

# Screening and identification of key biomarkers in lung squamous cell carcinoma by bioinformatics analysis

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**Abstract.** The high mortality rate of lung squamous cell carcinoma (LUSC) is in part due to the lack of early detection of its biomarkers. The identification of key molecules involved in LUSC is therefore required to improve clinical diagnosis and treatment outcomes. The present study used the microarray datasets GSE31552, GSE6044 and GSE12428 from the Gene Expression Omnibus database to identify differentially expressed genes (DEGs). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were conducted to construct the protein-protein interaction network of DEGs and hub genes module using STRING and Cytoscape. The 67 DEGs identified consisted of 42 upregulated genes and 25 downregulated genes. The pathways predicted by KEGG and GO enrichment analyses of DEGs mainly included cell cycle, cell proliferation, glycolysis or gluconeogenesis, and tetrahydrofolate metabolic process. Further analysis of the University of California Santa Cruz and ONCOMINE databases identified 17 hub genes. Overall, the present study demonstrated hub genes that were closely associated with clinical tissue samples of LUSC, and identified TYMS, CCNB2 and RFC4 as potential novel biomarkers of LUSC. The findings of the present study contribute to an improved understanding of the molecular mechanisms of carcinogenesis and progression of LUSC, and assist with the identification of potential diagnostic and therapeutic targets of LUSC.

## Introduction

Lung cancer is a disease with the highest morbidity and mortality rates worldwide. It is reported that non-small cell lung cancer (NSCLC) accounts for 85% of the total lung cancer cases worldwide, of which squamous cell lung cancer (LUSC), often with poor prognosis, accounted for 30% of NSCLC in 2017 (1,2). Data has demonstrated that more than 1 in 3 patients with lung adenocarcinoma (LUAD) benefit from molecular-targeted therapies (3). Inhibitors of epidermal growth factor receptor, v-ki-ras2 kirsten rat sarcoma viral oncogene homologue and anaplastic lymphoma receptor tyrosine kinase are some of the few molecules that are targeted in lung cancer therapy (4). However, the application of molecular-targeted therapies in the diagnosis and treatment of LUSC in the clinical setting is very limited. Thus, the identification of biomarkers that are associated with the diagnosis and treatment of LUSC has become one of the main focus areas in research. An increasing number of studies have revealed new genetic changes associated with LUSC, including the onco-genes baculoviral IAP repeat contain 5 (BIRC5) and GAPDH. BIRC5 is an important inhibitor of apoptosis, which serves an important role in carcinogenesis and progression of LUSC (5). Li *et al* (6) reported a significantly higher expression level of BIRC5 in LUSC tissues compared with normal tissues, indicating the potential of BIRC5 as a target for anti-tumor therapy. On the other hand, GAPDH has been reported to serve a crucial role in regulating glycolysis in cancer cells. GAPDH depletes ATP in cancer cells via the inhibition of glycolysis, which eventually kills cancer cells (7,8). Hence, GAPDH has become a therapeutic target of interest against cancer cells. LUSC accounts for more than 400,000 deaths worldwide each year (2); it is important to highlight that the mortality rate of LUSC is inevitably high, even at early stage, despite several discoveries of potential targets such as BIRC5 and GAPDH. Thus, the investigation of other potential molecular mechanisms associated with LUSC is important.

In recent years, gene microarray and gene chip technologies have developed rapidly, which has provided a theoretical basis for the detection of genetic alterations in cancer cells (9,10). These technologies can be applied to identify differentially expressed genes (DEGs), which can potentially be associated with the carcinogenicity and progression of LUSC. In the

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present study, in order to avoid false positive results from a single microarray gene expression dataset, three mRNA microarray datasets from the Gene Expression Omnibus (GEO) database platform were downloaded. LUSC tissues and non-cancerous tissues were analyzed in order to identify DEGs. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted and a protein-protein interaction (PPI) network was constructed in order to understand the molecular mechanisms underlying the generation and progression of LUSC. The associations between the hub genes and clinical tissue samples were identified using the University of California Santa Cruz (UCSC) Cancer Genomics Browser and ONCOMINE database. A total of 67 DEGs and 17 hub genes were identified as potential diagnostic and therapeutic biomarkers of LUSC. Five hub genes with the highest node value were selected via CentiScaPe, in which the results from the UCSC and ONCOMINE online clinical databases indicated all five hub genes to be associated with unfavorable prognosis of LUSC. Thymidylate synthetase (TYMS), cyclin B2 (CCNB2) and replication factor C subunit 4 (RFC4) were suggested as potential and novel target genes for the treatment of LUSC.

## Materials and methods

**Microarray data.** GEO (<http://www.ncbi.nlm.nih.gov/geo>) is an open database of gene expression abundance, consisting of high throughout gene expression data, gene microarrays and gene chips. Three gene expression datasets [GSE31552 (11), GSE6044 (12) and GSE12428 (13)] were downloaded from the GEO (Affymetrix GPL6244 platform, Affymetrix Human Gene 1.0 ST Array; Affymetrix GPL201 platform, Affymetrix Human HG-Focus Target Array; Affymetrix GPL1708 platform, Agilent-012391 Whole Human Genome Oligo Microarray G4112A). The GSE31552 dataset included 25 LUSC tissue samples and 25 non-cancerous samples. GSE6044 included 15 LUSC tissue samples and 5 non-cancerous samples. GSE12428 included 34 LUSC tissue samples and 28 non-cancerous samples.

**Identification of DEGs.** The DEGs between LUSC and non-cancerous tissue samples were selected by GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r>). GEO2R is a GEO online analysis tool, which analyzes differential gene expression among two or more datasets in GEO. The P-values were adopted to screen the DEGs accurately, and the probe sets without corresponding gene symbols during the screening process were removed. LogFC (fold change) >1 or logFC <-1 and P < 0.05 were considered as statistically significant.

