 23]. The expression level of TTK changes dynamically in the cell cycle, and it increases during the G1/S cell cycle phase and peaks in the G2/M phase [24]. Thus, the expression of TTK is closely related to the cell cycle. It is worth mentioning that the cell cycle was also demonstrated as the most significant signaling pathway of SCLC in this integrated investigation. TTK has been well demonstrated as an oncogene, and the dysregulation of TTK is linked to several cancers, including neuroendocrine lung cancer [25-27]. Du *et al.* believed that TTK could play a tumorigenic role in neuroendocrine lung cancer in combination with LMO1 [28]. Furthermore, TTK inhibitors have been used in antitumor therapy [29-31]. Zheng *et al.* showed that TTK inhibitors induced pronounced anticancer effects by augmenting polyploidy and apoptosis in NSCLC [32]. Herein, TTK was identified as a hub gene among the overlapping genes between the DEGs and DEM target genes. Importantly, the investigation not only demonstrated the overexpression of TTK in SCLC but also suggested a marked reduction in the expression of miRNAs (miR-445-3p and miR-140-5p) that regulate TTK.

MiR-455-3p is an important regulator of multiple tumors [33, 34]. MiR-455-3p has been suggested to serve as an anti-oncogene or oncogene in different cancers. For example, a previous study revealed that upregulated miR-455-3p enhanced the progression of breast cancer by regulating E124, indicating that miR-455-3p plays a role as a tumor promoter in breast cancer [35]. In contrast, miR-455-3p has been suggested to play an important role as a cancer inhibitor in NSCLC by directly targeting HOXB5, with decreased expression of miR-455-3p identified as associated with poorer overall survival in patients with NSCLC [36]. In addition, Ning *et al.* also found a significant correlation between the overall survival of patients with lung squamous cell carcinoma and miR-455-3p expression level [37]. In general, miR-455-3p is dysregulated in several human tumors, which is in accordance with our findings in SCLC.

MiR-140-5p, as a tumor-suppressor, has been extensively studied recently because it is involved in the tumorigenesis of multiple kinds of tumors, including gastric cancer, breast cancer, colorectal cancer, etc. [38-40]. Furthermore, numerous studies have been performed regarding miR-140-5p in lung cancer; in one study, *miR-140-5p* was shown to repress the proliferation of NSCLC cells through the MMD/Erk signaling pathway [41]. *Yang et al.* revealed that miR-140-5p regulates the invasion and migration of NSCLC by targeting VEGFA [40]. The miR-140-5p target genes can serve as biomarkers of NSCLC, contributing to diagnosis and prognostic prediction [42, 43]. Nevertheless, to the best of our knowledge, neither miR-455-3p nor miR-140-5p has yet been identified as associated with SCLC, and we speculate that the aberrant regulation of TTK by miR-455-3p and miR-140-5p may be an underlying regulator of the pathogenesis of SCLC.

Human cell division cycle associated 8 (CDCA8) was another hub gene among the overlapping DEGs. As a chromosomal passenger complex component [44], CDCA8 upregulation is related to the carcinogenesis of a variety of tumors [45, 46]. CDCA8 has been proven to be a tumor promoter that is overexpressed in various kinds of tumors and is essential for cancer cell survival and malignancy [47]. High expression levels of CDCA8 are associated with the development and poor survival of malignancies such as breast cancer, osteosarcoma, and melanoma [48-50]. Furthermore, Bidkhori G *et al.* indicated that CDCA8 is linked with the cell cycle progression of lung adenocarcinoma [51]. Hayama S *et al.* demonstrated that CDCA8 is phosphorylated and coactivated by AURKB in NSCLC cells and that phosphorylated CDCA8 contributes to the survival and growth of NSCLC cells [52]. Thus, CDCA8 is considered a novel diagnostic and therapeutic target of great promise.

Although there have been several studies on CDCA8, the role of CDCA8 in SCLC has not been explored. The present study revealed that although CDCA8 was overexpressed in SCLC, the expression of miR-133b, which regulates CDCA8 expression, was significantly decreased.

MiR-133b, as a particular member of myomiRs, was originally thought to be muscle-specific and played a key regulatory role in muscle development and remodeling [53]. However, more recent work shows that miR-133b is downregulated in various cancers, indicating that it is closely linked to oncogenesis [54]. In the field of lung cancer research, Crawford M *et al.* first reported miR-133b underexpression in lung adenocarcinoma and found that it targeted the Bcl-2 family to inhibit tumors [55]. Furthermore, Liu *et al.* reported that the expression of miR-133b was decreased in NSCLC tissues and that miR-133b suppressed the development of NSCLC through targeting EGFR [56]. Lin *et al.* showed that miR-133b reduces cisplatin resistance in NSCLC by targeting GSTP1, and its overexpression suppresses the invasion and malignant growth of cisplatin-resistant NSCLC cells [57]. Nevertheless, the expression level and carcinogenic mechanism of miR-133b associated with SCLC remains unclear. We can infer that miRNA-133b regulating CDCA8 may be another underlying mechanism of the pathogenesis of SCLC.

