Identification of potential diagnostic and therapeutic target genes for lung squamous cell carcinoma

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Abstract. The purpose of this study was to identify potential molecular markers of lung squamous cell carcinoma (LUSC). Three datasets containing LUSC mRNA sequencing data were downloaded from the Gene Expression Omnibus, The Cancer Genome Atlas and the Gene Expression Profiling Interactive Analysis databases. These datasets were used to identify significantly differentially expressed genes (DEGs) in LUSC. A protein-protein interaction network of the DEGs was constructed followed by Gene Ontology, Kyoto Encyclopedia of Genes and Genomes and overall survival analyses of the DEGs. A total of 37 DEGs between LUSC and normal tissues were identified, including 26 downregulated genes and 11 upregulated genes. Biological Process enrichment analysis revealed that the DEGs were mainly enriched in 'cell adhesion', 'cell-matrix adhesion', 'anatomical structure morphogenesis', 'ECM-receptor interaction' and 'focal adhesion'. Overall survival analysis demonstrated that transcription factor 21, α-2-macroglobulin, acyl-CoA synthetase long chain family member 5, integrin subunit β8, meiotic nuclear divisions 1 and secretoglobin family 1A member 1 were significantly associated with the occurrence and development of lung cancer, and these genes were selected as hub genes. The results obtained in the present study may aid the elucidation of the molecular mechanisms involved in the development of LUSC and may provide potential targets for LUSC treatment.

Introduction

Lung cancer is the leading cause of cancer-associated mortality worldwide (1). There are two main histological types of lung cancer: Small cell lung cancer and non-small cell lung cancer (NSCLC). The latter is further subdivided into lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and large cell lung cancer. The incidence and mortality rate of LUSC are high, with >400,000 new cases occurring worldwide each year (2). Novel therapeutic agents for the treatment of lung cancer have been developed, including bevacizumab and epidermal growth factor receptor (EGFR) tyrosine kinase, ALK receptor tyrosine kinase (ALK) and CD274 molecule inhibitors (3). However, these agents are not effective for the treatment of LUSC, which accounts for ~25% of NSCLC cases (4). The elucidation of the molecular mechanisms underlying the development of LUSC may aid in the identification of new treatment strategies. Microarray technology and bioinformatics analysis have emerged as powerful tools for the study of different types of cancer and may facilitate the discovery of novel biomarkers and potential therapeutic targets (5,6). However, the high false positive rate of single microarray analysis may confound results (7). The current study analyzed three datasets containing LUSC mRNA sequencing data downloaded from the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo), the Cancer Genome Atlas (TCGA; www.cancergenome.nih.gov) and the Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn) databases. Genes in the intersection of the three datasets were regarded as significantly differentially expressed genes (DEGs). Subsequently, Gene Ontology (GO; http://geneontology.org), Kyoto Encyclopedia of Genes and Genomes (KEGG; http://david.ncifcrf.gov), protein-protein interaction network (PPI) and overall survival analyses of DEGs were performed. Receiver operating characteristic (ROC) curves were generated to identify genes of potential diagnostic and therapeutic value for LUSC. A total of 37 DEGs were screened, of which six were selected as the hub genes. These hub genes were significant for LUSC prognosis and may be candidate biomarkers for lung cancer.

Materials and methods

Microarray data. The GEO is an international public repository that stores and freely distributes microarray, second-generation sequencing and other forms of high-throughput functional genomic datasets (8). TCGA is a large-scale cancer genome project that provides researchers with multidimensional maps of the key genomic changes and clinicopathological information in 33 types of cancer (9). GEPIA is a newly developed interactive web server for analyzing RNA sequencing expression

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data based on 9,736 tumors and 8,587 normal samples from the TCGA and Genotype-Tissue Expression databases. The gene expression dataset GSE31552 (10) was downloaded from GEO and included 25 nontumor tissues and 25 tumor tissues. The GPL6244 Affymetrix Human Gene 1.0 ST Array (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used. The TCGA dataset was derived from the 'Protein-coding Transcripts' of LUSC in the Cancer RNA-Seq Nexus (CRN; http://syslab4.nchu.edu.tw) database (11) and included 63 cancer samples and 51 normal samples. The GEPIA dataset was downloaded from the GEPIA online database including 486 cancer samples and 338 normal samples.

Data preprocessing and differential expression analysis. The original data were transformed into expression data using the affy package (http://www.bioconductor.org/packages/release/bioc/html/affy.html) in R (version 3.5.3; http://cran.r-project.org/bin/windows/base/rpatched.html) (12). Missing data were estimated with weighted K-nearest neighbors method (13), and all the expression profiles were normalized by the median normalization method (14). The t-test method of the limma package (http://master.bioconductor.org/packages/release/bioc/html/limma.html) in R was used to identify DEGs between LUSC and normal controls. In order to filter the DEGs of each dataset, set cut off criteria for each dataset. For GSE31552, DEGs were identified at $P<0.05$ and $|\log_2 FC|>1$, where FC is fold change; For the TCGA and GEPIA datasets, DEGs were identified at $P<0.05$ and $|\log_2 FC|>2$. Each probe name of GSE31552 was converted into a gene name using the hugene10sttranscriptcluster.db package (http://bioconductor.org/packages/release/data/annotation/html/hugene10sttranscriptcluster.db.html) in R and the corresponding platform file. The Spearman's correlation test was used to cluster the samples and to calculate the correlation coefficients between the samples, the results were subsequently visualized using pheatmap package (http://www.bioconductor.org/packages/release/bioc/html/heatmaps.html) in R. To verify the rationality of the DEGs of GSE31552, draw the volcano plot using ggplot2 package in R (http://cran.r-project.org/web/packages/ggplot2). Heatmaps of the DEGs (based on $|\log_2 FC|$) were generated using the heatmap.2 package (http://www.rdocumentation.org/packages/gplots/versions/3.0.1.1/topics/heatmap.2) in R to present the DEGs in each sample.

Screening and analysis of DEGs. Significant DEGs between LUSC and normal samples were screened using FunRich (version 3.1.3), which is an open-access standalone functional enrichment and interaction network analysis tool (15). A PPI network of DEGs was constructed using the Gene MANIA database (http://www.genemania.org). The Gene MANIA database is a useful tool for generating hypotheses about gene function, analyzing gene lists, prioritizing functionally analyzed genes and reporting weights (16). Subsequently, a hierarchical clustering of DEGs was constructed using an online analysis database of University of California, Santa Cruz Xena (UCSC Xena 2.0; http://xena.ucsc.edu/welcome-to-ucsc-xena) (17).

Functional enrichment analysis. GO is a common tool for annotating genes and their products (18). KEGG is a knowledge base for the systematic analysis of gene functions in terms of networks of genes and molecules (19). The Database for Annotation, Visualization and Integrated Discovery 6.8 (DAVID 6.8; http://david.ncifcrf.gov) (20) is a comprehensive database providing a complete set of functional annotation information of genes and proteins from which researchers can extract biological information. To analyze the DEGs at the functional level, GO and KEGG pathway enrichment analyses were performed using the DAVID online tool to obtain the enriched biological processes and pathways. $P<0.05$ was considered to indicate a statistically significant difference.

Hub gene selection and analysis. Overall survival analyses of DEGs were performed using GEPIA (21) and Kaplan-Meier plotter (version 2018.11.04; http://www.kmplot.com) (22). Genes with significant differences for the prognosis of LUSC were selected as hub genes ($P<0.05$). Analyzed the expression of Hub genes in different subtypes of lung cancer and different stages of LUSC based on GEPIA online analysis database (21). To understand the diagnostic value of the hub genes, ROC curves were plotted using the pROC package (pROC_1.12.1; http://master.bioconductor.org/packages/release/bioc/html/pROC.html) in R based on the GEO dataset. Through comprehensive analysis of Hub genes survival analysis, Hub gene expression in different lung cancer subtypes and LUSC stages, ROC curve of Hub genes and literature search. Firstly, six genes closely related to the survival of LUSC patients were screened using survival analysis of Hub genes. The expression of these six genes was validated with LUSC stages and in patients with LUAD and LUSC. The diagnostic efficacy of ROC was also evaluated, and the three methods revealed that secretoglobin family 1A member 1 (SCGB1A1) was closely related to survival and stages, and the diagnostic efficiency of SCB1A1 ROC was the highest among the screened genes. Therefore, SCB1A1 was selected as the target gene. The expression of SCGB1A1 in different LUSC cell lines based on The Cancer Cell Line Encyclopedia (CCLE; http://portals.broadinstitute.org/ccle) database was plotted using GraphPad Prism (version 7; GraphPad Software, Inc., La Jolla, CA, USA) (23). The Human Protein Atlas (Version 18.1; www.proteinatlas.org) (24) was used to show the expression of SCGB1A1 in different tissues. The Oncomine database (Version 4.5; https://www.oncomine.org) (25) was used to analyze Okayama (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31210) and Beer datasets (26) to identify the associations between SCGB1A1 and EGFR mutation status, echinoderm microtubule associated protein-like 4 (EML4) and anaplastic lymphoma kinase (ALK) gene fusion, expression of TP53 and smoking status.