**'KEGG pathway' and 'Gene Oncology (GO)' enrichment analyses of DEGs.** The Database for Annotation, Visualization and Integrated Discovery database (DAVID; <http://david.ncifcrf.gov>; version 6.8) is an online gene and pathway functional annotation database that contains biological information and also provides analysis tools (14). Biological information can be extracted from the comprehensive set of genes and proteins, which provides functional annotations. The KEGG database can be used to analyze genome information and

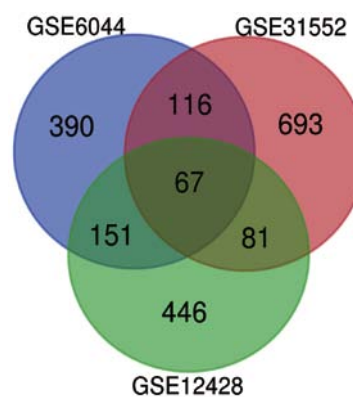


Figure 1. Venn diagram. Genes with  $|\log FC| > 1$  and  $P < 0.05$ , among the mRNA expression profiling datasets GSE31552, GSE6044 and GSE12428, were selected as DEGs. The overlap among the three datasets resulted in 67 DEGs.

gene function and study the gene expression information as a whole network (15). GO is a type of bioinformatics tool for annotating genes and analyzing their biological processes. GO enrichment analyses contain three modules of molecular function, cell composition and biological process (16). In order to analyze the function and cell signaling pathways of DEGs, KEGG and GO enrichment analyses were conducted using the DAVID database. KEGG and GO enrichment bubble plots were drawn using online graphics tools Image GP (<http://www.ehbio.com/ImageGP/>).  $P < 0.05$  was considered as statistically significant.

**PPI network and hub gene module construction.** The PPI network of DEGs was constructed by the online analysis website Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org>; version 11.0) (17) and the interaction of a combined score >0.4 was considered as statistically significant. Analyzing the function of PPI can provide insights into the mechanisms of disease occurrence and development. Cytoscape (version 3.6.1) is an open bioinformatics software platform that can be used to construct a visual network of molecular interactions (18). The plug-in Molecular Complex Detection (MCODE) (version 1.4.2) of Cytoscape is an APP for detecting densely correlated regions in the PPI networks (19). The gene modules were visualized and graphically displayed with the plug-in MCODE. The selection criteria were as follows: MCODE score >5; node score cut-off, 0.2; degree cut-off, 2; k-score, 2; and Max depth, 100. CentiScaPe (version 2.2), a Cytoscape APP specifically designed to calculate centrality indexes for the selection of the most critical nodes in a network (20). The plug-in CentiScaPe 2.2 was used to identify hub genes for functional analysis with interaction node degrees  $\geq 10$ .

**Hub genes selection and analysis.** The hub genes with interaction node degrees  $\geq 10$  were screened. The network of the genes and their co-expression genes was constructed by the online platform cBioPortal (<http://www.cbioportal.org>) (21,22). Hierarchical clustering of hub genes was constructed by online analysis website UCSC Cancer Genomics Browser (<http://genome-cancer.ucsc.edu>) (23). Heat maps of hub genes expression in three different studies of clinical LUSC

