Original Article

Weighted gene co-expression network analysis in identification of metastasis-related genes of lung squamous cell carcinoma based on the Cancer Genome Atlas database

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Background: Lung squamous cell carcinoma (lung SCC) is a common type of malignancy. Its pathogenesis mechanism of tumor development is unclear. The aim of this study was to identify key genes for diagnosis biomarkers in lung SCC metastasis.

Methods: We searched and downloaded mRNA expression data and clinical data from The Cancer Genome Atlas (TCGA) database to identify differences in mRNA expression of primary tumor tissues from lung SCC with and without metastasis. Gene co-expression network analysis, protein-protein interaction (PPI) network, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and quantitative real-time polymerase chain reactions (qRT-PCR) were used to explore the biological functions of the identified dysregulated genes.

Results: Four hundred and eighty-two differentially expressed genes (DEGs) were identified between lung SCC with and without metastasis. Nineteen modules were identified in lung SCC through weighted gene co-expression network analysis (WGCNA). Twenty-three DEGs and 26 DEGs were significantly enriched in the respective pink and black module. KEGG pathway analysis displayed that 26 DEGs in the black module were significantly enriched in bile secretion pathway. Forty-nine DEGs in the two gene co-expression module were used to construct PPI network. CFTR in the black module was the hub protein, had the connectivity with 182 genes. The results of qRT-PCR displayed that FIGF, SFTPD, DYNLRB2 were significantly down-regulated in the tumor samples of lung SCC with metastasis and CFTR, SCGB3A2, SSTR1, SCTR, ROPN1L had the down-regulation tendency in lung SCC with metastasis compared to lung SCC without metastasis.

Conclusions: The dysregulated genes including CFTR, SCTR and FIGF might be involved in the pathology of lung SCC metastasis and could be used as potential diagnosis biomarkers or therapeutic targets for lung SCC.

Keywords: Lung squamous cell carcinoma (lung SCC); genes expression profiling; gene regulatory network; neoplasm metastasis; biomarkers

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Introduction

Lung squamous cell carcinoma (lung SCC) is a histological subtype of non-small cell lung cancer (NSCLC), which is the second most frequent type of NSCLC after lung adenocarcinoma (1). Lung SCC is a multi-step progressive disease with high rates of morbidity and mortality worldwide.

The initiation of lung SCC is divided into the following five successive stages: normal bronchial epithelium, squamous metaplasia, mild-moderate dysplasia, carcinoma *in situ*, and invasive carcinoma (2). The prognosis of lung cancer is unfavorable, despite significant therapeutic improvements have been made in recent years. In current, there is no specific molecular targets for therapy have been identified (3), therefore, cisplatin plus gemcitabine is still the first-line treatment for lung SCC (4).

Currently, the tumorigenesis mechanism of lung SCC remains unclear. Numerous published articles demonstrate that dysregulated genes are essential for initiation, progression and development of lung cancer. SMC4 (structural maintenance of chromosome 4) is over-expressed in lung adenocarcinoma tissues and its inhibition significantly suppresses the proliferation and invasion of A549 cells (5). Knocking down the expression of WW45 (salvador family WW domain containing protein 1) promotes cell growth and migration of lung cancer and over-expression of WW5 improves the survival of mice model of lung cancer (6). INO80 (INO80 complex subunit), the SWI/SNF ATPase in the complex, is highly expressed in NSCLC cells compared with normal lung epithelia cells and its expression level is negatively correlated with the disease prognosis of patients with lung cancer (7).

Weighted gene co-expression network analysis (WGCNA) offers an effective approach to quantitatively assess the interconnectedness of genes, investigate the expression patterns of gene co-activity and evaluate the importance of genes within the network. It benefit to provide potential malignancy diagnostic molecular and connecting them together for disease (8,9). Shi *et al.* identifies four co-expression modules significantly correlated with clinic trait, the hub gene of each module including RPS15A, PTGDS, CD53 and MSI2, which might play a vital role in progress of uveal melanoma (10).

To our knowledge, this is the first report of WGCNA of lung SCC expression profiles. In this study, bioinformatics methods were applied to integrate mRNA expression data of lung SCC in The Cancer Genome Atlas (TCGA) database and construct gene co-expression module for pathogenesis mechanism elucidation and identification of the diagnostic biomarkers and therapeutic targets of lung SCC metastasis.

Methods

TCGA gene expression profiles

The level 3 mRNA expression data of lung SCC and the corresponding clinical records in TCGA database (Oct 26, 2015) were obtained from the data portal (https://tcga-data.nci.nih.gov/tcga/). Total of 504 lung SCC patients were available in TCGA. The inclusion criteria of expression profiling in our study were shown as below: (I) the dataset was from primary solid tumor of patients with lung SCC; (II) patients had no other malignancy history; (III) patients received no treatment before collection of tumor samples. The mRNA expression datasets of 163 lung SCC patients with lymph node metastasis or distant metastasis, and mRNA expression datasets of 222 patients without metastasis were available in TCGA database. The datasets contained 20,531 genes and the sequencing of expression profiles was based on the platform of IlluminaHiSeq_RNASeqV2.

Identification of differentially expressed genes (DEGs)

The raw expression data of lung SCC patients in our study were downloaded. The significantly DEGs were identified between lung SCC patients with metastasis and lung SCC patients without metastasis through DESeq2 repackage in R language (11). The false discovery rate (FDR) was performed for multiple testing corrections of raw P value through the Benjamin and Hochberg method (12,13). The threshold of DEGs was set as FDR <0.05.

Construct gene co-expression network

To explore the interactions between the genes, a system biology approach, WGCNA, which converts co-expression measure into connections weight or topology overlap measure, was applied for gene co-expression network construction (14). Co-expression methodology is typically used for explore correlation between gene expression level. Genes involved in the same pathway or same functional compound tend to demonstrate a similar expression pattern (15). Therefore, the construction of a gene co-expression network facilitates the identification of genes with similar biological functions (16). In our work, all of genes of

lung SCC patients with metastasis and lung SCC patients without metastasis were inputted to construct weighted co-expression modules using the WGCNA package in R language (17). The threshold of co-expression module was set as P<0.05.

Functional annotation

To obtain the biological function and signaling pathways of DEGs, the online software, GeneCodis3 was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment and Gene Ontology (GO) annotation of DEGs (18). The threshold of GO function and KEGG pathway of DEGs was set as FDR <0.0001 and FDR <0.05, respectively.

Protein-protein interaction (PPI)

In order to obtain insights into the interaction between DEGs of the black module and the pink module, PPI network was constructed by BioGRID, a database of known and predicted protein interactions (19). Then, PPI was visualized by Cytoscape software (http://cytoscape.org/) (20).

The expression level of DEGs in lung SCC tumor samples were validated by quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of fresh tumor samples from lung SCC patients with metastasis and lung SCC patients without metastasis were extracted by using TRIzol reagent (Invitrogen, CA, USA) according to the manual instructions. The SuperScript III Reverse Transcription Kit (Invitrogen, CA, USA) was used to synthesize the cDNA. qRT-PCR reactions were performed using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on the Applied Biosystems 7,500 (Applied Biosystems, Foster City, CA). 18s rRNA was used as internal control for mRNA detection. The relative expression of genes was calculated using the $2^{-\Delta\Delta CT}$ equation (21). The PCR primers used were shown as *Table S1*. The GraphPad Prism version 6.0 software packages (GraphPad Software, San Diego, CA, USA) was used to output figures.

Ethics statement

The study was approved by the ethics committee board of Linyi People's Hospital (No. lyll2015N67). Written informed consent was obtained from the patient for publication of this manuscript.