Results

Data preprocessing. Following the evaluation of missing data and normalization, the expression profiling data were plotted (Fig. 1A). The similar levels of data points indicate high consistency showing high accuracy of classification and comparison. The baseline level of Samples Clustering Analysis (Fig. 1B) revealed that the sample sources were reliable. For dataset GSE31552, a total of 1,712 DEGs were identified at $P<0.05$ and $|\log_2 FC|>1$, and the rationality of the values was verified by volcano plots (Fig. 1C), in which red dots represent upregulated

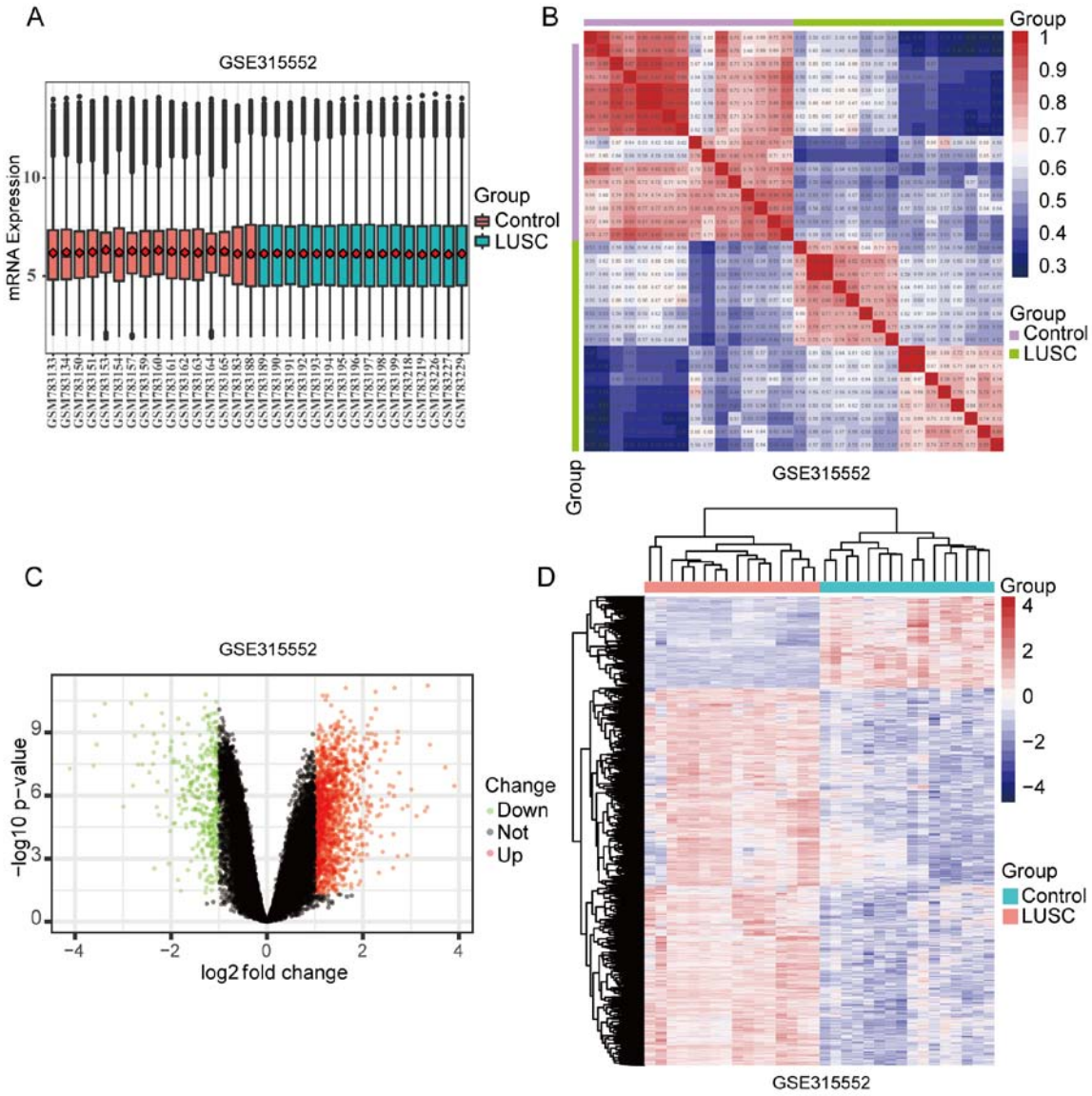

Figure 1. Data preprocessing and differential expression analysis. (A) A box-plot of standardized expression data of normal and LUSC tissues. The red symbols denote normal samples, and the blue symbols represent patients with LUSC. The red diamond in each frame represents the average level of gene expression in each sample. (B) Heatmap of the common DEGs between LUSC tissues and normal controls in the GSE31552 dataset. Blue represents downregulation and red represents upregulation. The normal groups were significantly separated from the LUSC groups, indicating that the sample source data are reliable. (C) Volcano plot used to verify the rationality of the P-values and corresponding fold changes of the expression data. The red circles denote upregulation, and the green symbols denote downregulation. (D) A hierarchical clustering diagram of the DEGs. Blue represents downregulation and red represents upregulation. LUSC, lung squamous cell carcinoma; DEGs, differentially expressed genes; down, downregulated genes; up, upregulated genes; not, not differentially expressed.

genes, green dots represent downregulated genes, and black dots represent unchanged genes. Hierarchical clustering analysis revealed that the gene expression patterns of the DEGs were similar among the array data of GSE31522 (Fig. 1D), indicating that the molecular changes in LUSC are consistent and may represent a novel genetic signature in LUSC.

Identification of DEGs in LUSC. Following standardization of the microarray results, DEGs (2,162 in GSE31552; 1,842 in TCGA database; and 1,691 in the GEPIA database) were identified. The three datasets shared 37 genes that were considered to be significant DEGs between LUSC and normal tissues (Fig. 2A). These genes included 26 downregulated and 11 upregulated genes.

KEGG and GO enrichment analyses of DEGs. To analyze the biological classifications of the DEGs, DAVID was used for functional and pathway enrichment analysis. The identified GO terms and pathways are presented in Table I. The GO terms enriched by DEGs were mainly associated with 'cell adhesion', 'cell-matrix adhesion' and 'anatomical structure morphogenesis', whereas the pathways enriched for DEGs were associated with 'ECM-receptor interaction' and 'focal adhesion'.

PPI network construction and module analysis. The PPI network of DEGs constructed using Gene MANIA (Fig. 2B) revealed the co-expression, genetic interactions and physical interactions among the DEGs and predicted genes. Red circles represent DEGs obtained by the above data set intersection;