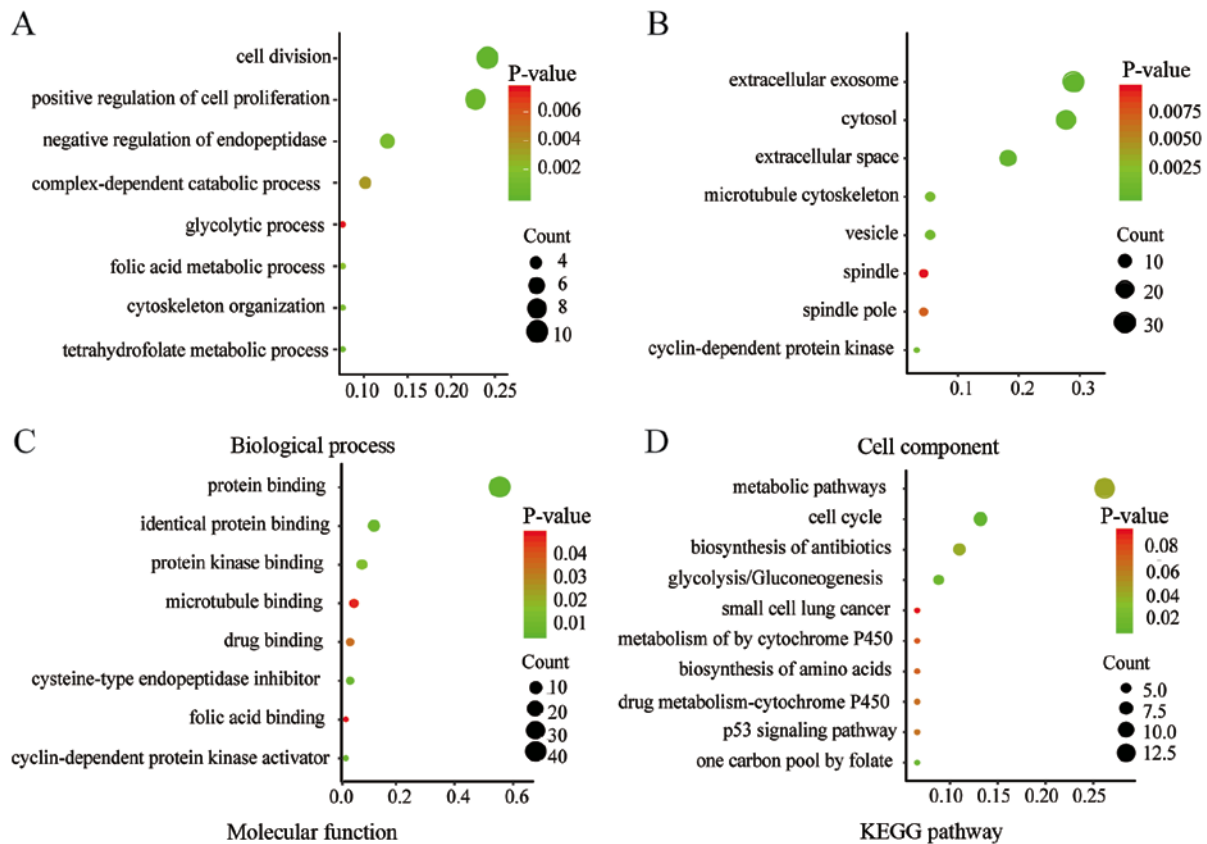


Figure 2. KEGG and GO enrichment plots of DEGs. The plots from the GO enrichment analysis of DEGs for (A) biological processes (B) cell and (C) molecular function were obtained using DAVID and drawn using Image GP. (D) KEGG enrichment analysis was performed using DAVID, the bubble plot of KEGG analysis was drawn using Image GP. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; DAVID, Database for Annotation, Visualization and Integrated Discovery.

samples vs. non-cancerous tissue samples (24-26), and the associations between the expression patterns and tumor stage, overall survival status (the survival rate of patients from diagnosis to the end of the study) and survival status at 5 years (the survival rate five years after diagnosis) were analyzed using the Hou Lung dataset (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19188>) (27), which was obtained from the Oncomine database (<http://www.oncomine.com>) (28,29).

## Results

**Identification of DEGs in LUSC.** The standard microarrays were obtained from the GEO database platform. Following further analysis using GEO2R, DEGs were identified from the GSE31552 (957), GSE6044 (724) and GSE12428 (745) datasets. The 67 DEGs between the three datasets are presented in a Venn diagram (Fig. 1), consisting of 42 upregulated genes and 25 downregulated genes between LUSC and non-cancerous tissues.

**KEGG and GO enrichment analyses of DEGs.** Functional and pathway enrichment analyses of DEGs were conducted by DAVID to obtain the biological classification. The GO enrichment analysis included biological processes (BP), cell component (CC) and molecular function (MF) terms of the DEGs. The results of the KEGG and GO enrichment analyses

are presented as bubble plots in Fig. 2. Changes in BP were significantly enriched in 'cell division', 'positive regulation of cell proliferation', 'negative regulation of endopeptidase activity' and 'tetrahydrofolate metabolic process' (Fig. 2A). Changes in CC were significantly enriched in 'extracellular exosome', 'extracellular space', 'cytosol' and 'vesicle' (Fig. 2B). Changes in MF were mainly enriched in 'protein binding', 'cysteine-type endopeptidase inhibitor activity', 'protein binding' and 'identical protein binding' (Fig. 2C). The KEGG pathway analysis was mainly enriched in 'cell cycle', 'glycolysis or gluconeogenesis', 'metabolic pathways' and 'one carbon pool by folate' (Fig. 2D).

**PPI network and hub gene module construction.** In order to identify the hub genes of LUSC, the PPI network of DEGs was analyzed by STRING. The results revealed that most genes interacted with each other and were located in the center of the network, and were closely associated with the surrounding proteins in the network (Fig. 3A). To enhance the accuracy of the results, the PPI network was also analyzed by Cytoscape. The obtained results were in correspondence with the results of STRING (Fig. 3B), and the hub gene module was obtained using MCODE (Fig. 3C).

**Hub gene selection and analysis.** In total, 17 genes were regarded as hub genes with degrees  $\geq 10$  using CentiScaPe. The names, abbreviations and functions for each of these hub genes