Results

DEGs between lung SCC patients with metastasis and lung SCC patients without metastasis

The raw expression profiles of lung SCC patients and corresponding clinical records were downloaded from the data portal of TCGA database. All of patients were divided into lung SCC with metastasis group or lung SCC without metastasis according to AJCC pathologic tumor stage. DEGs analysis was performed to the two groups. Total of 482 DEGs were identified as the threshold of FDR <0.05 (supplementary *Table S2*), consisting of 312 up-regulated DEGs and 170 down-regulated DEGs. As *Table 1* shown, VEG, VSIG10L and MFAP5 were the most significantly up-regulated DEGs; ZNF208, C8orf46 and TNF were the most significantly down-regulated DEGs.

Functional annotation of DEGs between lung SCC with and without metastasis

To explore the functional significance of the identified DEGs in lung SCC metastasis, 482 DEGs were performed to unbiased GO term and KEGG pathway enrichment analyses. For DEGs related to lung SCC metastasis, transmembrane transport (FDR =8.76E-06), calcium ion binding (FDR =5.18E-06) and extracellular region (FDR =1.20E-17) were the most significant enrichment in biological process, molecular function and cellular component, respectively (*Table 2*); PPAR signaling pathway (KEGG ID: hsa03320, FDR =1.64E-05), cytokine-cytokine receptor interaction (KEGG ID: hsa04060, FDR =1.05E-03) and neuroactive ligand-receptor interaction (KEGG ID: hsa04080, FDR =3.95E-03) were the most significantly enriched pathways (*Table 3*).

Construction of weighted gene co-expression modules

To explore the functional modules in lung SCC patients, the co-expression analysis of the 20,531 genes were performed in WGCNA. Modules for lung SCC were generated using the Scale-free Topology Criterion with a power cutoff of 12 and a minimum module size cutoff of 30. As *Figure 1* and *Table 4* shown, a total of 19 modules were identified. 23 DEGs and 26 DEGs between lung SCC with metastasis

Table 1 The top ten DEGs in lung SCC with metastasis

Gene ID	Gene symbol	Log_2FC	FDR
Up-regulated DEGs			
7425	VGF	6.302272	1.90E-06
147645	VSIG10L	5.870895	1.28E-05
8076	MFAP5	5.335057	0.000187
6927	HNF1A	5.184579	0.000382
57521	RPTOR	5.125631	0.000449
2147	F2	5.062773	0.000535
22809	ATF5	4.959337	0.000714
51083	GAL	4.944154	0.000714
3706	ITPKA	4.839489	0.001009
84057	MND1	4.835881	0.001009
Down-regulated DEGs			
7757	ZNF208	−6.99816	5.04E-08
254778	C8orf46	−6.37244	1.81E-06
7124	TNF	−5.93751	1.28E-05
2890	GRIA1	−5.90534	1.28E-05
339398	LINGO4	−5.8607	1.28E-05
338324	S100A7A	−5.42097	0.000144
4693	NDP	−5.33403	0.000187
116379	IL22RA2	−5.12306	0.000449
25833	POU2F3	−5.09145	0.000493
8190	MIA	−5.04813	0.000542

DEG, differentially expressed genes; lung SCC, lung squamous cell carcinoma; FC, fold change; FDR, false discovery rate.

Table 2 GO term enrichment analyses of DEGs

GO ID	GO term	Count	FDR
Biological process			
GO:0055085	Transmembrane transport	24	8.76E-06
GO:0006810	Transport	27	9.31E-06
GO:0050995	Negative regulation of lipid catabolic process	4	2.29E-05
GO:0007165	Signal transduction	4	4.67E-05
GO:0051091	Positive regulation of sequence-specific DNA binding transcription factor activity	4	4.67E-05
GO:0070374	Positive regulation of ERK1 and ERK2 cascade	4	4.67E-05
GO:0042493	Response to drug	4	4.67E-05
GO:0001666	Response to hypoxia	4	4.67E-05
Molecular function			
GO:0005509	Calcium ion binding	29	5.18E-06
GO:0005216	Ion channel activity	9	7.99E-05
Cellular component			
GO:0005576	Extracellular region	81	1.20E-17
GO:0005615	Extracellular space	43	3.18E-12
GO:0005576	Extracellular region	39	1.52E-11
GO:0005737	Cytoplasm	119	5.21E-07
GO:0016021	Integral to membrane	101	3.11E-06
GO:0005886	Plasma membrane	87	3.69E-06
GO:0016020	Membrane	70	7.56E-05

GO, Gene Ontology; FDR, false discovery rate; DEG, differentially expressed genes.

and lung SCC without metastasis were enriched in the respective pink and black modules through Chi-square test at the threshold of P<0.05, besides that, the expression pattern of pink modules, as well as black module, were significantly different between lung SCC with metastasis and lung SCC without metastasis through *t*-test at the threshold of P<0.05.

The functional annotation of DEGs persevered in the black module and pink module

DEGs and biological function preserved in each module were displayed in *Tables 5* and *6*, respectively. For the black module, Digestion (FDR =4.66E-05) and Extracellular region (FDR =3.96E-06) were the most significant enrichment in biological process and cellular component, respectively; bile secretion (hsa04976, FDR =1.91E-05) were the one significantly enriched pathways. For the pink module, Cilium axoneme (FDR =2.38E-06) was the most significant enrichment in biological process. There was no KEGG pathway was enriched from DEGs of the pink module.

PPI network

There are respective 26 and 23 DEGs related to lung SCC

Table 3 KEGG pathway enrichment analyses of DEGs

KEGG ID	KEGG term	Count	FDR	Genes
hsa03320	PPAR signaling pathway	9	1.64E-05	*ACSBG1, FABP7, UCP1, APOA2, RXRG, ACSL4, APOC3, MMP1, OLR1*
hsa04060	Cytokine-cytokine receptor interaction	13	1.05E-03	*IL1B, KIT, CRLF2, TNFRSF19, CX3CR1, IL22RA2, CCL17, PDGFA, FIGF, LEP, PF4, IL20, CCR6*
hsa04080	Neuroactive ligand-receptor interaction	12	3.95E-03	*DRD2, DRD5, NTSR1, SCTR, SSTR1, AGTR2, GRIA1, GRIK4, LEP, PRSS1, F2, GRIK1*
hsa04920	Adipocytokine signaling pathway	6	3.01E-03	*ACSBG1, G6PC2, AGRP, LEP, RXRG, ACSL4*
hsa04972	Pancreatic secretion	7	3.05E-03	*CFTR, SCTR, BST1, CPB2, PRSS1, PLA2G2F, ATP2B3*
hsa04976	Bile secretion	5	1.67E-02	*CFTR, SCTR, AQP1, ABCC2, BAAT*
hsa05160	Hepatitis C	3	1.50E-02	*CLDN2, CLDN19, CLDN23*
hsa04514	Cell adhesion molecules (CAMs)	3	1.53E-02	*CLDN2, CLDN19, CLDN23*
hsa04670	Leukocyte transendothelial migration	3	1.50E-02	*CLDN2, CLDN19, CLDN23*
hsa04530	Tight junction	3	1.50E-02	*CLDN2, CLDN19, CLDN23*
hsa04964	Proximal tubule bicarbonate reclamation	3	1.53E-02	*SLC25A10, AQP1, CA4*
hsa04724	Glutamatergic synapse	3	1.78E-02	*GRIA1, GRIK4, GRIK1*
hsa04150	mTOR signaling pathway	4	2.10E-02	*RICTOR, RPS6KB1, RPTOR, FIGF*
hsa05200	Pathways in cancer	3	2.18E-02	*KIT, PDGFA, FIGF*

KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate; DEGs, differentially expressed genes.