However, our work has limitations. As a bioinformatics analysis, the results are based on publically available data instead of laboratory experiments. Although this study verified the expression at the gene level and protein level, we did not conduct functional verification of the genes *in vitro*. In addition, due to

the lack of publicly available data on the clinical information of SCLC patients, we did not further analyze the association between the identified genes and patient survival, tumor recurrence, tumor stage, sex, age, etc.

Conclusions

Our study integrated the expression profiles of mRNAs and miRNAs in SCLC and identified miRNA-mRNA pairs associated with SCLC. Importantly, TTK regulated by miR-445-3p and miR-140-5p, together with CDCA8 regulated by miR-133b, might be crucial to the molecular mechanisms of SCLC, providing new insights into potential targets for the therapy of this disease.

Abbreviations

SCLC: small-cell lung cancer; NSCLC: non small-cell lung cancer; GEO: Gene Expression Omnibus; DEG: differentially expressed gene; DEM: differentially expressed microRNA; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; UTR: 3'-untranslated regions

Declarations

Acknowledgement

Not applicable

Author contributions

MT: designed the main study, performed the laboratory experiments, and completed the writing of the manuscript; XG: performed the bioinformatics analysis; ZX and LF assisted in modifying the manuscript; WL: supervised the study and critically revised the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The data for bioinformatics analyzed in this study were downloaded from the GEO database. The accession numbers of the mRNA microarray database are GSE1037, GSE6044, GSE11969, GSE43346, and GSE108055. The accession numbers of the miRNA microarray database are GSE19945 and

GSE74190. Other data and materials in the current study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

The present study was reviewed and approved by Ethics Committee of The First Hospital of Jilin University, Changchun, China (approval number 2019-279). All patients had signed written informed consent before sample collection.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Characteristics of the mRNA and miRNA datasets used in this study

Dataset	Type	Platform	Tumor Count	Normal Count	Contributors
GSE1037	mRNA	GPL962	15	19	Jones <i>et al.</i> (2004)
GSE6044	mRNA	GPL201	9	5	Rohrbeck <i>et al.</i> (2008)
GSE11969	mRNA	GPL7015	9	5	Takeuchi <i>et al.</i> (2009)
GSE43346	mRNA	GPL570	23	1	Sato <i>et al.</i> (2013)
GSE108055	mRNA	GPL13376	12	10	Asiedu <i>et al.</i> (2018)
GSE19945	miRNA	GPL9948	35	8	Ohba T <i>et al.</i> (2010)
GSE74190	miRNA	GLP19622	16	44	Jin Y <i>et al.</i> (2015)

Table 2 Primer list

miR-455-3p-RT	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACGTGTAC-3'
miR-455-3p-F	5'-GGCGCAGTCCATGGGCAT-3'
miR-140-5p-RT	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACCTACCA-3'
miR-140-5p-F	5'-GGGCCAGTGGTTTTACCCTA-3'
miR-133b-RT	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACTAGCTG-3'
miR-133b-F	5'-CCGTTTGGTCCCCTTCAAC-3'
miR-ty-R	5'-GTGCAGGGTCCGAGGT-3'
TTK-197-F	5'-GAACATGCCACCACAAGATG-3'
TTK-197-R	5'-CCAAATCTCGGCATTCTGAT-3'
CDCA8-195-F	5'-CCTGACACCCAGGTTTGACT-3'
CDCA8-195-R	5'-GCAATACTGTGCCTCTGCAA-3'
GAPDH-127F \square H \square	5'-CCAGGTGGTCTCCTCTGA-3'
GAPDH-127R \square H \square	5'-GCTGTAGCCAAATCGTTGT-3'
U6-F	5'-CTCGCTTCGGCAGCACA-3'
U6-R	5'-AACGCTTCACGAATTTGCGT-3'