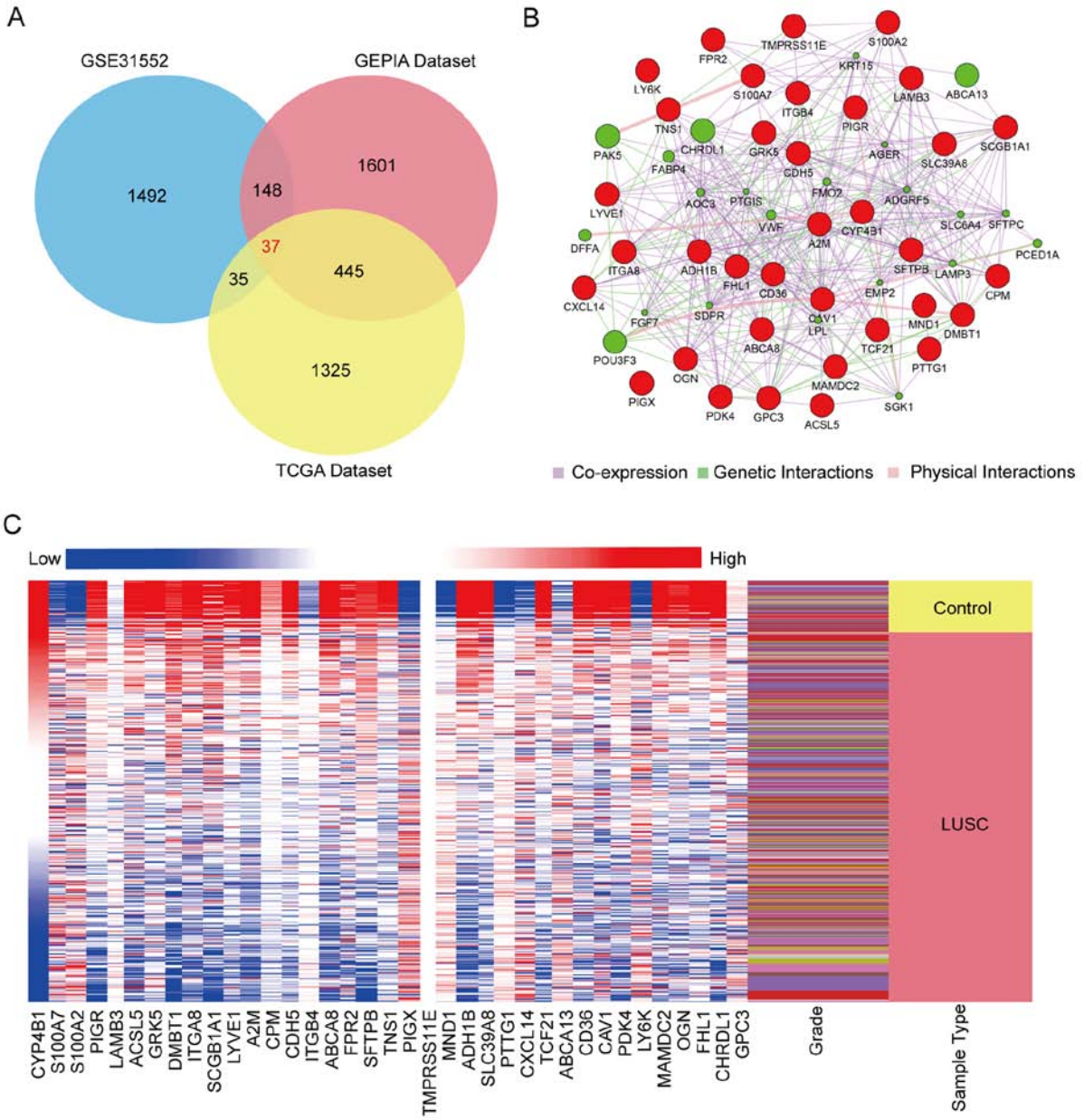

Figure 2. Venn diagram, PPI network and hierarchical clustering of significant DEGs. (A) Venn diagram drawn in FunRich (version 3.1.3). A total of 37 common significant DEGs were obtained. (B) PPI network of DEGs constructed using Gene MANIA. Red circles present DEGs and green circles present predicted genes. The size of the circle represents the level of connectivity. Purple lines represent co-expression, green lines represent genetic interactions and pink lines represent physical interactions. (C) Hierarchical clustering of DEGs was performed using the UCSC Xena database. Upregulation of genes is indicated by red and downregulation of genes is indicated by blue. The expression of DEGs in different grades and different sample types is presented (Note: TMPRSSIIE gene is not included in the lung cancer dataset of UCSC Xena database). PPI, protein-protein interactions; GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas; LUSC, lung squamous cell carcinoma; DEGs, differentially expressed genes; UCSC, University of California, Santa Cruz.

green circles represent predicted genes obtained from the above DEGs through the plug-in Gene MANIA. Dense networks indicated that DEGs were closely related to each other. In the hierarchical clustering plot (Fig. 2C), the DEGs distinguished the LUSC samples from the normal samples.

Screening for hub genes. To ensure the accuracy of the analyses, overall survival analysis of the DGEs was performed using GEPIA and Kaplan-Meier plotters. Genes were selected if they are meaningful (P<0.05) in both analyses. Six hub genes were ultimately selected based on their significant effects on survival (Fig. 3). The survival curves indicated that integrin subunit β8 (ITGB8) and SCGB1A1 were positively associated with overall survival, whereas TCF21, A2M, ACSL5 and meiotic nuclear divisions 1 (MND1) were negatively associated with overall survival. The hub genes and their functions are presented in Table II.

Hub gene analysis. Six genes were identified as hub genes and had significant effects on survival. The expression levels of the hub genes in different subtypes of NSCLC and different stages of LUSC were analyzed (Fig. 4). Compared with healthy

Table I. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of differentially expressed genes in lung squamous cell carcinoma samples.

Term	No. enriched genes	P-value
GO:0007155 'cell adhesion'	7	0.0003
GO:0007160 'cell-matrix adhesion'	3	0.0151
GO:0009653 'anatomical structure morphogenesis'	3	0.0157
GO:0048333 'mesodermal cell differentiation'	2	0.0227
GO:0031581 'hemidesmosome assembly'	2	0.0247
GO:0030301 'cholesterol transport'	2	0.0329
GO:2000811 'negative regulation of anoikis'	2	0.0348
GO:0019915 'lipid storage'	2	0.0489
hsa04512 'ECM-receptor interaction'	4	0.0014
hsa04510 'focal adhesion'	4	0.0153

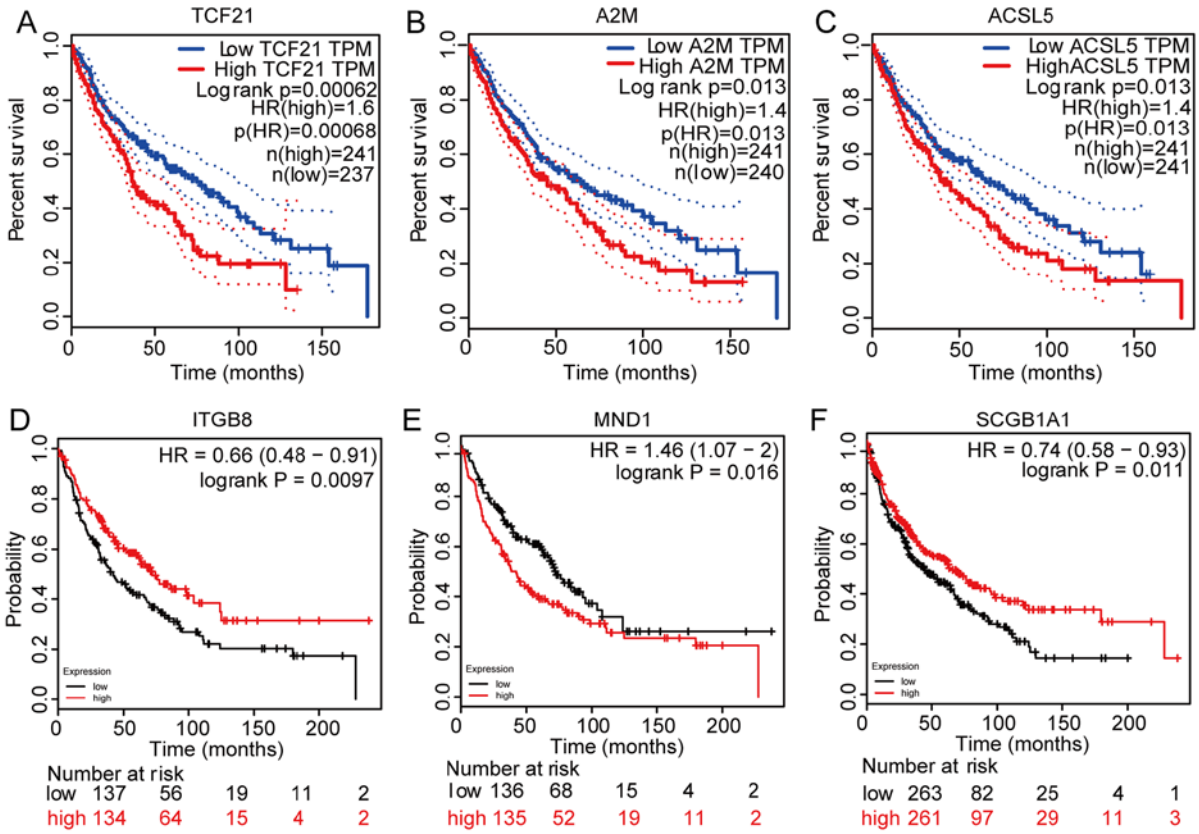

Figure 3. Overall survival analysis. (A-C) Overall survival analyses were performed using the GEPIA online platform and (D-F) Kaplan-Meier plotter online platform. The solid line represents the survival curve and the dotted line represents the 95% confidence interval. Log-rank P<0.05 was considered to indicate a statistically significant difference. Patients with expression above the median are indicated by red lines, and patients with expression below the median are indicated by (A-C) blue lines or (D-F) black lines. (A) TCF21 was negatively associated with the overall survival of patients with LUSC; (B) A2M was negatively associated with the overall survival of patients with LUSC; (C) ACSL5 was negatively associated with the overall survival of patients with LUSC; (D) ITGB8 was positively correlated with the overall survival of patients with LUSC; (E) MND1 was negatively associated with the overall survival of patients with LUSC; (F) SCGB1A1 was positively correlated with the overall survival of patients with LUSC. TCF21, transcription factor 21; A2M, α-2-macroglobulin; ACSL5, acyl-CoA synthetase long chain family member 5; ITGB8, integrin subunit beta 8; SCGB1A1, secretoglobin family 1A member 1; MND1, meiotic nuclear divisions 1; HR, hazard ratio; TMP, transcripts per million; LUSC, lung squamous cell carcinoma.