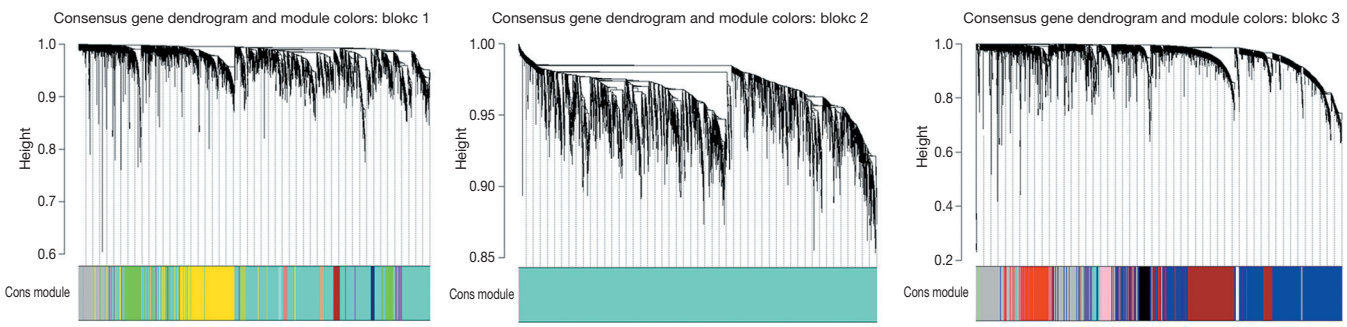

Figure 1 Network analysis of gene expression in lung SCC identifies 19 distinct modules of co-expression genes. The dendrogram produced by average linkage hierarchical clustering of 20,531 genes based on WGCNA package in R. Total of 19 modules were identified. The three panels were the whole dendrogram. lung SCC, lung squamous cell carcinoma; WGCNA, weighted gene co-expression network analysis.

metastasis in the black module and the pink module. To obtain the interaction between the DEGs in the both of modules, PPI network was explored and visualize by Cytoscape. As *Figure 2* shown, the network consisted of 396 nodes, 379 edges. In the PPIs network, the nodes with high degree are defined as hub protein. The most significant hub proteins were CFTR (degree =182), LRP2 (degree =37), HP (degree =20) and TEKT2 (degree =15). CFTR, LRP2, and HP were preserved in the black module; TEKT2 was preserved in the pink module.

Verification of the expression level of DEGs through qRT-PCR

To verify our bioinformatics analyses, the expression level of DEGs were quantified by qRT-PCR in three primary

tumor tissues of lung SCC patients with metastasis and three primary tumor tissues without metastasis. Seven DEGs including CFTR, FIGF, SSTR1, SFTPD, SCGB3A2, SCTR, DYNLRB2 were selected as candidate genes based on GO and KEGG annotation results from 49 genes in black and pink module, and literature review (22-26). One DEG, ROPN1L, was randomly selected as a candidate gene for qRT-PCR validation from 49 genes in black and pink module.

The biological roles of CFTR, FIGF, SSTR1, SFTPD and SCGB3A2 in lung cancer have been reported, but the biological roles of SCTR, DYNLRB2 an ROPN1L in lung SCC is unclear.

As shown in *Figure 3A-C*, FIGF (P<0.001), SFTPD (P<0.05), DYNLRB2 (P<0.05) were significantly down-regulated in the tumor samples of lung SCC with metastasis compared to those of lung SCC without metastasis; the expression levels of CFTR, SCGB3A2, SSTR1, SCTR and ROPN1L had no significance between lung SCC with metastasis and lung SCC without metastasis, but had the down-regulation tendency in lung SCC with metastasis (*Figure 3D-H*).

Table 4 The characteristics of gene co-expression modules

Module colors consensus	Number of genes	Number of DEGs	T.pval	Chi2.pval
Black	167	26	3.95E-03	5.48E-23
Blue	1,822	61	2.06E-01	1.23E-02
Brown	1,272	44	3.45E-01	1.92E-02
Cyan	56	2	8.23E-02	8.83E-01
Green	493	6	6.68E-01	1.43E-01
Green yellow	89	0	5.25E-01	2.76E-01
Grey	1,652	52	5.38E-01	5.71E-02
Grey60	35	0	6.57E-02	7.33E-01
Light cyan	51	0	9.71E-01	5.32E-01
Light green	31	1	3.05E-01	1.00E+00
Magenta	129	1	3.95E-01	3.87E-01
Midnight blue	54	2	6.33E-02	8.49E-01
Pink	160	23	3.42E-02	1.04E-18
Purple	93	0	3.45E-01	2.59E-01
Red	441	5	7.91E-01	1.38E-01
Salmon	56	3	3.54E-01	3.21E-01
Tan	75	4	1.26E-01	2.07E-01
Turquoise	8,538	101	5.16E-01	3.12E-10
Yellow	968	13	9.01E-01	5.89E-02

DEGs, differentially expressed genes; T.pval, P value of *t*-test; Chi2.pval, P value of Chi-square.

Discussion

Two gene co-expression modules involved in the process of lung SCC metastasis were identified in our study. The black module and pink module contained 26 and 23 DEGs in lung SCC with metastasis compared to lung SCC without metastasis, respectively. The DEGs in the black module including CFTR, SCTR and BAAT were significantly enriched in the bile secretion pathway (hsa04976). Bile secretion is essential for digestion and absorption of fats and fat-soluble vitamins in the small intestine and the pathway is closely related to cholangiocarcinoma, gall bladder disease and familial cholestasis (27). Bile secretion pathway might be involved in the process of lung SCC metastasis.

The official name of CFTR is cystic fibrosis (CF) transmembrane conductance regulator, is a member of the ATP-binding cassette (ABC) transporter superfamily.

Mutations in this gene are associated with the autosomal recessive disorders CF, which leads to the abnormal transport of chloride and sodium across the epithelium, resulting in chronic lung obstruction, infection and inflammation.

Table 5 DEGs preserved in the modules

Module	DEGs
Black [26]	BAAT, BMP3, C16orf89, CFTR, CLDN2, CRTAC1, FIGF, HP, IGFALS, KCNK17, KLHDC7A, KNDC1,LRP2, MBL1P, NRGN, RASGRF1, RRAD, SCGB3A1, SCGB3A2, SCN1A, SCTR, SFTPD, SLC46A2, SLC5A9, SSTR1, WFDC2
Pink [23]	EFCAB12, C1orf192, C22orf15, C5orf49,C6orf165, CCDC157, CCDC42B, CCDC65, DNAH6, DYDC2, DYNLRB2, EFCAB6, FAM166B, FAM183A, FAM92B, ROPN1L, SNTN, TEKT2, TEKT3, TMEM232, TSNAXIP1, WDR93, ZNF474

DEGs, differentially expressed genes.

Table 6 The function and pathway enrichment of DEGs of black module and pink module

ID	Terms	FDR	Genes
Black module-biological process			
GO:0007586	Digestion	4.66E-05	*SSTR1, BAAT, SCTR*
GO:0006811	Ion transport	0.00158761	*KCNK17, SCN1A, CFTR, SLC5A9*
GO:0055085	Transmembrane transport	0.00176614	*SCN1A, SLC46A2, CFTR, SLC5A9*
GO:0007165	Signal transduction	0.00987753	*RASGRF1, NRGN, IGFALS, RRAD*
Black module-cellular component			
GO:0005576	Extracellular region	3.96E-06	*C16orf89, SCGB3A2, HP, SFTPD, FIGF, WFDC2, SCGB3A1, IGFAL*
GO:0005615	Extracellular space	3.72E-05	*SFTPD, FIGF, WFDC2, SCGB3A1,IGFALS,BMP3*
GO:0005886	Plasma membrane	0.000342	*RASGRF1, SSTR1, KCNK17, SCN1A, SCTR, SLC46A2, CLDN2, LRP2, RRAD*
GO:0016021	Integral to membrane	0.013611	*KLHDC7A, KCNK17, SCN1A, SLC46A2, CLDN2, LRP2, CFTR, SLC5A9*
GO:0016020	Membrane	0.023071	*KLHDC7A, KCNK17, SCN1A, FIGF, LRP2, CFTR, SLC5A*
Black-KEGG pathway			
hsa04976	Bile secretion	1.91E-05	*CFTR, SCTR, BAAT*
Pink-biological process			
GO:0035085	Cilium axoneme	2.38E-06	*TEKT3, DNAH6, TEKT2*
GO:0005929	Cilium	1.34E-06	*SNTN, TEKT3, DNAH6, TEKT2*
GO:0005737	Cytoplasm	1.31E-05	*TEKT3, DNAH6, TEKT2, DYNLRB2*
GO:0005856	Cytoskeleton	1.31E-05	*TEKT3, DNAH6, TEKT2, DYNLRB2*
GO:0005874	Microtubule	1.31E-05	*TEKT3, DNAH6, TEKT2, DYNLRB2*

FDR, false discovery rate; DEG, differentially expressed genes.