Figures

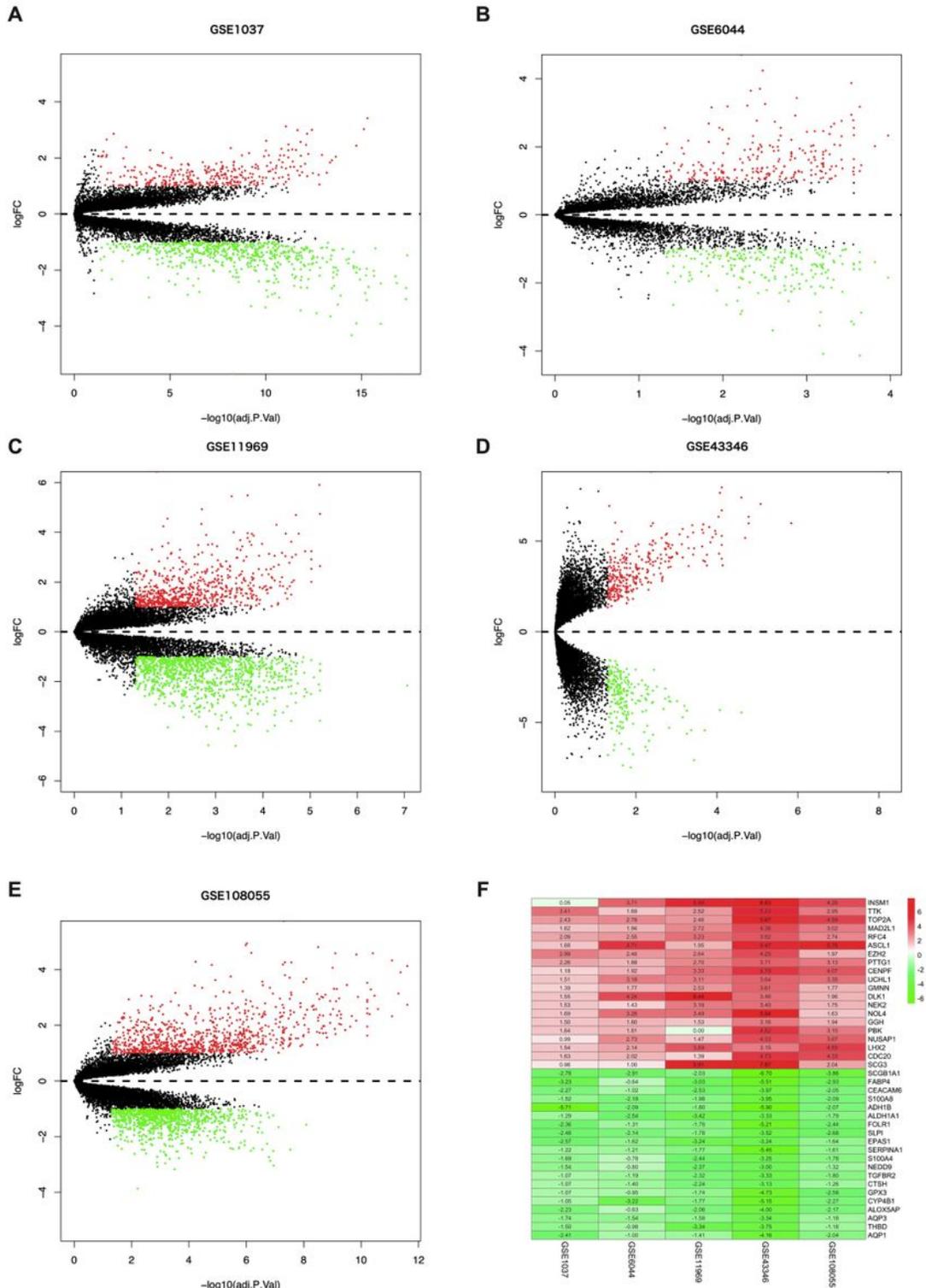


Figure 1

1 Identification of DEGs between two sets of samples. (a-e) Volcano maps for the DEGs of GSE1037, GSE6044, GSE11969, GSE43346, and GSE108055 datasets. The green and red dots indicate downregulated and upregulated genes, respectively. The black dots indicate genes with no significant difference. (f) The 20 common upregulated and downregulated DEGs with the lowest P values. Green and

red represent downregulation and upregulation, respectively. DEG, differentially expressed gene; FC, fold change