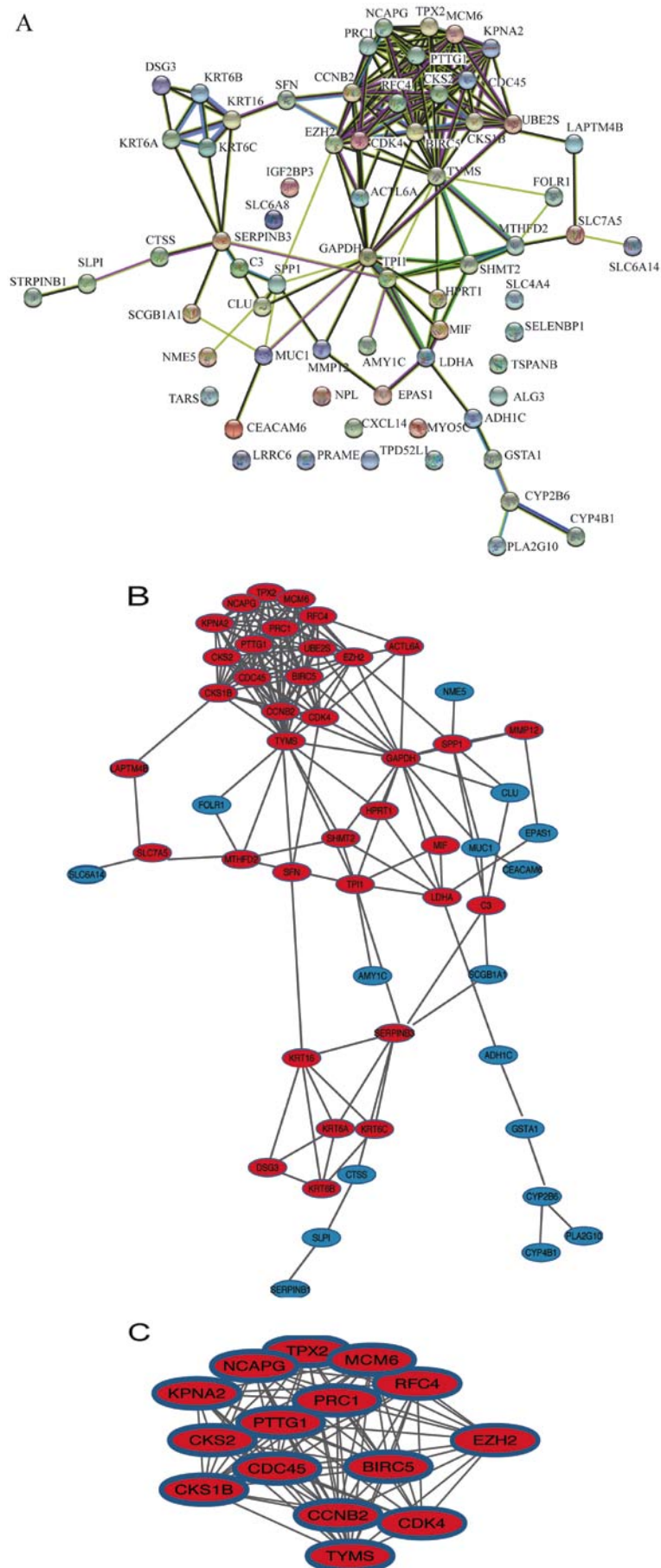


Figure 3. PPI network and hub gene module of DEGs. (A) The PPI network was established with the Search Tool for the Retrieval of Interacting Gene tool. (B) The PPI network of DEGs was established using Cytoscape. (C) The hub gene module was generated from the PPI network, consisting of 15 nodes and 84 edges. Upregulated genes are marked in red. Downregulated genes are marked in blue. DEGs, differentially expressed genes; PPI, protein-protein interaction.

Table I. Full name, abbreviation and function of 17 hub genes with node degree  $\geq 10$ .

No.	Gene symbol	Full name	Function
1	TYMS	Thymidylate synthetase	DNA synthesis, DNA repair and proliferation of cancer cells.
2	CCNB2	Cyclin B2	Correlated with invasion, metastasis and poor prognosis of various cancer types.
3	RFC4	Replication factor C subunit 4	Associated with poorly differentiated and advanced Tumor-Node-Metastasis stage in multiple cancer types.
4	BIRC5	Baculoviral IAP repeat containing 5	Plays a key role in proliferation, apoptosis and angiogenesis of LUSC.
5	GAPDH	Glyceraldehyde-3-Phosphate dehydrogenase	Elevated GAPDH significantly promotes cell proliferation and migration in LUSC.
6	CKS1B	CDC28 protein kinase regulatory subunit 1B	Attributes to prognosis, chemoresistance and chemosensitivity in cancer.
7	MCM6	Minichromosome maintenance complex component 6	Initiation of DNA replication and a marker for proliferating cells.
8	EZH2	Enhancer of Zeste 2 polycomb repressive complex 2 subunit	Invasion, cell proliferation and adverse prognosis in LUSC.
9	PTTG1	Pituitary tumor-transforming 1	Carcinogenesis, migration, invasion and prognosis.
10	CDK4	Cyclin dependent kinase 4	Regulates cell cycle positively, overexpressed and gene amplified in LUSC.
11	TPX2	TPX2, microtubule nucleation factor	Overexpression associated with differentiation grade, stage and metastasis of LUSC.
12	PRC1	Protein regulator of cytokinesis 1	Promotes progression and migration in LUSC.
13	CKS2	CDC28 protein kinase regulatory subunit 2	Inhibit DNA damage response and contribute to tumor cell proliferation in breast cancer
14	CDC45	Cell division cycle 45	Regulator of cell proliferation and associated with S-phase DNA damage.
15	KPNA2	Karyopherin subunit alpha 2	Attributed to cancer cell proliferation and metastasis.
16	NCAPG	Non-SMC condensin I complex subunit G	Associated with cell cycle, apoptosis and migration in human hepatocellular carcinoma.
17	UBE2S	Ubiquitin conjugating enzyme E2 S	Involved in the malignant characteristics, mitosis and survival of various types of cancer cells.

are summarized in Table I. The genes associated with the hub genes and their co-expression network were obtained using the cBioPortal online platform by performing interaction analysis (Fig. 4A). The expression of 17 hub genes in LUSC tissues and its association with the severity and prognosis among LUSC patients were further explored using the UCSC and ONCOMINE online databases. Furthermore, a heat map of hierarchical clustering obtained using UCSC demonstrated that the expression of hub genes in LUSC tissues was higher compared with that of non-cancerous samples. However, the expression of hub genes showed no differences with gender (Fig. 4B). The heat map of hub genes expression in clinical LUSC tissue samples and normal tissue samples were analyzed using three different datasets, using the ONCOMINE online platform. The results revealed that most of the hub genes were significantly upregulated in clinical LUSC samples in all the datasets (Fig. 5). The hub genes whose interaction node degree was among the top five were TYMS, CCNB2, RFC4, BIRC5 and GAPDH, indicating their potential role in the processes

of carcinogenesis, development and unfavorable prognosis of LUSC. The associations between the upregulated hub genes and tumor stage, overall survival status and survival status at 5 years were also analyzed. The top five genes were associated with high tumor stage, poor overall survival status and poor survival status at 5 years, which suggests their upregulation to be involved in the promotion of tumor progression and poor prognosis (Fig. 6).