CF affects not only the physiological function of lungs, but also the pancreas, liver and intestines (22,28). In our study, CFTR was the hub protein in the PPI network, had the highest connectivity with 182 genes (*Figure 2*). It was down-regulated in lung SCC metastasis and its expression level was validated through qRT-PCR (*Figure 3D*), the result was accordance with the previous study (29). Several articles demonstrate that abnormal CFTR expression is related to the tumorigenesis and development of NSCLC. Low CFTR expression is significantly associated with advanced stage, lymph node metastasis and poor prognosis in NSCLC patients (22). The *in vivo* and *in vitro* experiments present knockdown of CFTR promotes epithelial-mesenchymal transition, invasion and migration of NSCLC cells; conversely, overexpression of CFTR suppresses cancer progression of NSCLC (22). Methylation of the CFTR gene is significantly greater in lung SCC than in lung adenocarcinomas and CFTR gene methylation is associated with significantly poorer survival in young patients, but not in elderly patients (30). Based on the above, low expression of CFTR might play pivotal roles in the process of lung SCC metastasis.

SCTR encodes secretin receptor, is a G protein-coupled receptor and belongs to the glucagon-VIP-secretin receptor family. It has been observed to be upregulated or down-regulated in several tumor types, and functions as promoting or suppressing the proliferation of tumor cells. SCTR was significantly underexpressed in primary pancreatic neuroendocrine tumors, nodal and liver metastases (31). Down-regulation of SCTR by promoter methylation promotes the cell proliferation and migration of breast cancer (32). In our study, the verification results of SCTR through qRT-PCR were accordance with our bioinformatics analysis. In addition to the bile secretion pathway, SCTR was significantly enriched in neuroactive ligand-receptor interaction and pancreatic secretion

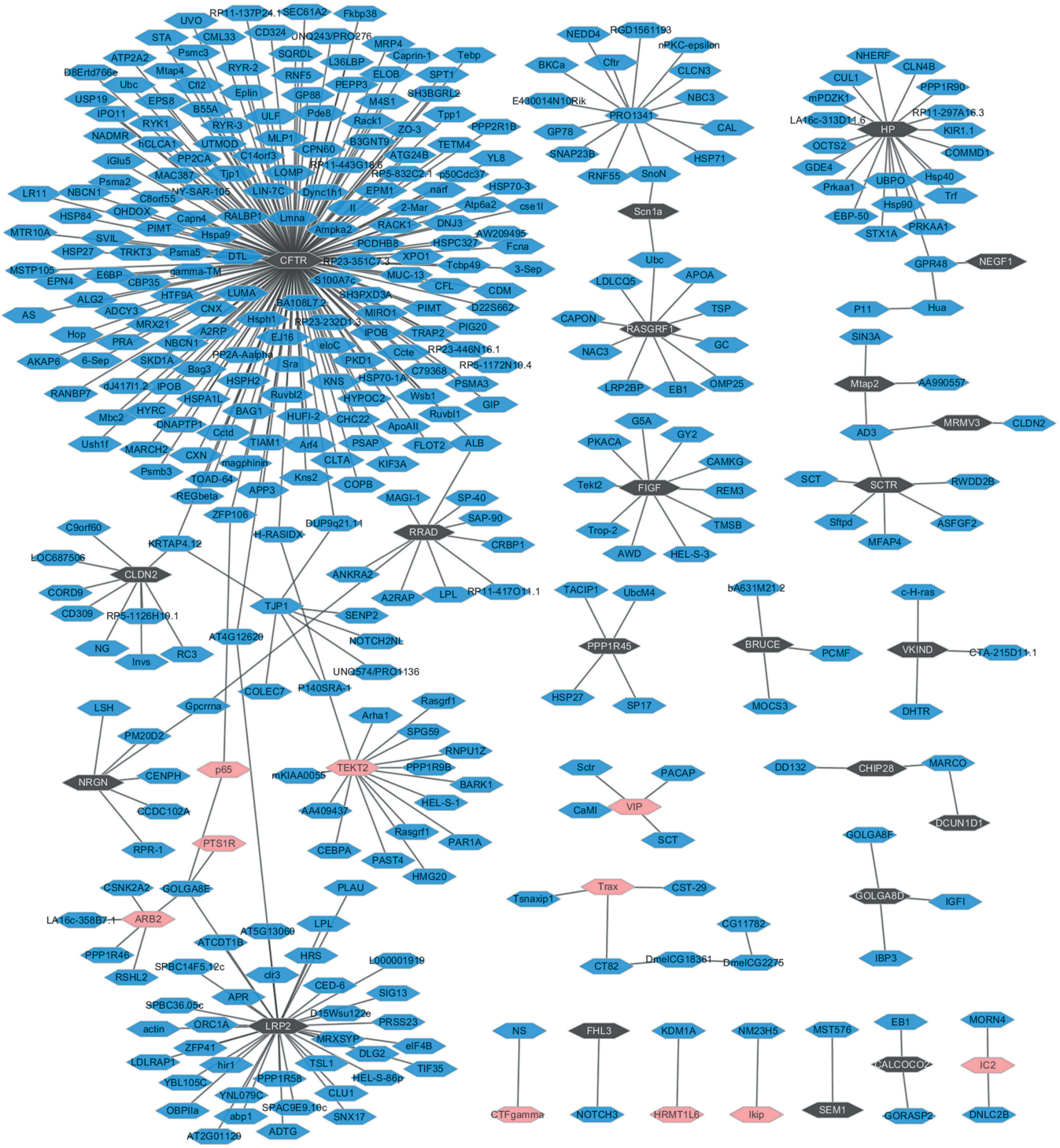

Figure 2 The constructed PPI networks of the DEGs in black module and the pink module. Pink nodes and black nodes represent DEGs in the pink module and black module, respectively. The blue nodes denote products of genes predicted to interact with the DEGs. The solid line means PPI correlation. PPI, protein-protein interaction; DEGs, differentially expressed genes.