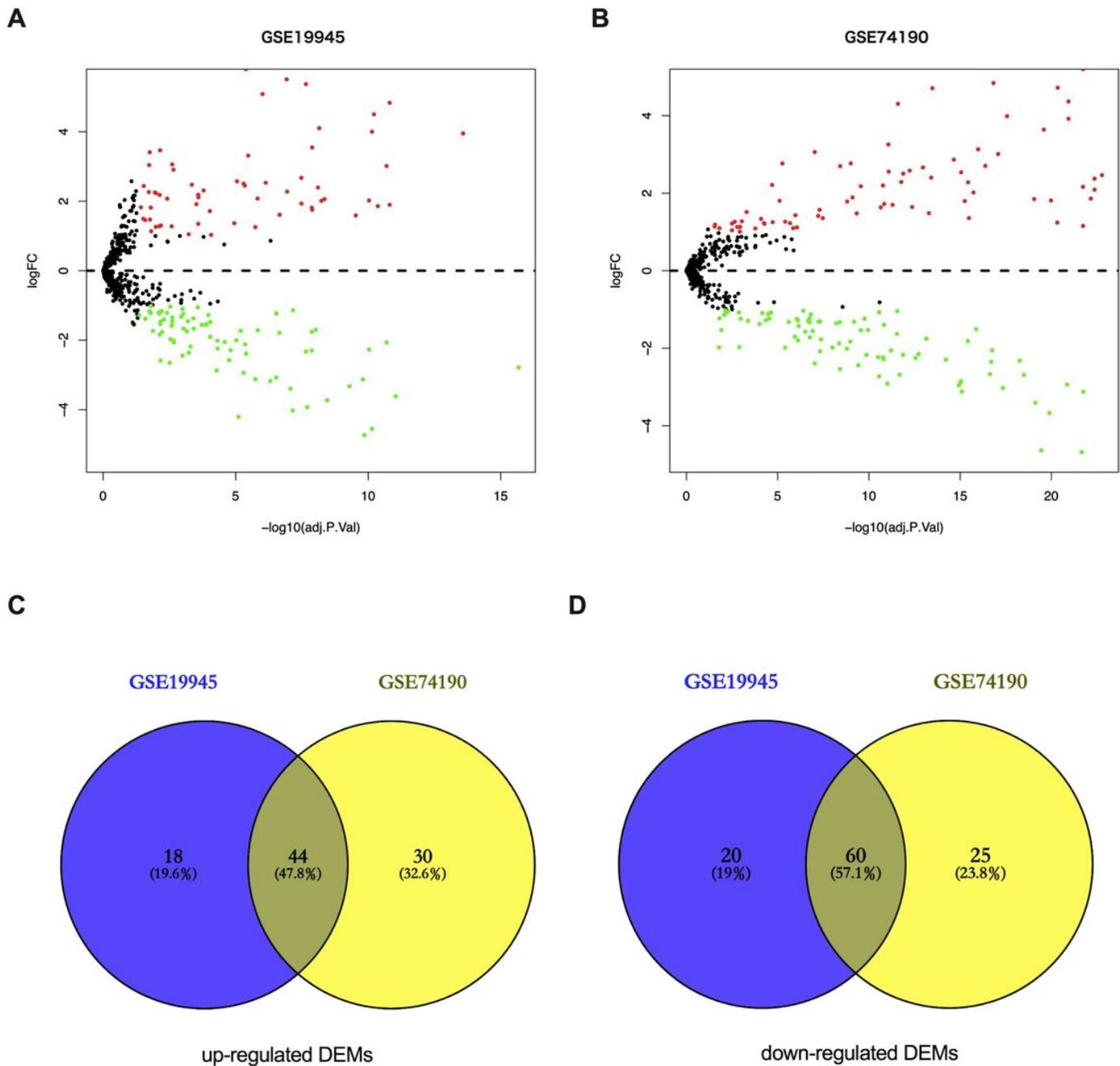


Figure 2

2 Identification of DEMs between two sets of samples. (a and b) Volcano maps for the DEMs of GSE19945 and GSE74190 datasets. The green and red dots indicate downregulated and upregulated genes, respectively. The black dots indicate genes with no significant difference. (c and d) Venn diagrams of the upregulated and downregulated DEMs from the two datasets. DEM, differentially expressed miRNA