controls, TCF21, A2M and SCGB1A1 were downregulated in LUSC and LUAD, ITGB8 and MND1 were upregulated in LUAD and LUSC, and ACSL5 was downregulated in LUSC and upregulated in LUAD. In addition, expression of TCF21, ITGB8, MND1 and SCGB1A1 was significantly different among different stages of LUSC. SCGB1A1 was closely associated with LUSC stage [$Pr(>F)=8.06x10^{-7}$]. ROC curves revealed that the hub genes had a good diagnostic value for LUSC [area under the curve (AUC)>0.7], particularly SCGB1A1 (AUC=0.922; Fig. 5A).

Table II. Function of the hub genes (GeneCards; https://www.genecards.org).

Gene	Gene name	Function
TCF21	Transcription factor 21	RNA polymerase II transcription factor activity, sequence-specific DNA binding
A2M	α-2-Macroglobulin	Tumor cell adhesion, migration and growth
ACSL5	Acyl-CoA synthetase long chain family member 5	Pro-apoptotic sensing of enterocytes
ITGB8	Integrin subunit β8	Cell-adhesion to extra cellular matrix or to other cells
MND1	Myeloid cell nuclear differentiation antigen	Meiosis and recombination
SCGB1A1	Secretoglobin family 1A member 1	Inhibition of phospholipase A2 and arachidonic acid release, prostaglandin D2 receptor antagonism in the lung

AA, arachidonic acid.

In summary, downregulation of SCGB1A1 was significantly associated with overall survival of LUSC patients (log-rank P=0.011; Fig. 3F), and SCGB1A1 downregulation was significantly associated with LUSC stages [Pr(>F)=$8.06x10^{-7}$; Fig. 4C]. Additionally, ROC curve analysis demonstrated that SCGB1A1 had high diagnostic value (AUC=0.922). Thus, SCGB1A1 was screened as a target gene for LUSC. Further analysis based on GEPIA Database revealed that SCGB1A1 expression was lower in LUSC compared with normal lung tissue (Fig. 5C). The Human Protein Atlas database analysis demonstrated that SCGB1A1 was overexpressed in normal lung tissues compared with other tissues (Fig. 5D). The expression of SCGB1A1 in lung cancer cell lines was analyzed using the CCLE online platform; the results revealed that SCGBA1 was downregulated in the majority lung cancer cell lines (Fig. 5B). Oncomine analysis of Okayama and Beer datasets revealed that lower mRNA levels of SCGB1A1 were associated with EGFR mutation, EML4-ALK fusion, expression of TP53 and smoking (Fig. 6A-D).

Discussion

Lung cancer is a leading cause of cancer-associated mortality worldwide (1). The five-year relative survival rate of lung cancer is lower than the corresponding rates of breast cancer, colon cancer and kidney cancer (27). This low rate is partly due to a late-stage diagnosis in >50% of cases (28). Although chemotherapy and improvements in supportive care have improved overall survival and quality of life, the prognosis of patients with advanced NSCLC remains poor (27). Individualized targeted therapies for lung cancer are being investigated, and progress has been applied to the clinic (29), including in TP53, EGFR, KRAS proto-oncogene, GTPase, EML4-ALK rearrangement and MET signal transduction targeted therapies (30). The majority of these treatments are effective in LUAD and no front-line targeted therapies are currently clinically available for LUSC (31,32). Therefore, effective diagnostic and therapeutic markers are required for LUSC. Microarray technology may allow the identification of the genetic changes implicated in LUSC and is an effective method for identifying new biomarkers in other diseases, such as vascular diseases (33) and oral squamous cell carcinoma (34).

The present study analyzed three datasets, including GSE31552, and TCGA and GEPIA datasets, and revealed DEGs between LUSC and normal tissues. A total of 37 DEGs were identified, including 26 downregulated genes and 11 upregulated genes. To understand the functions and associations of these DEGs, GO and KEGG analyses were performed. The DEGs were mainly enriched in 'cell adhesion', 'cell-matrix adhesion', 'anatomical structure morphogenesis', whereas KEGG pathway enrichment was mainly concentrated in 'ECM-receptor interaction' and 'focal adhesion'. Previous studies have reported that cell adhesion, cell-matrix adhesion and anoikis are closely associated with tumorigenesis and development (35-37). Additionally, studies of anatomical structure and morphogenesis have revealed cholesterol levels to be associated with tumor metastasis and invasion (38-40). These studies indicated that DEGs may be closely related to the occurrence, development, metastasis and invasion of tumors. The pathways of ECM-receptor interaction and focal adhesion are important mediators of cell adhesion, growth, proliferation, survival, angiogenesis and migration (41,42). The results obtained in the aforementioned studies are consistent with the results obtained in the current study. The enriched modules and pathways identified in the current study may have genetic effects on LUSC and the identified genes may interact within a network.

Survival analysis was performed using the DEGs and six genes with significant impacts on overall survival were identified as hub genes. SCGB1A1, also known as the Clara cell secretory protein (43,44), had a good diagnostic value for LUSC (AUC=0.922) and was significantly associated with poor overall survival and tumor stage. Previous studies have demonstrated that SCGB1A1 demonstrated anti-inflammatory, immunomodulatory and antitoxin properties (45-49). SCGB1A1 mRNA expression is abundant throughout the airway (50), however, its expression level is very low in lung tumors (51), which is consistent with the results obtained in the current study. Expression levels of SCGB1A1 are decreased in diffuse goblet cell hyperplasia and squamous metaplasia, whereas alveolar expression is elevated in alveolar cell bronchitis (52). SCGB1A1 knockout mice are more susceptible to lung injury (by bacterial or viral infection, ozone and cigarette smoke) or sensitization, exhibit increased inflammation and remodeling reactions, have more frequent lung tumors, and