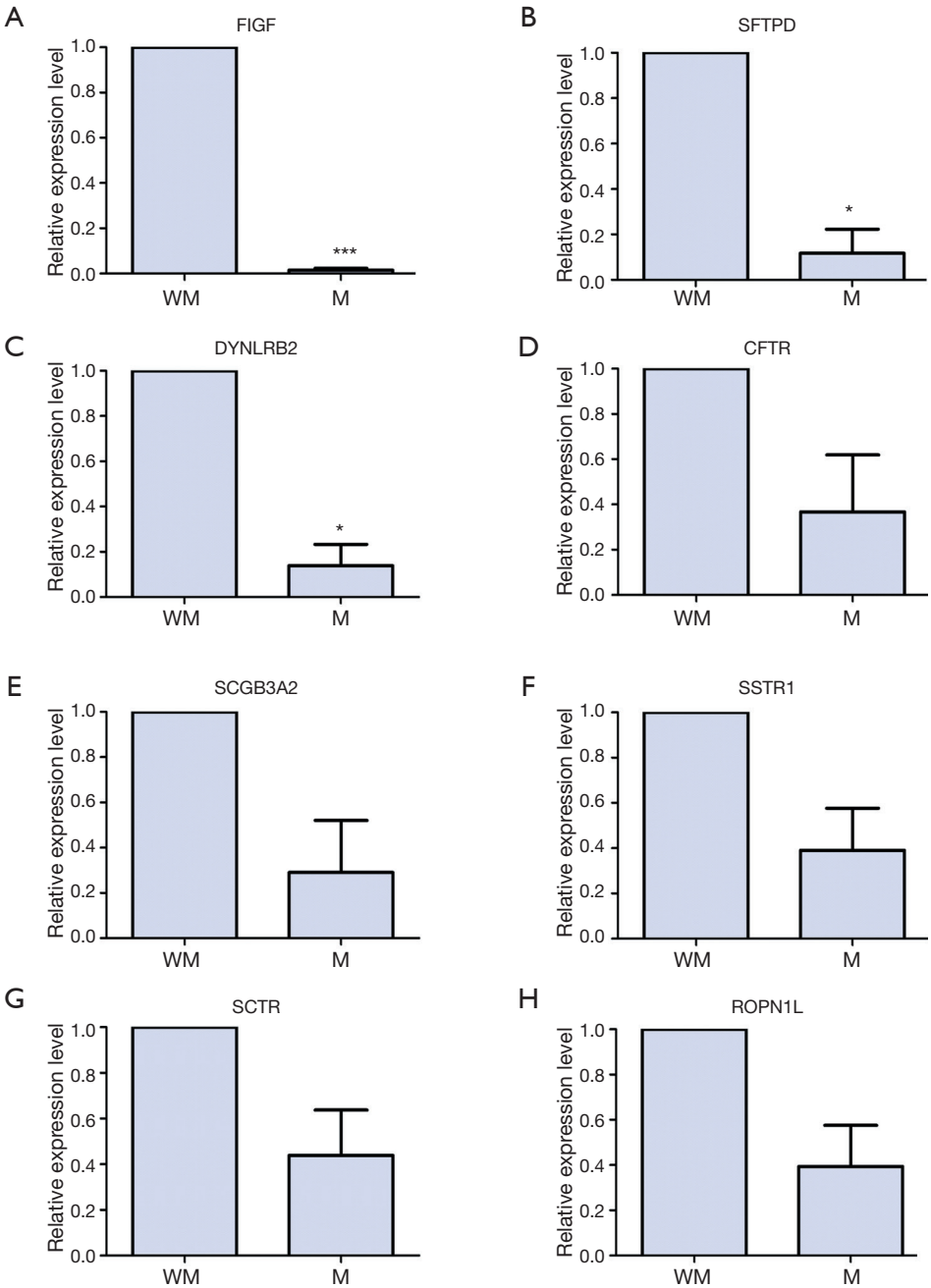

Figure 3 The verification of mRNA expression level of DEGs between of lung SCC with and without metastasis in primary tumor tissues through qRT-PCR. (A) FIGF; (B) SFTPD; (C) DYNLRB2; (D) CFRT; (E) SCGB3A2; (F) SSTR1; (G) SCTR; (H) ROPN1L. M means patients with lung SCC metastasis; MW means patients without lung SCC metastasis. *, means P<0.05; and ***, means P<0.001. lung SCC, lung squamous cell carcinoma; DEGs, differentially expressed genes; qRT-PCR, quantitative real-time polymerase chain reactions.

pathway (*Table 3*). SCTR had the connectivity with six genes in the PPI network (*Figure 2*). To our knowledge, this is the first reports presented SCTR was down-regulated in patients with lung SCC metastasis compared to those patients with lung SCC without metastasis. The biological function of SCTR in process of lung SCC needs to be further elucidated.

FIGF is also called VEGF-D or VEGFD. It encodes c-fos induced growth factor, a member of the platelet-derived growth factor/vascular endothelial growth factor family and is involved in angiogenesis, lymphangiogenesis and endothelial cell growth. VEGFD is a ligand for the VEGF receptor tyrosine kinases and activates it (33). The qRT-PCR shown FIGF was significantly down-regulated ($P<0.001$) in lung SCC metastasis compared to lung SCC without metastasis (*Figure 3A*). It was significantly enriched in cytokine-cytokine receptor interaction, mTOR signaling pathway and pathways in cancer. FIGF was a number of the black module and it had the connectivity with ten genes in the PPI network (*Figure 2*). In line with the previous article, FIGF is down-regulated in lung SCC (23). The mechanism of FIGF in lung SCC metastasis was unknown and further studies need to be investigated.

DYNLRB2 and ROPN1L were members of the pink module, encodes dynein light chain roadblock-type 2 and rhophilin associated tail protein 1 like, respectively.

ROPN1L variants were significantly associated with breast cancer risk in Korean women and Caucasian (34,35). The frequent amplification and copy number loss of DYNLRB2 is correlated with primary ductal carcinoma *in situ* (DCIS) and mixed DCIS, respectively (36). This is the first report displayed DYNLRB2 and ROPN1L were dysregulated in lung SCC, and might be related to the lung SCC metastasis.

SCGB3A2, SSTR1 and SFTPD were down-regulated in lung SCC metastasis compared to lung SCC without metastasis. SCGB3A2 encodes secretoglobin family 3A member 2. In lung cancer, SCGB3A2 were predominantly expressed in lung adenocarcinoma, compared with lung SCC and small cell carcinoma (24). It is a potentially useful marker for primary pulmonary tumors both in mice and humans (37). SSTR1 encodes somatostatin receptor 1, the expression level of SSTR1 mRNA was higher in both small cell lung cancer and lung SCC than in adenocarcinoma cell lines (26). SFTPD encodes surfactant protein D, is a lung-specific anti-inflammatory factor that antagonizes inflammation by inhibiting oxidative stress and stimulating innate immunity (38). In line with the previous article, SFTPD is down-regulated in NSCLC (25). The pathophysiology mechanism of dysregulated SCGB3A2, SSTR1 and SFTPD in lung SCC metastasis need further investigation.

In conclusion, we identified 482 DEGs in lung SCC without metastasis compared to lung SCC metastasis. Two gene co-expression modules including the black module and the pink module involved in the process of lung SCC metastasis were identified. Respective 26 and 23 dysregulated genes were enriched in the black module and the pink module. In the two of co-expression modules, CFTR, SCTR, FIGF might play key roles in the lung SCC metastasis. Our findings may contribute to the identification of early diagnosis biomarker for lung SCC metastasis and prognosis.

There are limitations in our study. Firstly, the biological roles of the key DEGs including SCTR and FIGF were unknown. In the future work, the *in vivo* and *in vitro* experiments were essential for exploring the function of genes mentioned above in the process of lung SCC metastasis. Secondly, additional studies with large cohorts of lung SCC with and without metastasis patients are needed to demonstrate the diagnostic value of identified genes as practical biomarkers.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the ethics committee board of Linyi People's Hospital (No. lyll2015N67) and written informed consent was obtained from all patients.

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Supplementary

Table S1 List of primers designed for the qRT-PCR verification

Primer	Primer sequence (5' to 3')
CFTR Forward	GATGGGGGCTGTGTCCTAAG
CFTR Reverse	GCATTGCTTCTATCCTGTGTTCA
FIGF Forward	ATCTAATCCAGCACCCCAAAAACT
FIGF Reverse	CTGGTATGAAAGGGGCATCTGTC
SCGB3A2 Forward	CTCTGGACAACATTCTTCCCTTTAT
SCGB3A2 Reverse	CCACCTCCGCTCTTTATCTTGA
SSTR1 Forward	GGGGCTATCTGCCTGTGCTAC
SSTR1 Reverse	CAAACACCATCACCACCATCAT
SCTR Forward	CCGTCCTCTACTGCTTCCTCAAC
SCTR Reverse	GGCTCTGCTCCAAGTGGCTG
SFTPD Forward	GCTACCTGGAAGCAGAAATGAA
SFTPD Reverse	AACAGAGCCATTGTCCCCTTT
DYNLRB2 Forward	CACCTGACAATGAAAGCCAAAAG
DYNLRB2 Reverse	TCACATGGATTCTGAATGACGAT
ROPN1L Forward	GTGCCTGCCGAAGGAAAA
ROPN1L Reverse	AAAACGTCTTGAAGGGGATGC
18srRNA Forward	GTAACCCGTTGAACCCCATT
18srRNA Reverse	CCATCCAATCGGTAGTAGCG

qRT-PCR, quantitative real-time polymerase chain reaction.