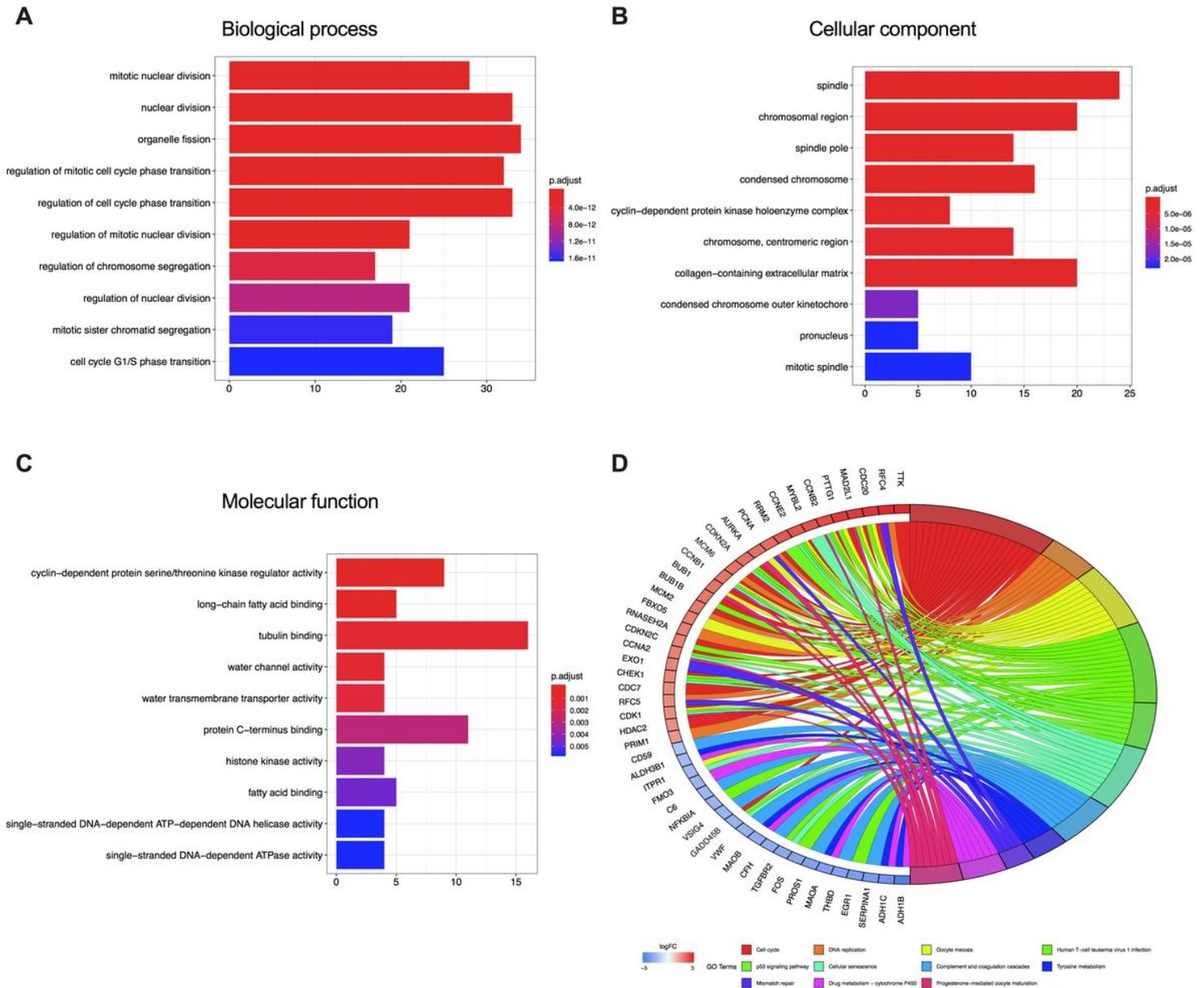


Figure 3

GO and KEGG analyses of the common DEGs. Enriched GO terms in the (a) biological processes, (b) cellular components, (c) molecular functions. (d) KEGG pathway analysis. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, differentially expressed gene