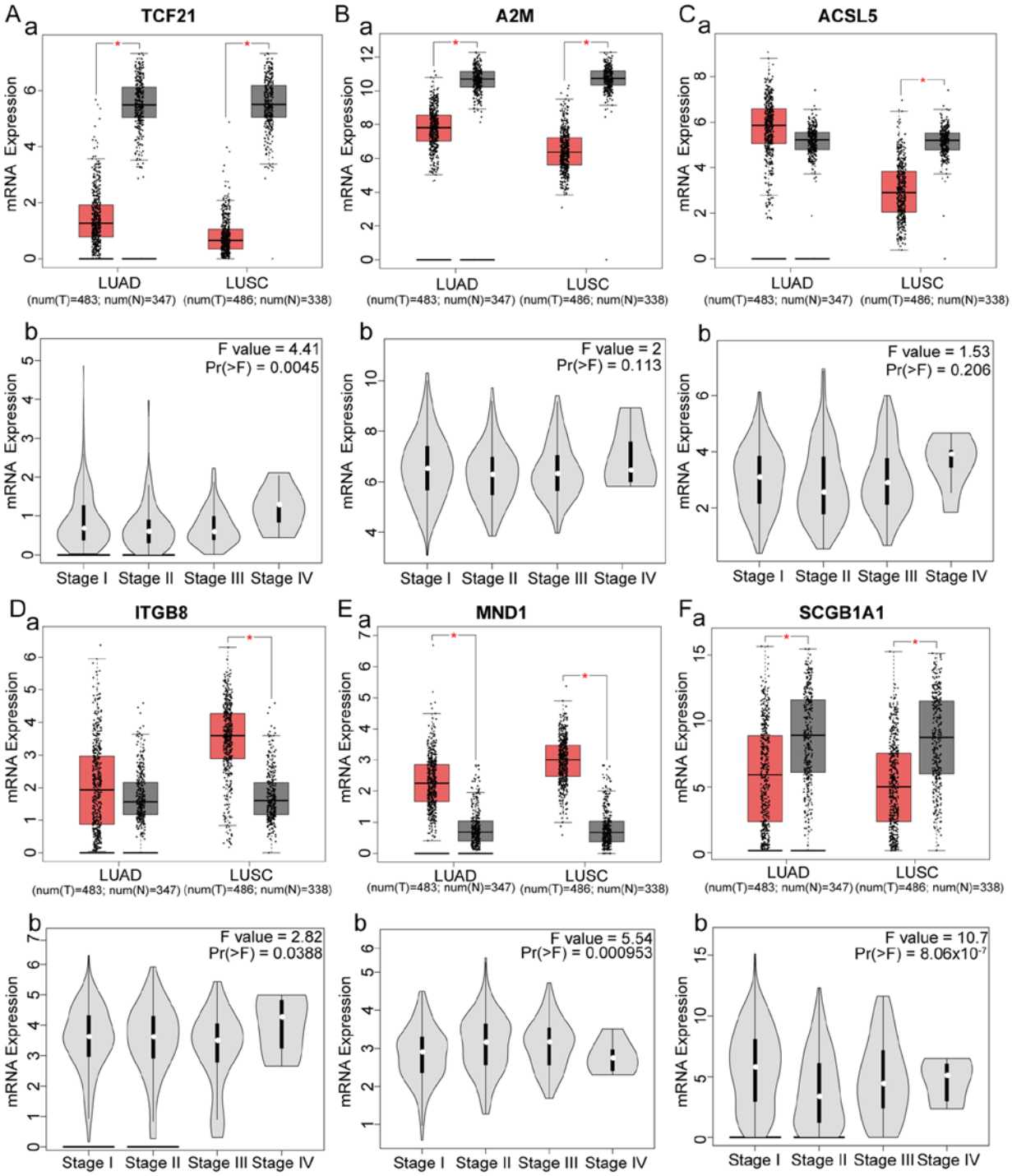

Figure 4. Box and violin plots were produced using the Gene Expression Profiling Interactive Analysis online platform. The boxplots present the expression of hub genes in LUSC and LUAD; red, tumor tissue; gray, normal tissue. Violin plots demonstrate the association between hub gene expression and LUSC staging. (Aa) TCF21 was downregulated in LUSC and LUAD; (Ab) TCF21 was associated with LUSC stages [Pr(>F)=0.0045]. (Ba) A2M was downregulated in both LUSC and LUAD; (Bb) A2M was not significantly different among the different stages of LUSC; (Ca) ACSL5 was downregulated in LUSC but upregulated in LUAD; (Cb) ACSL5 was not significantly different among the different stages of LUSC. (Da) ITGB8 was upregulated in LUAD and LUSC; ITGB8 was associated with stages of LUSC [Pr(>F)=0.0388] ; (Ea) MND1 was upregulated in LUAD and LUSC; MND1 was associated with stages of LUSC [Pr(>F)=0.000953] (Fa) SCGB1A1 was downregulated in both LUSC and LUAD; SCGB1A1 was closely related to stages of LUSC [Pr(>F)=8.06×10^{-7}]. *P<0.01 tumor group vs. control group. LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TCF21, transcription factor 21; A2M, α-2-macroglobulin; ACSL5, acyl-CoA synthetase long chain family member 5; ITGB8, integrin subunit beta 8; SCGB1A1, secretoglobin family 1A member 1; MND1, meiotic nuclear divisions 1; T, tumor; N, normal.

have a stronger T-helper 2-directed immune response compared with control mice (53). In the present study, SCGB1A1 was associated with tumor stage, EGFR mutation, ALK gene fusion and smoking history. A previous study has revealed that EGFR tyrosine kinase inhibitors have the same effect on the prognosis of patients with LUSC as chemotherapy, with fewer complications and higher quality of life (54). Therefore, SCGB1A1 may serve a protective role in lung tissue. Inducing SCGB1A1 expression may inhibit the expression of c-MYC and C-RAF, which may further inhibit the metastasis of

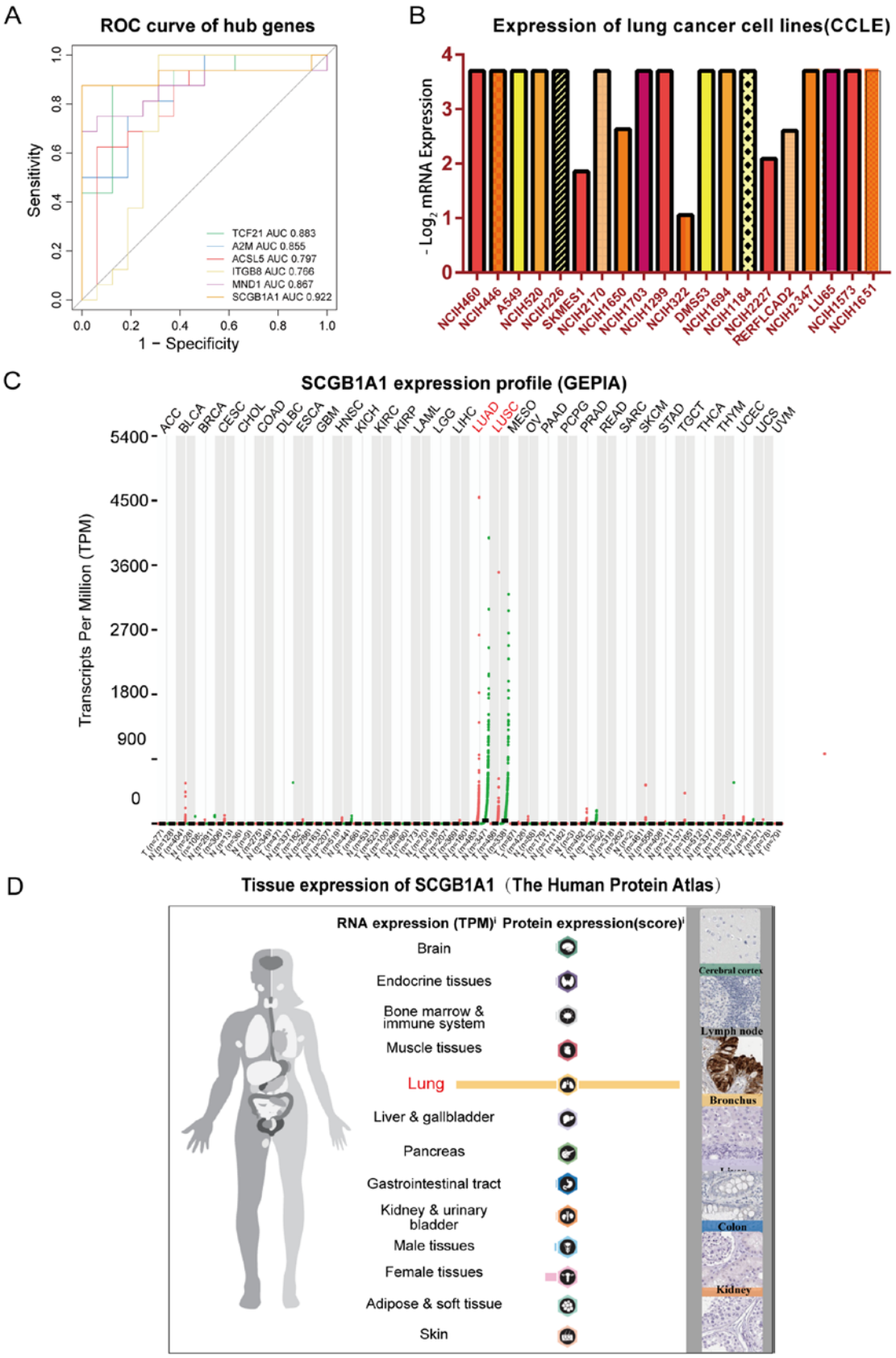

Figure 5. ROC curves of hub genes and SCGB1A1 expression in different tumors, cell lines and tissues. (A) ROC curves of hub genes were plotted based on the Gene Expression Omnibus dataset. The closer AUC value is to one, the higher the diagnostic value of the gene. (B) SCGB1A1 gene expression profiles of 20 common lung cancer cell lines based on the CCLE database. (C) Dot plot of SCGB1A1 gene expression profile across different tumor samples and paired normal tissues. Each dot represents sample expression; red denotes tumor samples and green denotes normal samples. (D) SCGB1A1 mRNA and protein expression in normal human tissues based on The Human Protein Atlas. ROC, receiver operating characteristic; SCGB1A1, secretoglobin family 1A member 1; MND1, meiotic nuclear divisions 1; TCF21, transcription factor 21; A2M, α-2-macroglobulin; ACSL5, acyl-CoA synthetase long chain family member 5; ITGB8, integrin subunit beta 8; CCLE, Cancer Cell Line Encyclopedia; GEPIA, Gene Expression Profiling Interactive Analysis; TPM, transcripts per million; AUC, area under the curve; T, tumor; N, normal.