## Discussion

In recent years, the incidence and mortality of lung cancer has continued to increase rapidly worldwide (30). LUAD and LUSC are the two common types of lung cancer. The incidence of LUSC was reported to be associated with smoking, whereas the treatment of LUSC remains limited compared with that of LUAD. The underlying pathological mechanisms of LUSC at the molecular level are still at the exploration stage (31). Mutations or amplifications of phosphatidylinositol-3

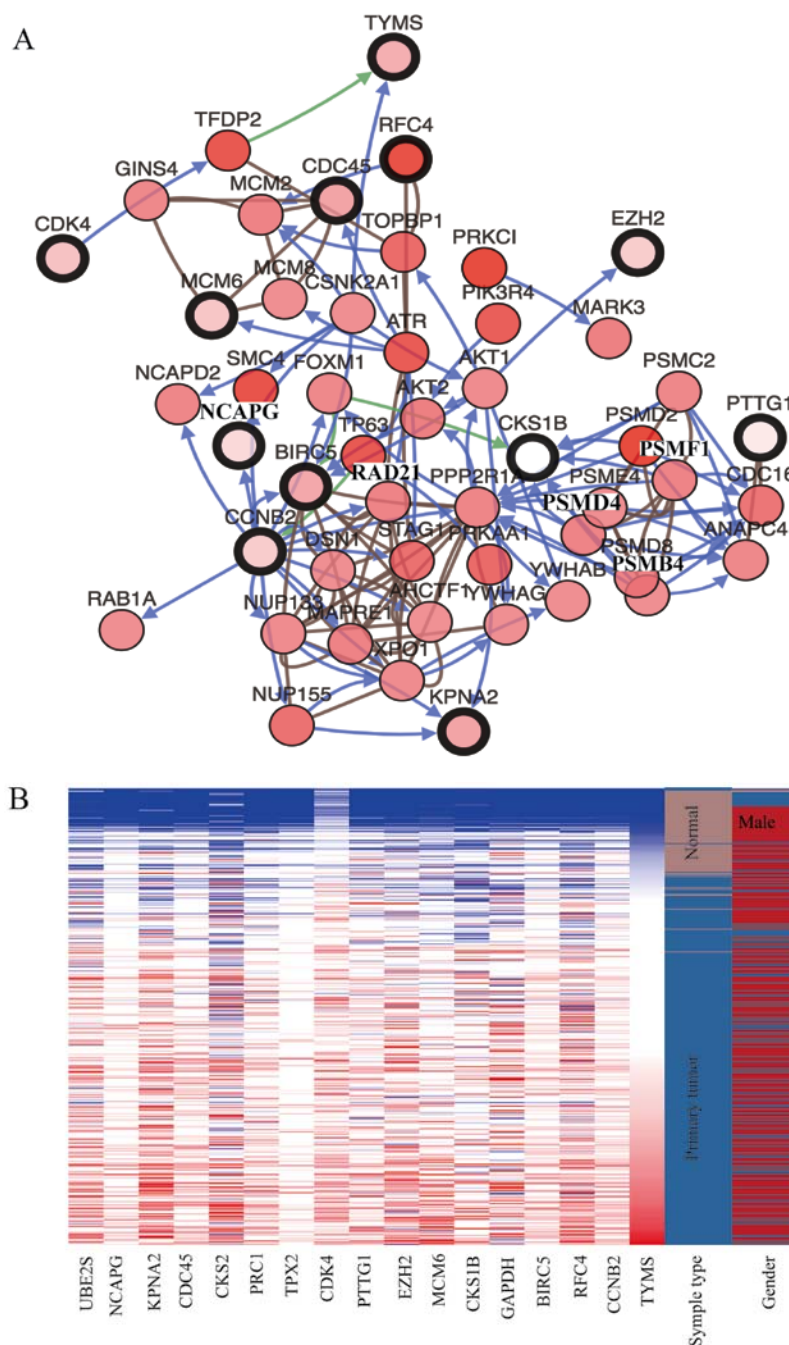


Figure 4. Co-expression network and heat map of hub genes. (A) The network of hub genes and their co-expression genes were constructed using cBioPortal. Nodes with bold black outline represent hub genes. Nodes with thin black outline represent the co-expression genes. (B) The heat map of hierarchical clustering of hub genes was created with the University of California Santa Cruz Cancer Genomics browser. The samples under the blue bar are lung squamous cell carcinoma samples, whereas the samples under the brown bar are normal samples. The sex under the red bar is male and the sex under the blue bar is female. High expression of genes in samples is marked in red and low expression of genes is marked in blue. PPI, protein-protein interaction.

kinases (PI3K), phosphatase and Tensin homolog (PTEN), erythropoietin-producing hepatocellular A2 (EphA2) and liver kinase B1 (LKB1) were reported to be associated with the incidence, progression and prognosis of LUSC (32,33). A study conducted using the Cancer Genome Atlas Research Network demonstrated the dysfunction of NFE2L2, KEAP1, CDKN2A and RB1, and the abnormal structures of their products are associated with the occurrence and development of LUSC (34). The high mortality rate of LUSC is in part due to the lack of early detection of LUSC biomarkers (35). As a result, the identification of key molecules involved in LUSC

is required and important for improving clinical efficacy. Microarray is a high-throughput technology in obtaining novel biomarkers, which can provide the basis for further studies on the mechanism of LUSC and clinical targeted therapies at the molecular level.