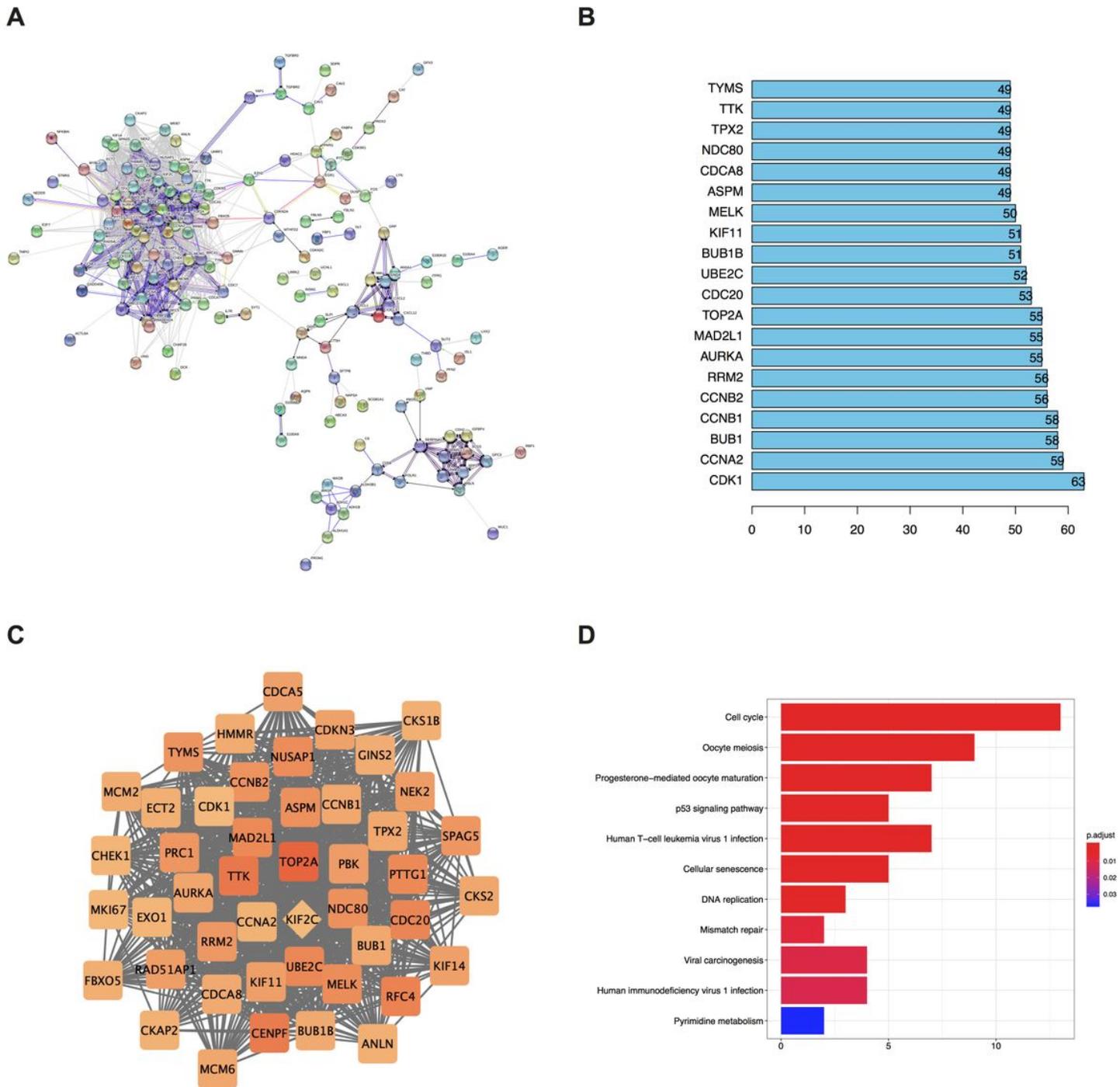


Figure 4

Construction of the PPI network and identification of hub genes and significant modules. (a) The PPI network was constructed using Cytoscape. (b) The top 20 hub genes in