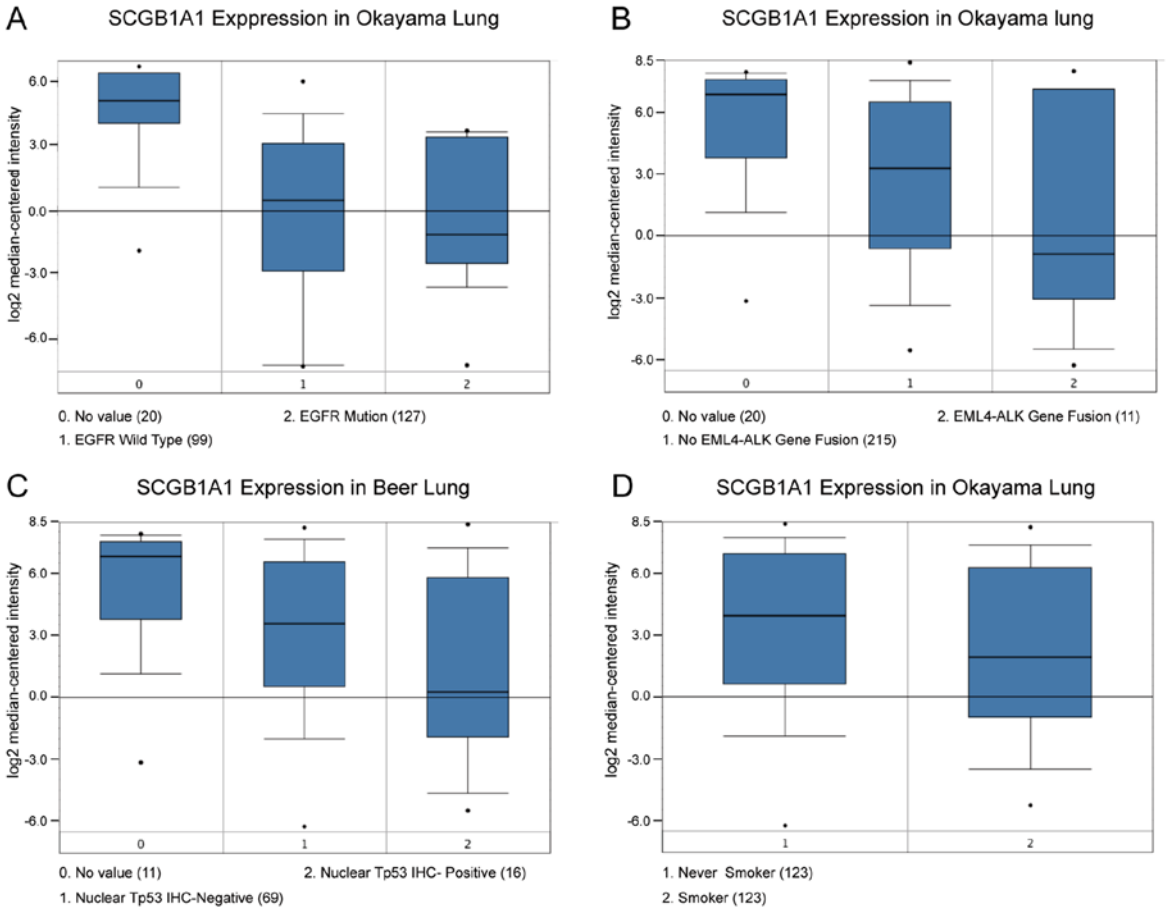

Figure 6. Associations between the expression of SCGB1A1 and (A) EGFR mutation, (B) EML4-ALK gene fusion, (C) expression of TP53 and (D) smoking in the Okayama and Beer dataset of the Oncomine database. No value, patients without the phenotype. SCGB1A1, secretoglobin family 1A member 1; EGFR, epidermal growth factor receptor; EML4, microtubule associated protein-like 4; ALK, anaplastic lymphoma kinase; IHC, immunohistochemistry.

tumors (55). However, the mechanism remains unclear, and future studies are required to elucidate the pathways involved in SCGB1A1 and lung cancer.

TCF21, located on chromosome 6q23-q24, encodes a basic helix-loop-helix transcription factor essential for epithelial cell differentiation (56,57). It can be readily methylated and subsequently cause tumorigenesis (58,59). A previous study has indicated that hypermethylation and decreased expression of TCF21 are tumor-specific and are frequently observed in NSCLC (60). The protein encoded by A2M is a protease inhibitor and cytokine transporter (61). A previous study has revealed that a progressive A2M deficiency may promote tumor development in nude mice (62). A2M regulated tumor cell adhesion, migration and growth by inhibiting tumor-promoting signaling pathways, including the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway and mothers against decapentaplegic homolog (SMAD) and upregulating phosphatase and tensin homolog via downregulation of microRNA-21 *in vitro* and in tumor xenografts (62). The level of A2M in human blood decreases with age (63). ACSL5, a mitochondria-localized enzyme that catalyzes the synthesis of long-chain fatty acid thioesters, is physiologically involved in the induction of apoptosis in intestinal cells (64). Studies have revealed that ACSL5 isozymes serve leading roles in the biosynthesis of mitochondrial cardiolipin and may participate in the survival of cancer cells (65-67). ITGB8, a member of the integrin β chain family, is increased in different types of cancer, including breast, lung, throat and stomach cancer (68). High expression of ITGB8 serves an important role in the metastasis of human lung cancer cells. When ITGB8 is silenced, the expression of E-cadherin and cystatin B is increased, whereas the expression of C-X-C motif chemokine ligand CXCL1, CXCL2, CXCL5, matrix metalloproteinase (MMP)-2 and MMP-9 is decreased (69). Furthermore, changes in the cell cycle, the expression of metastasis-associated genes and metastatic potential may be accompanied by decreased tumor cell signal transduction and molecular activity (69,70). The products of the MND1 gene bind to PSMC3 interacting protein to form stable heterodimer complexes that bind to DNA and stimulate the activities of RAD51 recombinase and DNA meiotic recombinase 1, which are required for meiotic recombination (71). MND1 was significantly upregulated in ovarian cancer compared with ovarian tissue samples from healthy controls (72). However, to the best of our knowledge, MND1 upregulation has not been previously reported in human lung cancer.

The hub genes identified in the current study are associated with the occurrence and development of tumors. The involvement of TCF21 and ITGB8 in lung cancer has been previously documented. However, there are fewer reports of SCGB1A1, A2M, ACSL5 and MND1 in lung cancer. *In vitro* overexpression of TCF21 may inhibit tumor growth and chemoresistance possibly

through the AKT signaling pathway (73,74). Upregulation of ITGB8 may promote the expression of tumor metastasis genes and enhance the invasive ability of tumor cells in LUSC by regulating the phosphorylation levels of mitogen-activated protein kinase/extracellular signal-regulated kinase and AKT. An increased incidence of lung injury and lung tumors was reported following SCGB1A1 knockout (52). A previous study has reported that SCGB1A1 may serve an anti-inflammatory role by inhibiting phospholipase A2 (75); therefore, the down-regulation of SCGB1A1 may lead to an imbalance of T cell subsets, which in turn affects the antitumor activity of serum peripheral blood mononuclear cells in patients with LUSC (76). Transcriptome analysis of A2M-treated tumor cells, xenografts and mouse liver revealed that A2M modulates tumor cell adhesion, migration and proliferation by inhibiting tumor-promoting signaling pathways, such as PI3K/AKT and SMAD, and by upregulating PTEN via downregulation of miR-21 *in vitro* and in tumor xenografts (77). ASCL5 is closely associated with cancer cell apoptosis (64). The hub genes in the current study were associated with poor overall survival rates, and ROC curves revealed high diagnostic values (AUC>0.7). The results obtained in the current study suggest that these genes may serve important roles in the occurrence and development of LUSC and may be used as biomarkers for the diagnosis of LUSC.

In conclusion, the purpose of the current study was to identify genes that may be involved in the development or progression of LUSC. A total of 37 DEGs were identified, of which 6 were identified as hub genes and may be used as biomarkers for the diagnosis and prognosis evaluation of LUSC. However, the results of this study were obtained through big data analysis, and validation of the results via animal experiments and clinical trials is required.

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Availability of data and materials

The dataset of GSE31522 analyzed during the present study are available in the Gene Expression Omnibus repository (https://www. ncbi.nlm.nih.gov/gds); The dataset of TCGA analyzed during the current study are available in Cancer RNA-Seq Nexus (https://syslab4.nchu.edu.tw); The dataset of GEPIA analyzed during the current study are available in the Gene Expression Profiling Interactive Analysis databases (https://gepia.cancer-pku.cn).