Table S2 DEGs between lung SCC with and without metastasis

Gene	Gene ID	FC	FDR
Up-regulated genes			
VGF	7425	6.302272	1.90E-06
VSIG10L	147645	5.870895	1.28E-05
MFAP5	8076	5.335057	0.000187
HNF1A	6927	5.184579	0.000382
RPTOR	57521	5.125631	0.000449
F2	2147	5.062773	0.000535
ATF5	22809	4.959337	0.000714
GAL	51083	4.944154	0.000714
ITPKA	3706	4.839489	0.001009
MND1	84057	4.835881	0.001009
TSPAN5	10098	4.772975	0.001176
DRP2	1821	4.672904	0.001748
DHCR7	1717	4.524834	0.003136
C19orf76	199800	4.502532	0.003261
KRT71	112802	4.393386	0.004713
UPK2	7379	4.335088	0.00555
SMARCD2	6603	4.324975	0.005592
C4orf49	84709	4.297849	0.005983
SRP68	6730	4.237439	0.007321
TEX13B	56156	4.221298	0.007491
NDUFC1	4717	4.102	0.009792
PRB3	5544	4.098234	0.009792
PIWIL2	55124	4.084314	0.010104
KRT18	3875	4.064585	0.010387
C9orf70	84850	4.058666	0.010503
TAC3	6866	4.049676	0.010714
NPSR1	387129	4.036762	0.011201
SLC9A2	6549	4.011459	0.011298
GRIK1	2897	4.008088	0.011298
ABCC2	1244	4.007931	0.011298
C4BPB	725	3.986718	0.011558
TCHP	84260	3.966038	0.011919
KCNH6	81033	3.96427	0.011919
TRIM54	57159	3.951173	0.012284
PROCR	10544	3.941815	0.012469
NEB	4703	3.92073	0.013004
ZNF787	126208	3.920271	0.013004
FOXH1	8928	3.920242	0.013004
ZWINT	11130	3.918314	0.013004
GPR155	151556	3.916905	0.013004
SYNPO2L	79933	3.904368	0.01311
FA2H	79152	3.894921	0.01344
NCAPG2	54892	3.873486	0.014273
NBAS	51594	3.86964	0.014273
POLR3G	10622	3.868808	0.014273
COG1	9382	3.868597	0.014273
PRR4	11272	3.856872	0.014627
SFRS9	8683	3.839756	0.015322
SCN3A	6328	3.826201	0.015533
ADI1	55256	3.826146	0.015533
DDX54	79039	3.825855	0.015533
CPS1	1373	3.80012	0.016528
SAPS1	22870	3.77652	0.017456
MAPT	4137	3.766167	0.017892
APOC3	345	3.76264	0.018047
MIPOL1	145282	3.757985	0.018235
HRC	3270	3.752649	0.018276
IL1RAPL1	11141	3.749319	0.018375
PLK4	10733	3.747044	0.018375
SMAGP	57228	3.732997	0.018471
DKK4	27121	3.730844	0.018471
CADPS	8618	3.723286	0.01859
USP32	84669	3.722682	0.01859
NPNT	255743	3.711312	0.019352
SYNGR4	23546	3.708512	0.019391
PSMC5	5705	3.706852	0.019391
USP7	7874	3.694849	0.020068
NR2E1	7101	3.686376	0.020569
ONECUT1	3175	3.683024	0.020731
ASPSCR1	79058	3.669242	0.021581
SAP30BP	29115	3.667889	0.021597
TESK1	7016	3.651205	0.022574
PCDHGB4	8641	3.640394	0.022895
RAD51C	5889	3.63531	0.02311
HES7	84667	3.632468	0.02311
APOA2	336	3.627873	0.023268
C19orf48	84798	3.626093	0.023293
NADSYN1	55191	3.62274	0.023306
NAA15	80155	3.62065	0.023398
GALNT13	114805	3.608216	0.024172
DCXR	51181	3.606123	0.024193
ING2	3622	3.605638	0.024193
C9orf82	79886	3.59792	0.024535
MED13	9969	3.596717	0.024552
FAM104A	84923	3.590437	0.024911
PITPNC1	26207	3.589103	0.024911
SMC6	79677	3.588884	0.024911
CYP3A5	1577	3.581118	0.025177
NTSR1	4923	3.579779	0.025198
TRAP1	10131	3.57807	0.025198
OR51E2	81285	3.575338	0.025228
KPNA2	3838	3.570615	0.025532
MELK	9833	3.565422	0.025758
RRM2	6241	3.539854	0.027316
C4orf41	60684	3.539614	0.027316
FUT6	2528	3.531912	0.028026
HIST1H3H	8357	3.525661	0.0283
C4orf48	401115	3.524516	0.028325
CELA1	1990	3.521018	0.028538
COL25A1	84570	3.519272	0.028538
TSPO2	222642	3.517829	0.028538
MRPL38	64978	3.516225	0.028538
DUSP13	51207	3.499272	0.029593
CLTC	1213	3.49865	0.029593
GAR1	54433	3.489856	0.03009
SLC25A10	1468	3.489392	0.03009
ABCE1	6059	3.488184	0.03009
CCDC124	115098	3.476216	0.031069
XRCC6BP1	91419	3.467209	0.031602
FDXR	2232	3.465684	0.031616
KLC2	64837	3.459955	0.032099
C21orf45	54069	3.45586	0.032196
ARHGDIA	396	3.433486	0.034049
HBQ1	3049	3.428947	0.034321
CIT	11113	3.419155	0.035096
CENPE	1062	3.409378	0.035835
SNX31	169166	3.407213	0.036019
MRPL21	219927	3.404929	0.036117
DCTD	1635	3.399858	0.03628
KRTAP5-7	440050	3.399706	0.03628
C17orf53	78995	3.398381	0.03628
CLTA	1211	3.377629	0.038227
FOXK2	3607	3.368591	0.039039
C14orf80	283643	3.361435	0.039747
PWP2	5822	3.36065	0.039755
FIBCD1	84929	3.358374	0.039889
UBTF	7343	3.356782	0.039896
UCP1	7350	3.354004	0.040174
LOC92659	92659	3.352846	0.040174
FAM84B	157638	3.342063	0.041043
DDN	23109	3.339528	0.041043
MTL5	9633	3.336217	0.041112
CST4	1472	3.335754	0.041112
CENPO	79172	3.33492	0.041132
SLC5A5	6528	3.326312	0.04147
RPS6KB1	6198	3.325852	0.04147
H2AFB1	474382	3.320782	0.042023
VWA5B2	90113	3.319972	0.042044
C16orf75	116028	3.318308	0.042194
LOC127841	127841	3.313993	0.042443
LSM6	11157	3.312778	0.042527
LMBR1	64327	3.30957	0.042815
MUC6	4588	3.307915	0.042882
PGP	283871	3.306565	0.042882
DDX4	54514	3.306498	0.042882
VRK1	7443	3.303783	0.043098
PCGF2	7703	3.302845	0.043105
GINS2	51659	3.302439	0.043105
RWDD4A	201965	3.300758	0.043155
HIST1H2BM	8342	3.298546	0.043306
C9orf140	89958	3.295731	0.043642
C12orf43	64897	3.285002	0.044826
ALDH1A1	216	3.283336	0.04499
DHX8	1659	3.277351	0.045645
UBE2O	63893	3.273996	0.045794
HSPBP1	23640	3.27391	0.045794
NPPC	4880	3.263528	0.047087
THOC4	10189	3.258293	0.047551
AKR7A3	22977	3.255861	0.047642
GGNBP1	449520	3.253542	0.047824
BCAT2	587	3.252302	0.047828