SCLC. (c and d) The most significant module and its pathway enrichment analysis. PPI, protein-protein interaction; SCLC, small-cell lung cancer

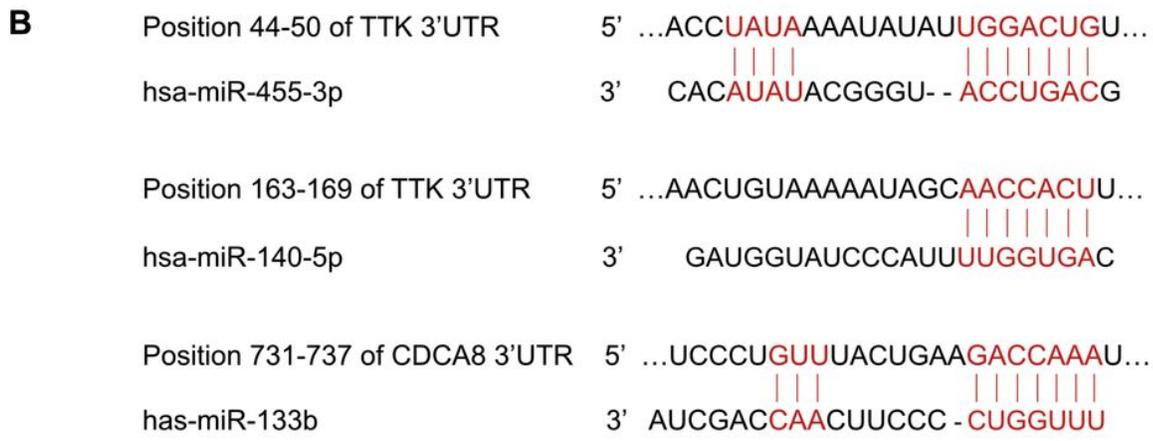
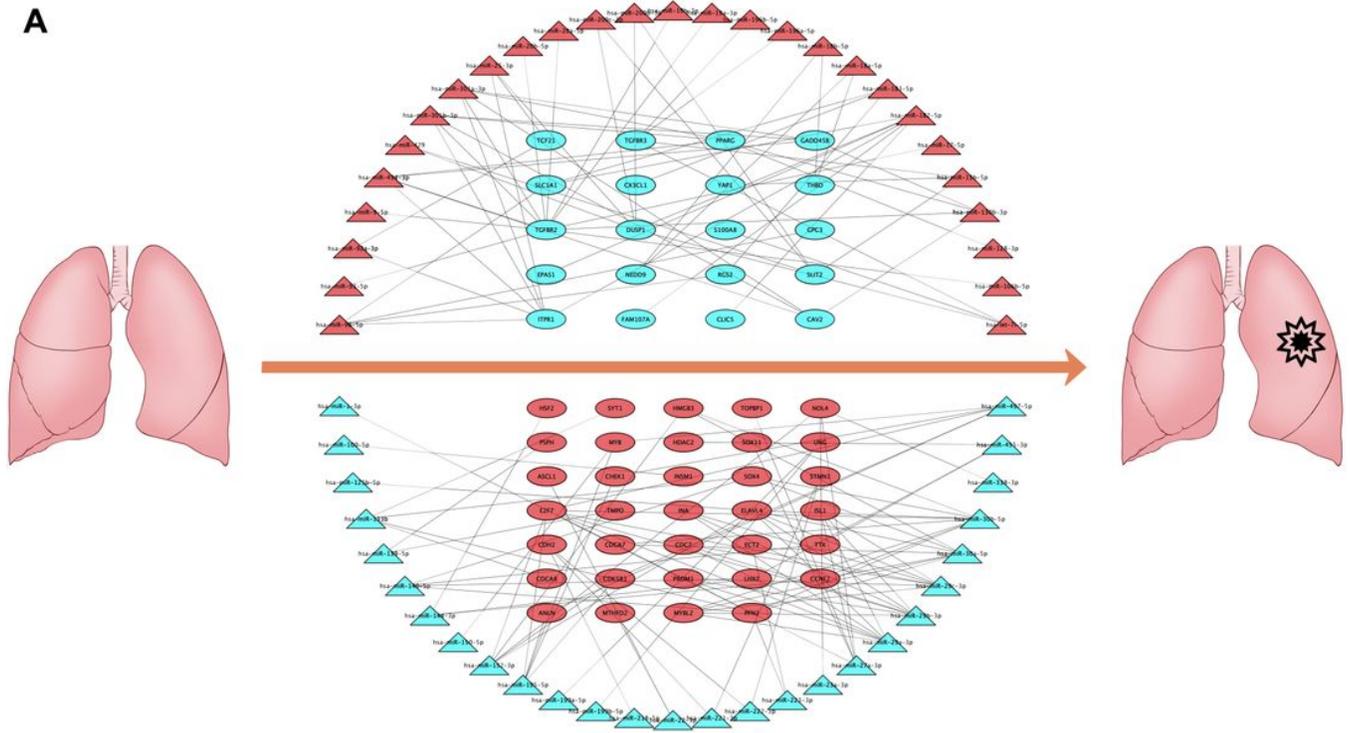