Authors' contributions

YXW, NNZ and QQX designed and conceived the study. NNZ performed the bioinformatics analysis and wrote the manuscript. HW, HC, FQW and DBDW contributed to data collection, data analysis and revised the manuscript. All the authors read and approved the 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.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
2. Gandara DR, Hammerman PS, Sos ML, Lara PN Jr and Hirsch FR: Squamous cell lung cancer: From tumor genomics to cancer therapeutics. Clin Cancer Res 21: 2236-2243, 2015.
3. Perezmoreno P, Brambilla E, Thomas R and Soria JC: Squamous cell carcinoma of the lung: Molecular subtypes and therapeutic opportunities. Clin Cancer Res 18: 2443-2451, 2012.
4. Kuribayashi K, Funaguchi N and Nakano T: Chemotherapy for advanced non-small cell lung cancer with a focus on squamous cell carcinoma. J Cancer Res Ther 12: 528-534, 2016.
5. Morrey ME, Abdel MP, Riester SM, Dudakovic A, van Wijnen AJ, Morrey BF and Sanchez-Sotelo J: Molecular landscape of arthrofibrosis: Microarray and bioinformatic analysis of the temporal expression of 380 genes during contracture genesis. Gene 610: 15-23, 2017.
6. Liu C, Fei HD, Sun ZY and Tian JW: Bioinformatic analysis of the microarray gene expression profile in degenerative intervertebral disc cells exposed to TNF-α. Eur Rev Med Pharmacol Sci 19: 3332-3339, 2015.
7. Meltzer EB, Barry WT, D'Amico TA, Davis RD, Shu SL, Onaitis MW, Morrison LD, Sporn TA, Steele MP and Noble PW: Bayesian probit regression model for the diagnosis of pulmonary fibrosis: Proof-of-principle. BMC Med Genomics 4: 70, 2011.
8. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, *et al*: NCBI GEO: Archive for functional genomics data sets-update. Nucleic Acids Res 39 (Database Issue): D991-D1005, 2013.
9. Tomczak K, Czerwińska P and Wiznerowicz M: The cancer genome atlas (TCGA): An immeasurable source of knowledge. Contemp Oncol (Pozn) 19: A68-A77, 2015.
10. Lin J, Marquardt G, Mullapudi N, Wang T, Han W, Shi M, Keller S, Zhu C, Locker J and Spivack SD: Lung cancer transcriptomes refined with laser capture microdissection. Am J Pathol 184: 2868-2884, 2014.
11. Li JR, Sun CH, Li W, Chao RF, Huang CC, Zhou XJ and Liu CC: Cancer RNA-Seq Nexus: A database of phenotype-specific transcriptome profiling in cancer cells. Nucleic Acids Res 44 (D1): D944-D951, 2016.
12. Core Team R: R: A language and environment for statistical computing. Computing 1: 12-21, 2015.
13. Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, Botstein D and Altman RB: Missing value estimation methods for DNA microarrays. Bioinformatics 17: 520-525, 2001.
14. Hogan SJ, Feltz KT, Murdock DR, Goodman TA, Vercande DJ, Tangeman MR, Busch EM, Kripakaran R, Jayasimha MG, Smith KE, *et al*: Call-processing system and method, 2003.
15. Pathan M, Keerthikumar S, Ang CS, Gangoda L, Quek CY, Williamson NA, Mouradov D, Sieber OM, Simpson RJ, Salim A, *et al*: FunRich: An open access standalone functional enrichment and interaction network analysis tool. Proteomics 15: 2597-2601, 2015.
16. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, *et al*: The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 38 (Web Server Issue): W214-W220, 2010.

17. Goldman M, Craft B, Zhu J and Haussler D: Abstract 2584: The UCSC Xena system for cancer genomics data visualization and interpretation. Cancer Res 77: 2584, 2017.
18. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al*: Gene ontology: Tool for the unification of biology. The gene ontology consortium. Nat Genet 25: 25-29, 2000.
19. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H and Kanehisa M: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 27: 29-34, 1999.
20. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA: The DAVID gene functional classification tool: A novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 8: R183, 2007.
21. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z: GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 45 (W1): W98-W102, 2017.
22. Piao J, Sun J, Yang Y, Jin T, Chen L and Lin Z: Target gene screening and evaluation of prognostic values in non-small cell lung cancers by bioinformatics analysis. Gene 647: 306-311, 2018.
23. Mitteer DR, Greer BD, Fisher WW and Cohrs VL: Teaching behavior technicians to create publication-quality, single-case design graphs in graphpad prism 7. J Appl Behav Anal 51: 998-1010, 2018.
24. Pontén F, Jirström K and Uhlen M: The human protein atlas-a tool for pathology. J Pathol 216: 387-393, 2010.
25. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A and Chinnaiyan AM: ONCOMINE: A cancer microarray database and integrated data-mining platform. Neoplasia 6: 1-6, 2004.
26. Beer DG, Kardia SL, Huang CC, Giordano TJ, Levin AM, Misek DE, Lin L, Chen G, Gharib TG, Thomas DG, *et al*: Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat Med 8: 816-824, 2002.
27. Non-Small Cell Lung Cancer Collaborative Group: Chemotherapy and supportive care versus supportive care alone for advanced non-small cell lung cancer. Cochrane Database Syst Rev 12: CD007309, 2010.
28. Blandin KS, Crosbie PA, Balata H, Chudziak J, Hussell T and Dive C: Progress and prospects of early detection in lung cancer. Open Biol 7: pii:170070, 2017.
29. Mayekar MK and Bivona TG: Current landscape of targeted therapy in lung cancer. Clin Pharmacol Ther 102: 757-764, 2017.
30. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, Shafi S, Johnson DH, Mitter R, Rosenthal R, *et al*: Tracking the evolution of non-small-cell lung cancer. N Engl J Med 376: 2109-2121, 2017.
31. Youlden DR, Cramb SM and Baade PD: The international epidemiology of lung cancer: Geographical distribution and secular trends. J Thorac Oncol 3: 819-831, 2008.
32. Cheng TY, Cramb SM, Baade PD, Youlden DR, Nwogu C and Reid ME: The international epidemiology of lung cancer: Latest trends, disparities, and tumor characteristics. J Thorac Oncol 11: 1653-1671, 2016.
33. Raghavachari N: Microarray technology: Basic methodology and application in clinical research for biomarker discovery in vascular diseases. Methods Mol Biol 1027: 47-84, 2013.
34. Li G, Li X, Yang M, Xu L, Deng S and Ran L: Prediction of biomarkers of oral squamous cell carcinoma using microarray technology. Sci Rep 7: 42105, 2017.
35. Albelda SM and Buck CA: Integrins and other cell adhesion molecules. FASEB J 4: 2868-2880, 1990.
36. Zaman MH, Trapani LM, Siemeski A, Mackellar D, Gong H, Kamm RD, Wells A, Lauffenburger DA and Matsudaira P: Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. Proc Natl Acad Sci USA 103: 10889-10894, 2006.
37. Uccelli A, Moretta L and Pistoia V: Mesenchymal stem cells in health and disease. Nat Rev Immunol 8: 726-736, 2008.
38. Wang D, Song Z and Wang Z: Common mechanism of pathogenesis in various types of metastatic osteosarcoma. Oncol Lett 14: 6307-6313, 2017.
39. Laval S, Laklai H, FanJul M, Pucelle M, Susini C, Pyronnet S and Bousquet C: Abstract 5181: Forced hemidesmosome assembly blocks pancreatic cancer cell invasiveness. Cancer Res 70: 5181, 2011.
40. Zamanian-Daryoush M and DiDonato JA: Apolipoprotein A-I and cancer. Front Pharmacol 6: 265, 2015.
41. Zhang H, Tao J, Sheng L, Hu X, Rong R, Xu M and Zhu T: RETRACTED: Twist2 promotes kidney cancer cell proliferation and invasion via regulating ITGA6 and CD44 expression in the ECM-Receptor-Interaction pathway. Biomed Biomed Pharmacother 81: 453-459, 2016.
42. Golubovskaya VM: Focal adhesion kinase as a cancer therapy target. Anticancer Agents Med Chem 10: 735-741, 2010.
43. Randell SH: Airway epithelial stem cells and the pathophysiology of chronic obstructive pulmonary disease. Proc Am Thorac Soc 3: 718-725, 2006.
44. Giangreco A, Reynolds SD and Stripp BR: Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am J Pathol 161: 173-182, 2002.
45. Antico G, Lingen MW, Sassano A, Melby J, Welch RW, Fiore S, Pilon AL and Miele L: Recombinant human uteroglobin/CC10 inhibits the adhesion and migration of primary human endothelial cells via specific and saturable binding to fibronectin. J Cell Physiol 207: 553-561, 2010.
46. Singh G and Katyal SL: Clara cell proteins. Ann N Y Acad Sci 923: 43-58, 2000.
47. Yang Y, Zhang Z, Mukherjee AB and Linnoila RI: Increased susceptibility of mice lacking Clara cell 10-kDa protein to lung tumorigenesis by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a potent carcinogen in cigarette smoke. J Biol Chem 279: 29336-29340, 2004.
48. Wang SZ, Rosenberger CL, Bao YX, Stark JM and Harrod KS: Clara cell secretory protein modulates lung inflammatory and immune responses to respiratory syncytial virus infection. J Immunol 171: 1051-1060, 2003.
49. Watson TM, Reynolds SD, Mango GW, Boe IM, Lund J and Stripp BR: Altered lung gene expression in CCSP-null mice suggests immunoregulatory roles for Clara cells. Am J Physiol Lung Cell Mol Physiol 281: L1523-L1530, 2001.
50. Laucho-Contreras ME, Polverino F, Tesfaigzi Y, Pilon A, Celli BR and Owen CA: Club cell protein 16 (CC16) augmentation: A potential disease-modifying approach for chronic obstructive pulmonary disease (COPD). Expert Opin Ther Targets 20: 869-883, 2016.
51. Ludovini V, Bianconi F, Siggillino A, Piobbico D, Vannucci J, Metro G, Chiari R, Bellezza G, Puma F, Della Fazia MA, *et al*: Gene identification for risk of relapse in stage I lung adenocarcinoma patients: A combined methodology of gene expression profiling and computational gene network analysis. Oncotarget 7: 30561-30574, 2016.
52. Linnoila RI, Szabo E, DeMayo F, Witschi H, Sabourin C and Malkinson A: The role of CC10 in pulmonary carcinogenesis: From a marker to tumor suppression. Ann N Y Acad Sci 923: 249-267, 2000.
53. Bourdin A, Kotsimbos T, Nguyen K, Vachier I, Mainprice B, Farce M, Paganin F, Marty-Ané C, Vernhet H, Godard P and Chanez P: Non-invasive assessment of small airway remodelling in smokers. COPD 7: 102-110, 2010.
54. Sun Y, Yin X, Wen MM, Zhang J, Wang XJ, Xia JH, Zhang YN, Zhang ZP and Li XF: EGFR mutations subset in Chinese lung squamous cell carcinoma patients. Mol Med Rep 17: 7575-7584, 2018.
55. Thakur C, Rapp UR and Rudel T: Cysts mark the early stage of metastatic tumor development in non-small cell lung cancer. Oncotarget 9: 6518-6535, 2017.
56. Robb L, Mifsud L, Hartley L, Biben C, Copeland NG, Gilbert DJ, Jenkins NA and Harvey RP: Epicardin: A novel basic helix-loop-helix transcription factor gene expressed in epicardium, branchial arch myoblasts, and mesenchyme of developing lung, gut, kidney, and gonads. Dev Dyn 213: 105-113, 1998.
57. Smith LT, Lin M, Brena RM, Lang JC, Schuller DE, Otterson GA, Morrison CD, Smiraglia DJ and Plass C: Epigenetic regulation of the tumor suppressor gene TCF21 on 6q23-q24 in lung and head and neck cancer. Proc Natl Acad Sci USA 103: 982-987, 2006.
58. Arab K, Smith LT, Gast A, Weichenhan D, Huang JP, Claus R, Hielscher T, Espinosa AV, Ringel MD, Morrison CD, *et al*: Epigenetic deregulation of TCF21 inhibits metastasis suppressor KISS1 in metastatic melanoma. Carcinogenesis 32: 1467-1473, 2011.
59. Costa VL, Henrique R, Danielsen SA, Eknaes M, Patrício P, Morais A, Oliveira J, Lothe RA, Teixeira MR, Lind GE and Jerónimo C: TCF21 and PCDH17 methylation: An innovative panel of biomarkers for a simultaneous detection of urological cancers. Epigenetics 6: 1120-1130, 2011.