In the present study, three mRNA microarray datasets were analyzed to identify 67 common DEGs. The DEGs consisted of 42 upregulated and 25 downregulated genes between LUSC tissue samples and normal tissue samples. GO terms and KEGG pathway enrichments were analyzed in order to investigate interactions among the DEGs. The results indicated that

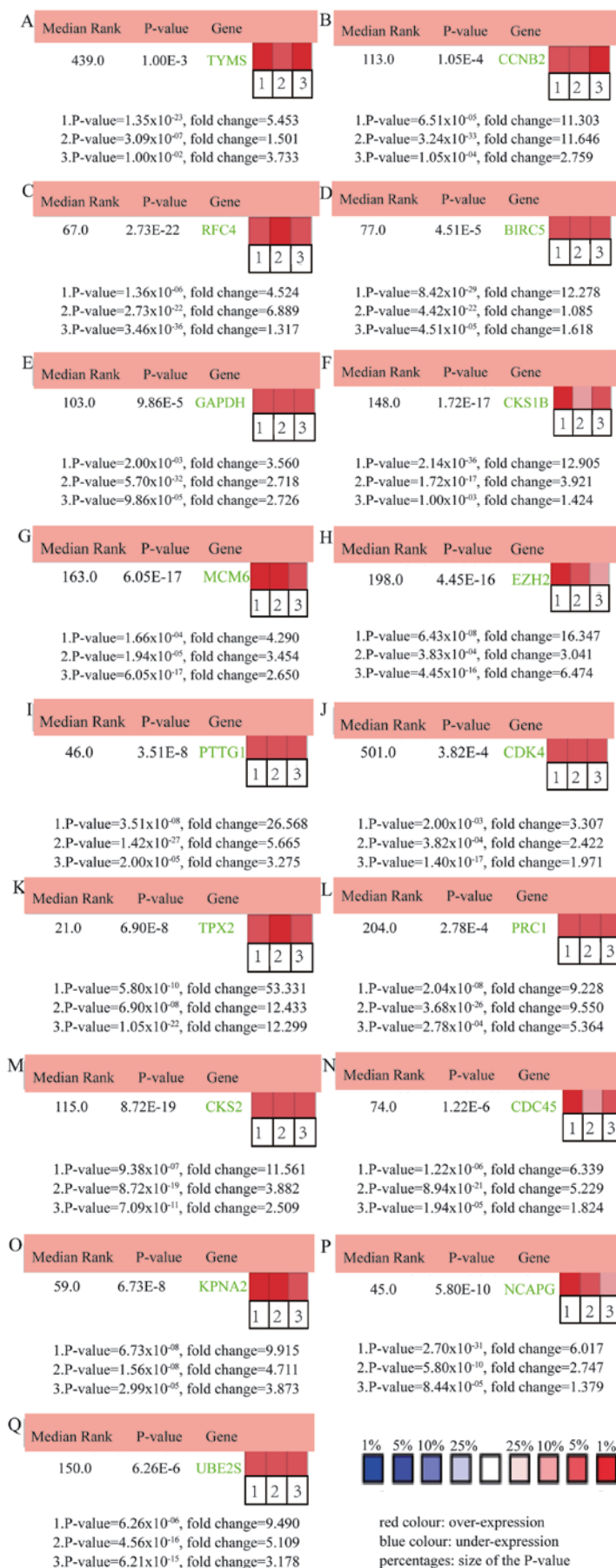


Figure 5. ONCOMINE analysis of LUSC vs. normal tissue of hub genes. Heat maps of hub genes expression in clinical LUSC samples vs. normal tissue samples. Hub genes: (A) TYMS, (B) CCNB2, (C) RFC4, (D) BIRC5, (E) GAPDH, (F) CKS1B, (G) MCM6, (H) EZH2, (I) PTTG1, (J) CDK4, (K) TPX2, (L) PRC1, (M) CKS2, (N) CDC45, (O) KPNA2, (P) NCAPG and (Q) UBE2S. The rank for a gene is the median rank for that gene across each of the analyses and the P-value for a gene is its P-value for the median-ranked analysis. LUSC, lung squamous cell carcinoma.