LOC81691	81691	3.251006	0.047944
TLK2	11011	3.247125	0.048489
CLGN	1047	3.24657	0.048489
HSD17B1	3292	3.243008	0.04858
CKM	1158	3.237947	0.049187
LONP1	9361	3.237664	0.049187
GDF11	10220	3.236492	0.049227
FGF3	2248	3.232167	0.049726
Down-regulated genes			
ZNF208	7757	−6.99816	5.04E-08
C8orf46	254778	−6.37244	1.81E-06
TNF	7124	−5.93751	1.28E-05
GRIA1	2890	−5.90534	1.28E-05
LINGO4	339398	−5.8607	1.28E-05
S100A7A	338324	−5.42097	0.000144
NDP	4693	−5.33403	0.000187
IL22RA2	116379	−5.12306	0.000449
POU2F3	25833	−5.09145	0.000493
MIA	8190	−5.04813	0.000542
FOXI2	399823	−5.02927	0.000562
PCDHGA4	56111	−5.01861	0.000562
SYCE1	93426	−4.94241	0.000714
FCER1A	2205	−4.88237	0.000926
C7orf16	10842	−4.82702	0.001009
TRIM58	25893	−4.82627	0.001009
C2orf73	129852	−4.82333	0.001009
KLHDC7A	127707	−4.81194	0.001009
SCG3	29106	−4.81048	0.001009
FAM169B	283777	−4.7216	0.001467
LOC283731	283731	−4.68577	0.001693
CCDC42B	387885	−4.6414	0.001977
FAIM2	23017	−4.61988	0.002131
SLC47A2	146802	−4.56176	0.002738
PCDHGA9	56107	−4.52173	0.003136
UPP2	151531	−4.50633	0.003261
PDC	5132	−4.48382	0.003474
C1orf192	257177	−4.4712	0.003598
HTR3A	3359	−4.44092	0.004047
HSD17B2	3294	−4.42747	0.004129
EMID1	129080	−4.4268	0.004129
S100B	6285	−4.38458	0.004789
WBSCR17	64409	−4.38066	0.004789
DPYS	1807	−4.37219	0.004877
CD1A	909	−4.36288	0.004988
HP	3240	−4.32821	0.005592
CDH20	28316	−4.30398	0.005983
TMPRSS5	80975	−4.28326	0.006278
NDN	4692	−4.27326	0.006453
C5orf13	9315	−4.26551	0.006567
OLR1	4973	−4.23143	0.007383
MSTN	2660	−4.22817	0.007383
C1orf186	440712	−4.20012	0.008098
GPR17	2840	−4.18922	0.008366
C9orf44	158314	−4.1585	0.00943
C4orf7	260436	−4.1494	0.009666
HEPACAM2	253012	−4.14051	0.009791
CRLF1	9244	−4.13588	0.009791
LOC284749	284749	−4.12985	0.009791
IGFN1	91156	−4.1266	0.009791
DYNLRB2	83657	−4.12242	0.009791
ART3	419	−4.12232	0.009791
MAGEE2	139599	−4.12076	0.009791
CCDC129	223075	−4.11658	0.009791
G6PC2	57818	−4.11556	0.009791
CORO2B	10391	−4.11263	0.009791
RBM44	375316	−4.11151	0.009791
CNGA3	1261	−4.10112	0.009792
MBL1P	8512	−4.09773	0.009792
HPD	3242	−4.09711	0.009792
OR2W3	343171	−4.08612	0.010104
GRIK4	2900	−4.07796	0.010114
AGTR2	186	−4.07697	0.010114
FAM55D	54827	−4.07446	0.010114
MOGAT2	80168	−4.07341	0.010114
PNMA5	114824	−4.05686	0.010503
CCDC141	285025	−4.0289	0.011298
ACOT12	134526	−4.02808	0.011298
WDR93	56964	−4.02511	0.011298
TPTE2	93492	−4.02483	0.011298
PCDHGA6	56109	−4.01945	0.011298
C13orf15	28984	−4.01794	0.011298
IL1B	3553	−4.01286	0.011298
CTCFL	140690	−4.01208	0.011298
SSTR1	6751	−4.00644	0.011298
HEPHL1	341208	−3.99112	0.011558
WFDC12	128488	−3.9907	0.011558
HTR3C	170572	−3.9881	0.011558
PADI2	11240	−3.98802	0.011558
STAC2	342667	−3.98702	0.011558
ACSL4	2182	−3.98589	0.011558
CLDN2	9075	−3.98299	0.011566
RASD2	23551	−3.98158	0.011566
MYO1H	283446	−3.97564	0.011756
GPIHBP1	338328	−3.97226	0.011822
TRPC2	7221	−3.96666	0.011919
CA4	762	−3.961	0.011984
FOXI1	2299	−3.95747	0.012063
CPB2	1361	−3.94544	0.012448
TEX9	374618	−3.94412	0.012448
SHC4	399694	−3.93515	0.012719
FAM123A	219287	−3.9236	0.013004
PAGE5	90737	−3.91727	0.013004
PSAPL1	768239	−3.91368	0.013026
C1orf150	148823	−3.91293	0.013026
LOC646851	646851	−3.91116	0.013026
ROPN1L	83853	−3.90859	0.013031
CRABP1	1381	−3.90756	0.013031
AMZ1	155185	−3.90189	0.013151
ADAMTS8	11095	−3.88394	0.013963
C1QL2	165257	−3.88144	0.01401
PLD5	200150	−3.87838	0.014089
TCTEX1D4	343521	−3.86347	0.014479
DRGX	644168	−3.8586	0.014627
TMPRSS3	64699	−3.85615	0.014627
CYB561D1	284613	−3.84443	0.015243
CLEC4C	170482	−3.83844	0.015322
FOLR3	2352	−3.83843	0.015322
ACRV1	56	−3.83238	0.015533
TMEM100	55273	−3.83079	0.015533
GPR128	84873	−3.82961	0.015533
FNDC5	252995	−3.82395	0.015558
CLDN23	137075	−3.81787	0.015702
DYDC2	84332	−3.81611	0.015702
KLK7	5650	−3.81601	0.015702
DRD2	1813	−3.81539	0.015702
SLC5A9	200010	−3.81427	0.015702
FAM166B	730112	−3.7975	0.016606
CFP	5199	−3.79313	0.016803
COL9A2	1298	−3.78771	0.016969
ALDH1A3	220	−3.7876	0.016969
SERPINA3	12	−3.78639	0.016969
RYR3	6263	−3.78106	0.017238
IQSEC3	440073	−3.77321	0.017563
HPR	3250	−3.7722	0.017563
RIMS3	9783	−3.75729	0.018235
CYP2B6	1555	−3.75556	0.018261
RNF122	79845	−3.75386	0.018276
DNAH6	1768	−3.74677	0.018375
HAPLN4	404037	−3.74597	0.018375
DENND3	22898	−3.74354	0.018391
FAM183A	440585	−3.74312	0.018391
TRPM5	29850	−3.7399	0.018471
SLC6A13	6540	−3.73831	0.018471
CHIT1	1118	−3.73584	0.018471
FIGF	2277	−3.73417	0.018471
RXRG	6258	−3.73254	0.018471
DBC1	1620	−3.73111	0.018471
MS4A8B	83661	−3.72991	0.018471
TTTY2	60439	−3.72925	0.018471
RHO	6010	−3.72784	0.018478
PTX3	5806	−3.72667	0.018478
CUX2	23316	−3.70606	0.019391
C22orf15	150248	−3.70595	0.019391
SPRR2G	6706	−3.70009	0.019751
CHI3L1	1116	−3.6862	0.020569
TNFRSF19	55504	−3.6782	0.021029
CRTAC1	55118	−3.67011	0.021581
C1orf175	374977	−3.66343	0.021827
ACSBG1	23205	−3.66287	0.021827
KRT31	3881	−3.65343	0.022543
C6orf217	1E+08	−3.6508	0.022574
NEDD9	4739	−3.64821	0.022701