60. Richards KL, Zhang B, Sun M, Dong W, Churchill J, Bachinski LL, Wilson CD, Baggerly KA, Yin G, Hayes DN, *et al*: Methylation of the candidate biomarker TCF21 is very frequent across a spectrum of early-stage nonsmall cell lung cancers. Cancer 117: 606-617, 2011.
61. Chen X, Kong X, Zhang Z, Chen W, Chen J, Li H, Cao W, Ge Y and Fang S: Alpha-2-macroglobulin as a radioprotective agent: A review. Chin J Cancer Res 26: 611-621, 2014.
62. Kurz S, Thieme R, Amberg R, Groth M, Jahnke HG, Pieroh P, Horn LC, Kolb M, Huse K, Platzer M, *et al*: Correction: The anti-tumorigenic activity of A2M-A lesson from the naked mole-rat. PLoS One 13: e0195169, 2018.
63. Birkenmeier G, Müller R, Huse K, Forberg J, Gläser C, Hedrich H, Nicklisch S and Reichenbach A: Human alpha2-macroglobulin: Genotype-phenotype relation. Exp Neurol 184: 153-161, 2003.
64. Klaus C, Kaemmerer E, Reinartz A, Schneider U, Plum P, Jeon MK, Hose J, Hartmann F, Schnölzer M, Wagner N, *et al*: TP53 status regulates ACSL5-induced expression of mitochondrial mortalin in enterocytes and colorectal adenocarcinomas. Cell Tissue Res 357: 267-278, 2014.
65. Mashima T, Oh-hara T, Sato S, Mochizuki M, Sugimoto Y, Yamazaki K, Hamada J, Tada M, Moriuchi T, Ishikawa Y, *et al*: p53-defective tumors with a functional apoptosome-mediated pathway: A new therapeutic target. J Natl Cancer Inst 97: 765-777, 2005.
66. Ding S, Tang S, Wang M, Wu D and Guo H: Acyl-CoA synthetase 5 promotes the growth and invasion of colorectal cancer cells. Can J Gastroenterol Hepatol 2017: 7615736, 2017.
67. Pitule P, Vycital O, Bruha J, Novak P, Hosek P, Treska V, Hlavata I, Soucek P, Kralickova M and Liska V: Differential expression and prognostic role of selected genes in colorectal cancer patients. Anticancer Res 33: 4855-4865, 2013.
68. Ni R, Shen X, Wu H, Zhu W, Ni J, Huang Z, Song Y and Gao X: Expression and significance of integrins subunits in laryngeal squamous cell carcinoma. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 24: 686-689, 2010 (In Chinese).
69. Xu Z and Wu R: Alteration in metastasis potential and gene expression in human lung cancer cell lines by ITGB8 silencing. Anat Rec (Hoboken) 295: 1446-1454, 2012.
70. Huang L, Cai JL, Huang PZ, Kang L, Huang MJ, Wang L and Wang JP: miR19b-3p promotes the growth and metastasis of colorectal cancer via directly targeting ITGB8. Am J Cancer Res 7: 1996-2008, 2017.
71. Tsubouchi H and Roeder GS: The Mnd1 protein forms a complex with hop2 to promote homologous chromosome pairing and meiotic double-strand break repair. Mol Cell Biol 22: 3078-3088, 2002.
72. Yeganeh PN, Richardson C, Bahrani-Mostafavi Z, Tait DL and Mostafavi MT: Dysregulation of AKT3 along with a small panel of mRNAs stratifies high-grade serous ovarian cancer from both normal epithelia and benign tumor tissues. Genes Cancer 8: 784-798, 2017.
73. Yang Z, Jiang X, Li D, Dong Q, Zhao H and Jiang X: TCF21 inhibits proliferation and chemoresistance through the AKT pathway in human gastric cancer. Gene 682: 42-49, 2019.
74. Dai Y, Duan H, Duan C, Zhu H, Zhou R, Pei H and Shen L: TCF21 functions as a tumor suppressor in colorectal cancer through inactivation of PI3K/AKT signaling. Onco Targets Ther 10: 1603-1611, 2017.
75. Shijubo N, Itoh Y and Abe S: Anti-inflammatory molecule, Clara cell 10 kilodalton protein and respiratory diseases. Rinsho Byori 50: 370-373, 2002 (In Japanese).
76. Joyce JA and Fearon DT: T cell exclusion, immune privilege, and the tumor microenvironment. Science 348: 74-80, 2015.
77. Kurz S, Thieme R, Amberg R, Groth M, Jahnke HG, Pieroh P, Horn LC, Kolb M, Huse K, Platzer M, *et al*: The anti-tumorigenic activity of A2M-A lesson from the naked mole-rat. PLoS One 12: e0189514, 2017.