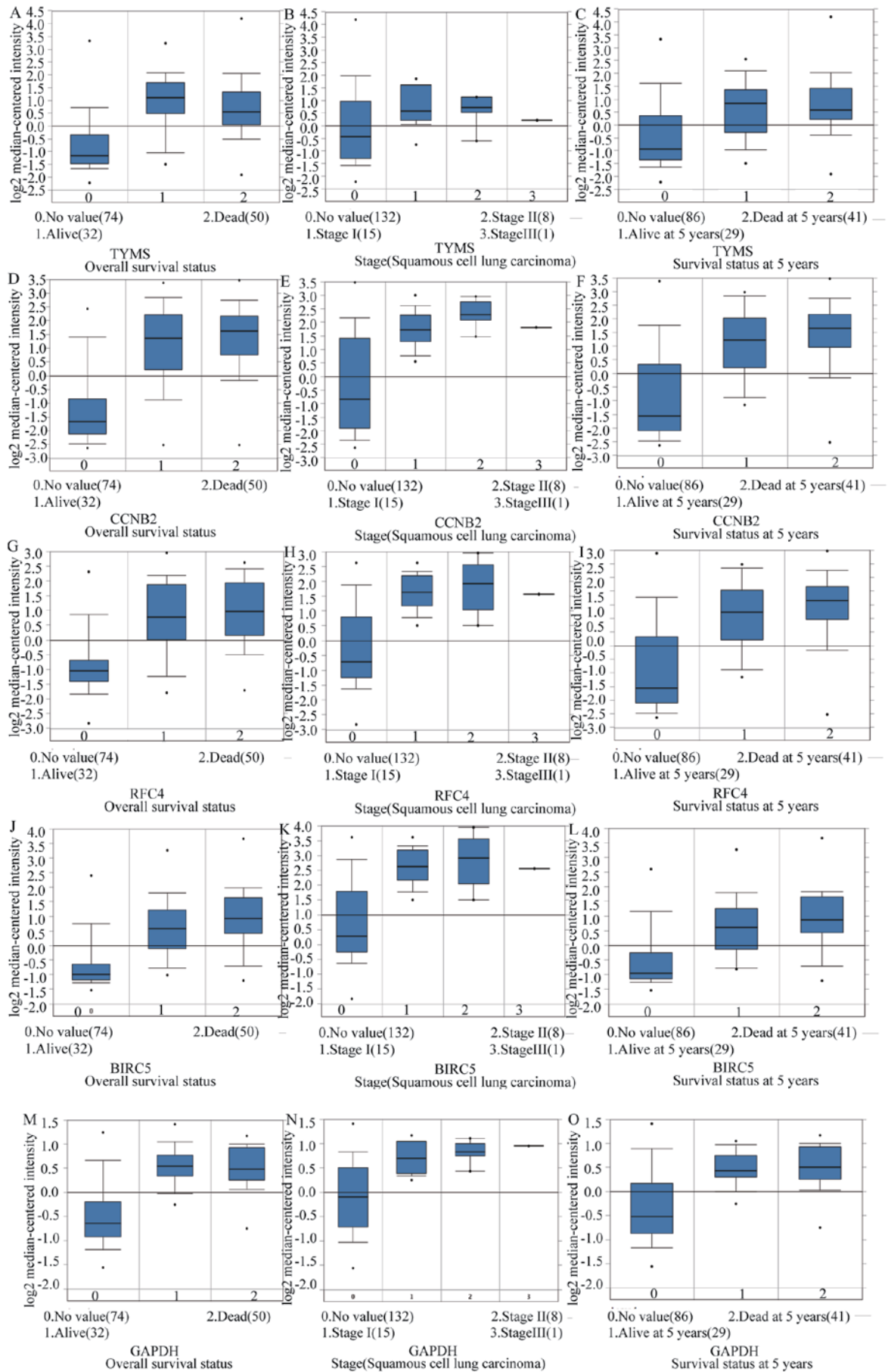


Figure 6. The associations between upregulation of TYMS, CCNB2, RFC4, BIRC5 and GAPDH and tumor stage, overall survival status and survival status at 5 years in the Hou Lung dataset which was obtained from the ONCOMINE database. (A-C) TYMS, (D-F) CCNB2, (G-I) RFC4, (J-L) BIRC5 and (M-O) GAPDH mRNA expression in LUSC vs. normal lung tissues. LUSC lung squamous cell carcinoma.



the 67 DEGs were significantly enriched in cell cycle, cell proliferation, glycolysis or gluconeogenesis, and tetrahydrofolate metabolic process. Previous studies have illustrated that dysregulations of cell cycle and cell proliferation serve roles in the carcinogenesis and malignant change of LUSC (36-38). In addition, multiple studies have also shown that glycolysis or gluconeogenesis serve important roles in tumor initiation, progression and unfavorable prognosis in cancer (39,40). Furthermore, gene polymorphism of tetrahydrofolate induces a decreased activity of tetrahydrofolate reductase, which affects the normal metabolism of folate in cells, where tetrahydrofolate metabolic disorder is closely associated with tumorigenesis (41,42). The findings of the present study were in accordance with the conclusions of previous studies and showed that GO and KEGG enrichment analyses were significantly enriched in cell cycle, cell proliferation, glycolysis or gluconeogenesis, and tetrahydrofolate metabolic process. A total of 17 DEGs were identified as hub genes with an interaction node degree  $\geq 10$ . The hub genes whose degrees were among the top five were TYMS, CCNB2, RFC4, BIRC5 and GAPDH, and the PPI network showed that they were directly interacting with each other.

Several studies have suggested that TYMS is a predictive biomarker to test for the effectiveness of pemetrexed used in chemotherapy for treating NSCLC (43,44). Lu *et al* (45) reported that the expression of TYMS was significantly upregulated among patients with lymph node metastasis. The expression of TYMS was also higher among patients with 5-year recurrence rate. Besides, high expression of TYMS was found in the case of breast cancer, which resulted in increased susceptibility of an individual to the progression of the disease. Gene polymorphisms of TYMS have been reported to have potential in improving the diagnosis, prevention and treatment of breast cancer (46). Hence, several studies are now focusing on the association between gene polymorphic variations of TYMS with various types of cancer (47-49). BIRC5 was upregulated in 76% LUSC samples and the expression in LUSC tissues was significantly higher compared with that in non-cancerous tissues (50). BIRC5 is a potential biomarker or therapeutic target of smoking-associated LUSC (51). The expression of BIRC5 was higher among patients who are smokers compared with non-smokers, and in squamous vs. non-squamous lung tumor ( $P < 0.001$ ). The present study also demonstrated that BIRC5 expression level was negatively associated with the expression of tumor suppressor gene Tp53 (52). GAPDH serves a critical role in inhibiting the process of glycolysis in tumor cells (53). The expression of GAPDH was notably upregulated in LUSC tissues, and an increased level of GAPDH significantly promotes the cell proliferation and migration in LUSC (54). CCNB2 as a member of the cell cyclin protein family, was significantly associated with different staging and metastatic statuses of tumors ( $P < 0.001$ ). Thus, CCNB2 is a potential biomarker for evaluating metastatic status and therapeutic efficacy for cancer patients (55). Additionally, the upregulation of CCNB2 was closely associated with the degree of differentiation, progression, lymph node metastasis, invasion and adverse prognosis in NSCLC (56). CCNB2 has also been found to be upregulated in patients with bladder and colorectal cancer (57). Thus, CCNB2 is a potential diagnostic biomarker and a therapeutic target for LUSC. RFC4 was

involved in DNA replication and regulation of cell proliferation and cell cycle. Studies reported an association of RFC4 with cancer progression and worse survival outcome, and the ability to predict response to radiotherapy and neoadjuvant radiotherapy in rectal cancer (58,59). RFC4 has been demonstrated to be associated with several types of cancer, however the underlying carcinogenic mechanism needs to be further explored. The top five hub genes reported in the present study were associated with various types of cancer. Multiple studies have demonstrated that GAPDH and BIRC5 are associated with LUSC (50,54). However, to our knowledge, no previous studies reported TYMS, CCNB2 and RFC4 to be directly associated with LUSC. In the present study, ONCOMINE and UCSC analysis confirmed that the top five hub genes from clinical LUSC samples were significantly upregulated and were all associated with different staging of cancer and survival rate compared to that of other samples. Therefore, TYMS, CCNB2 and RFC4 are potential novel biomarkers of LUSC for further investigation.