Figure 5

(a) The miRNA-mRNA regulatory network of SCLC contains 54 genes and 153 miRNA-mRNA pairs. Triangles represent the DEMs, and rectangles represent the DEGs targeted by the DEMs. Red and blue colors represent upregulation and downregulation, respectively. (b) The predicted binding sites of the three DEM-hub gene pairs. DEM, differentially expressed miRNA; DEG, differentially expressed gene

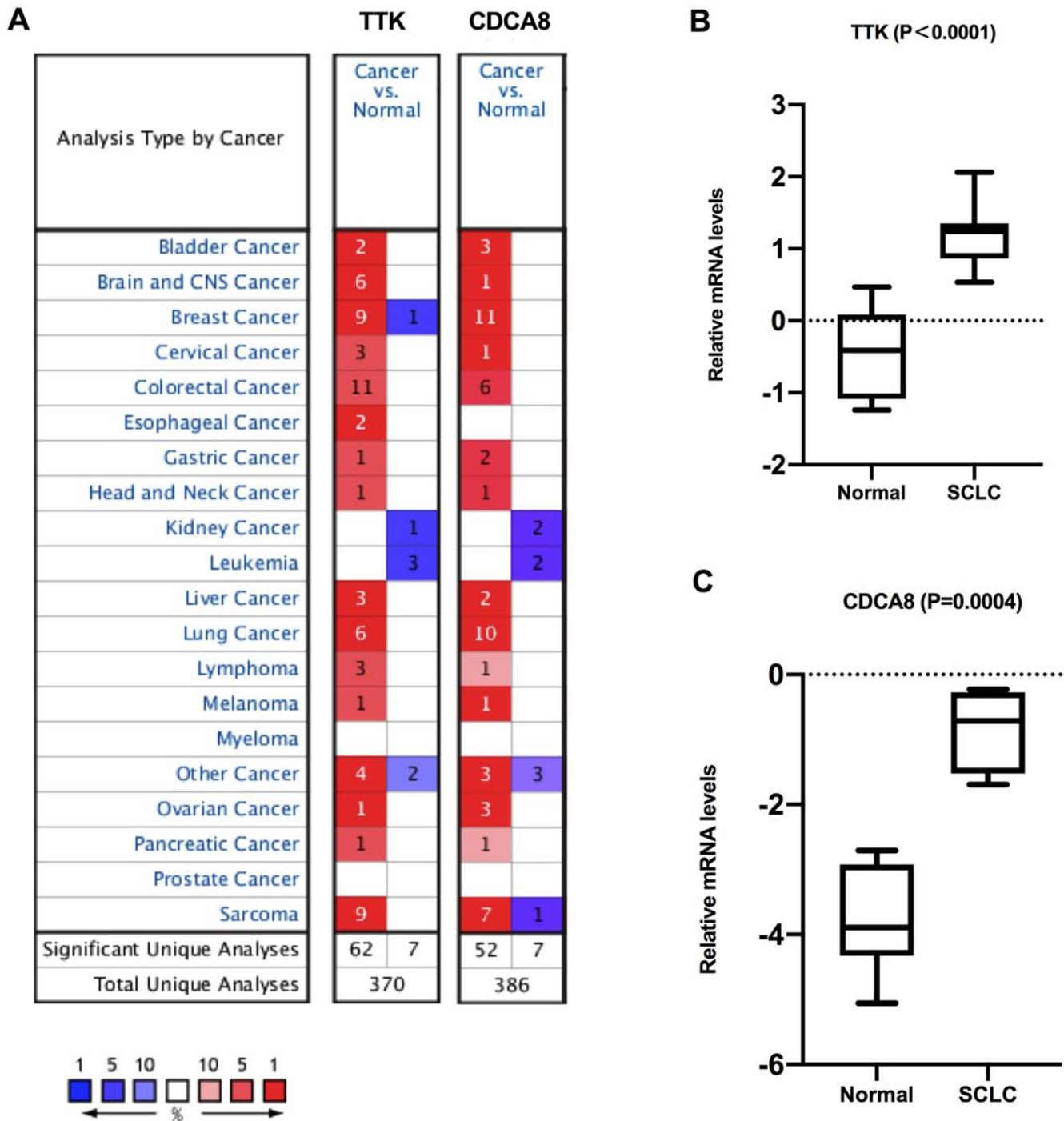


Figure 6

The expression of TTK and CDCA8 in the Oncomine database. (a) Expression of TTK and CDCA8 in various tumors. Red and blue represent upregulation and downregulation, respectively. The threshold value of statistical significance was a P value of 0.05 and a fold change of 2. (b-c) Comparison of TTK and CDCA8 expression levels between normal and SCLC tissues. The expression levels were preprocessed to log₂-normalized and median-centered. TTK, Thr/Tyr kinase; CDCA8, human cell division cycle associated 8; SCLC, small-cell lung cancer

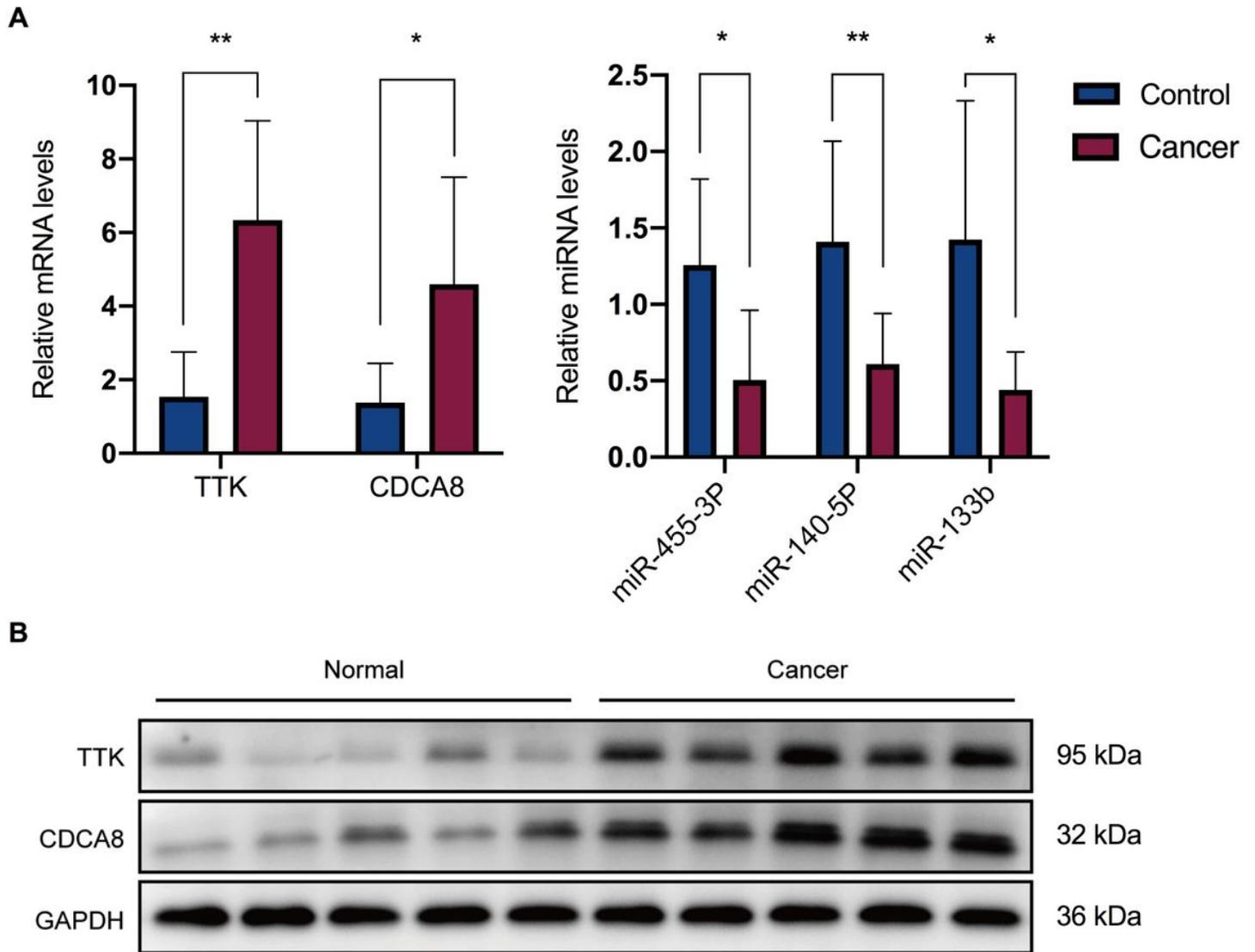


Figure 7

(a) Gene level expression validation of two mRNAs and three miRNAs in five pairs SCLC clinical tissues and their corresponding normal samples by RT-qPCR. * $P < 0.05$. ** $P < 0.01$. (b) Protein level expression validation of two mRNAs in five pairs SCLC clinical tissues and their corresponding normal samples by Western blotting. SCLC, small-cell lung cancer