BST1	683	−3.64606	0.022722
C5orf49	134121	−3.64571	0.022722
TSNAXIP1	55815	−3.64271	0.022888
OCA2	4948	−3.64101	0.022895
ARHGAP6	395	−3.63432	0.02311
SCGB3A2	117156	−3.63351	0.02311
NANOS1	340719	−3.63266	0.02311
PCDHB18	54660	−3.62948	0.023268
CACNA1G	8913	−3.62744	0.023268
DRD5	1816	−3.62405	0.023306
BMP3	651	−3.62323	0.023306
TRY6	154754	−3.61309	0.023993
CELF3	11189	−3.60886	0.024172
PSG8	440533	−3.60801	0.024172
ERVFRDE1	405754	−3.60466	0.024193
FBN3	84467	−3.60111	0.02435
PTCHD2	57540	−3.60091	0.02435
CHI3L2	1117	−3.59413	0.0247
PRY2	442862	−3.58729	0.024968
C16orf89	146556	−3.58584	0.025011
FAM151A	338094	−3.58328	0.02516
PDK4	5166	−3.58162	0.025177
C3orf16	389161	−3.57796	0.025198
MMP3	4314	−3.57631	0.025228
PAK3	5063	−3.57472	0.025228
POLB	5423	−3.56716	0.025758
BIRC2	329	−3.56631	0.025758
SCGB3A1	92304	−3.56338	0.025865
TSPAN2	10100	−3.56094	0.026012
C4A	720	−3.55936	0.026075
COL29A1	256076	−3.55698	0.02616
LEP	3952	−3.55605	0.02616
TCTEX1D1	200132	−3.55567	0.02616
TMEM217	221468	−3.55251	0.026382
TMEM232	642987	−3.54522	0.027026
CPAMD8	27151	−3.54242	0.027217
MMP1	4312	−3.53001	0.028088
LOC148824	148824	−3.52948	0.028088
SMYD1	150572	−3.52739	0.028213
PDGFA	5154	−3.51856	0.028538
ADHFE1	137872	−3.51763	0.028538
DCLK1	9201	−3.51695	0.028538
KNDC1	85442	−3.50966	0.028964
C19orf59	199675	−3.5085	0.028964
CCR6	1235	−3.50812	0.028964
NRGN	4900	−3.5079	0.028964
CCL17	6361	−3.50786	0.028964
SPOCK2	9806	−3.50282	0.029391
OBP2B	29989	−3.50221	0.029391
SCN1A	6323	−3.49743	0.029631
PDYN	5173	−3.49134	0.03009
FABP7	2173	−3.48896	0.03009
LMOD3	56203	−3.48822	0.03009
SCTR	6344	−3.4856	0.030285
SNTN	132203	−3.48335	0.030444
SLC25A27	9481	−3.47904	0.030841
GNAO1	2775	−3.47505	0.031107
KCNK17	89822	−3.47269	0.031284
PCDHA9	9752	−3.47128	0.031351
LRP2	4036	−3.46908	0.03151
CCDC157	550631	−3.46663	0.031602
C21orf62	56245	−3.46092	0.032082
KAL1	3730	−3.45759	0.032196
RASGEF1B	153020	−3.45638	0.032196
NPPA	4878	−3.45595	0.032196
LCE3E	353145	−3.45363	0.032366
GAS2	2620	−3.45174	0.032496
FAM92B	339145	−3.44844	0.032797
TDRD6	221400	−3.44545	0.033063
MEGF11	84465	−3.441	0.033388
CX3CR1	1524	−3.44075	0.033388
CYP2D6	1565	−3.44039	0.033388
CLDN19	149461	−3.43628	0.033799
CLLU1	574028	−3.42963	0.034321
CAT	847	−3.42928	0.034321
BOC	91653	−3.42237	0.03506
IRX1	79192	−3.4209	0.035093
TMEM190	147744	−3.42053	0.035093
SLC46A2	57864	−3.41846	0.035096
RASGRF1	5923	−3.41816	0.035096
KANK4	163782	−3.41106	0.035835
KCNE1L	23630	−3.41057	0.035835
BAAT	570	−3.41002	0.035835
KIAA1644	85352	−3.40608	0.036066
OR4N4	283694	−3.40208	0.03628
PCDHA10	56139	−3.40013	0.03628
DNAH5	1767	−3.39858	0.03628
MT1F	4494	−3.39841	0.03628
SH2D7	646892	−3.39276	0.03693
CFTR	1080	−3.3898	0.037229
TAL1	6886	−3.38671	0.037548
IGFALS	3483	−3.38561	0.037597
EFCAB12	90288	−3.38423	0.037683
CTSH	1512	−3.38248	0.037822
ZNF98	148198	−3.37939	0.038146
CCDC65	85478	−3.37732	0.038227
C15orf37	283687	−3.3738	0.038615
CLEC3B	7123	−3.37133	0.03886
RGS13	6003	−3.36932	0.039039
SFTPD	6441	−3.36378	0.03962
TEKT3	64518	−3.36159	0.039747
KLHDC8B	200942	−3.35827	0.039889
ROPN1	54763	−3.35679	0.039896
CRLF2	64109	−3.35271	0.040174
EFCAB6	64800	−3.34864	0.040663
RASL12	51285	−3.34363	0.041043
IL20	50604	−3.3433	0.041043
APOL4	80832	−3.34189	0.041043
CAMK1G	57172	−3.34104	0.041043
DNAI1	27019	−3.3407	0.041043
COL14A1	7373	−3.33932	0.041043
CNTN4	152330	−3.33921	0.041043
PRSS1	5644	−3.339	0.041043
BMF	90427	−3.33803	0.041084
TMEM151B	441151	−3.33632	0.041112
LOC283050	283050	−3.33424	0.041132
KLF17	128209	−3.3305	0.04147
RRAD	6236	−3.32852	0.04147
GP5	2814	−3.32848	0.04147
CLDN25	644672	−3.32741	0.04147
SOX11	6664	−3.32658	0.04147
WDR38	401551	−3.3261	0.04147
SRD5A2	6716	−3.32582	0.04147
C1orf228	339541	−3.32106	0.042023
NAV3	89795	−3.31762	0.042197
SPRR2B	6701	−3.31648	0.042268
C14orf139	79686	−3.31408	0.042443
TKTL1	8277	−3.31158	0.042608
HAND1	9421	−3.30775	0.042882
RICTOR	253260	−3.30458	0.043076
CACNG1	786	−3.30069	0.043155
RDH16	8608	−3.30017	0.043155
PVALB	5816	−3.2924	0.044062
SEC14L3	266629	−3.29154	0.044096
HPCAL4	51440	−3.28873	0.044437
DIRC3	729582	−3.2857	0.044817
AGRP	181	−3.28238	0.045042
FAM81B	153643	−3.27818	0.045613
PLA2G2F	64600	−3.27614	0.04574
CCDC30	728621	−3.27408	0.045794
PF4	5196	−3.27303	0.045836
CSDC2	27254	−3.26912	0.046371
RNF112	7732	−3.26788	0.046472
SLC17A8	246213	−3.26127	0.04736
PURG	29942	−3.25821	0.047551
TG	7038	−3.25766	0.047551
KIT	3815	−3.25764	0.047551
DES	1674	−3.25617	0.047642
TEKT2	27285	−3.25453	0.047762
ZNF474	133923	−3.25294	0.047824
CDX1	1044	−3.24595	0.048491
C6orf165	154313	−3.24447	0.048565
ATP2B3	492	−3.24396	0.048565
WFDC2	10406	−3.2437	0.048565
LPO	4025	−3.24181	0.048682
WDR91	29062	−3.23624	0.049227
SGK223	157285	−3.23513	0.049315
AQP1	358	−3.2312	0.049791
TMEM114	283953	−3.23056	0.049798

Lung SCC, lung squamous cell carcinoma; DEG, differentially expressed genes; FC, fold change; FDR, false discovery rate; ABC, ATP-binding cassette.