Among the other 12 hub genes identified in the present study, MCM6, EZH2, CDK4, TPX2 and PRC1 were previously reported to be associated with LUSC. Minichromosome maintenance (MCM) proteins serve a critical role in cell proliferation and cell cycle. Meanwhile, MCM6 is often associated with poor prognosis, particularly among male patients with LUSC and with a history of smoking (60). The presence of EZH2 was associated with the aggressiveness of cancer development. Behrens *et al* (61) analyzed 221 LUSC samples and 320 lung adenocarcinomas samples, which revealed significantly higher expression of EZH2 in LUSC compared with that of LUAD ( $P < 0.0001$ ). Cell cycle protein CDK4 has an established association with neoplasia and cancer progression. Recent studies have found that pathways including that of CDK4/6 were frequently altered in LUSC via diverse mechanisms, suggesting CDK4/6 inhibitors as potential target for the treatment of LUSC (62,63). TPX2, which actively participates in the formation of spindle microtubules during mitosis, was significantly associated with cell differentiation and metastatic status of LUSC cells, suggesting its potential as a prognostic predictor of LUSC (64). PRC1, an important protein involved in cytokinesis, plays an important role in microtubule organization in eukaryotes (65). A recent study has implicated the overexpression of PRC1 in LUSC to be associated with increased susceptibility to lymph node metastasis and shorter survival time in patients with LUSC (66).

Literature review revealed that the interaction among LUSC and hub genes CKS1B, PTTG1, CKS2, CDC45, KPNA2, NCAPG and UBE2S has not been widely reported. These genes are potential novel biomarkers and therapeutic targets of LUSC. CKS1B, an adaptor for cyclin-dependent kinases, was shown to be associated with chemoresistance, low chemosensitivity and poor prognosis in cancer. An elevated level of CKS1B has been reported to result in the resistance of cancer cells to bortezomib, and activation of the NEDD8 pathway, which in turn leads to further advancement of cancer. Thus, CKS1B is a potential novel target in multiple myeloma (67). In addition, CKS1B promotes proteasomal degradation and ubiquitination of p27Kip1. Overexpression of CKS1B contributes to an increased turnover rate of p27Kip1 and promotion of cancer cell proliferation, resulting in poor

prognosis in many types of cancer (68). PTTG1 functions to regulate transcription, the G-M phase of mitosis and the repair of DNA. Overexpression of PTTG1 has been reported in multiple types of cancer, in which PTTG1 is associated with metabolic processes, such as carcinogenesis, migration, invasion and epithelial-mesenchymal transition in squamous cell carcinomas (69). Recent studies demonstrated that various non-coding RNAs lead to cancer cell growth and metastasis via PTTG1 (70,71). Therefore, PTTG1 is a potential and novel therapeutic target of LUSC tumor growth and metastasis. CKS2, as a cyclin-dependent kinase-interacting protein, serves important roles in regulating cell cycle, inducing apoptosis, as well as regulating cancer cell invasion and metastasis. Upregulation of CKS2 can lead to DNA damage in cells, which can increase the proliferation of cancer cell (72). Therefore, the molecular mechanism by which CKS2 regulates cell cycle and induces cell apoptosis may be critical in investigating diagnostic methods and treatment methods for LUSC. CDC45 is an essential regulator of cell proliferation. An elevated expression of CDC45 is correlated with DNA damage in the S-phase, in which the anti-cancer effect of CDC45 suppressor is mediated by limiting DNA damage during S phase (73). KPNA2, as a member of the Karyopherin  $\alpha$  family, actively participates in the process of signal transduction from the extracellular space to the nucleus. Furthermore, the upregulation of KPNA2 is correlated with cancer cell proliferation and metastasis (74). Moreover, several studies reported that KPNA2 is involved in the progression of cancer by regulating nuclear translocation of cancer-associated proteins; which may explain the significantly upregulated expression of KPNA2 in LUSC. NCAPG is a novel mitotic gene and provides novel therapeutic targets for cancer. Goto *et al* (75) reported that NCAPG, as a target of miR-145-3p, could predict the survival rate of patients with prostate cancer. UBE2S is a central protein in the process of ubiquitination and is associated with the malignancy of various types of cancer (76). Studies have reported the upregulation of UBE2S to enhance the nuclear translocation of  $\beta$ -catenin and induced expression of c-Myc and cyclin D1, suggesting UBE2S as a potential prognostic factor and oncogene in LUSC (77-78).

In conclusion, the present study was conducted in order to identify potential DEGs that may be associated with carcinogenesis or adverse progression of LUSC. A total of 67 DEGs and 17 hub genes were identified, in which hub genes were regarded as promising targets for the diagnosis and treatment of LUSC. Meanwhile, TYMS, CCNB2 and RFC4 were identified as potential novel biomarkers of LUSC. However, the present study was based on bioinformatics methods and no experiments were performed to validate the findings. Therefore, further studies are required to explore the biological association between the genes identified in the present study in LUSC, in order to improve treatments and clinical outcomes of LUSC.

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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

JM, XZ, HD and SL designed the study. JM and XZ analyzed and interpreted the microarray datasets, and wrote the manuscript. XY and LM made substantial contributions to acquisition and interpretation of data. JM, XG, HY, JC and YL analyzed the data. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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