

Identification of immunohistochemical markers for distinguishing lung adenocarcinoma from squamous cell carcinoma

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Background: Immunohistochemical staining has been widely used in distinguishing lung adenocarcinoma (LUAD) from lung squamous cell carcinoma (LUSC), which is of vital importance for the diagnosis and treatment of lung cancer. Due to the lack of a comprehensive analysis of different lung cancer subtypes, there may still be undiscovered markers with higher diagnostic accuracy.

Methods: Herein first, we systematically analyzed high-throughput data obtained from The Cancer Genome Atlas (TCGA) database. Combining differently expressed gene screening and receiver operating characteristic (ROC) curve analysis, we attempted to identify the genes which might be suitable as immunohistochemical markers in distinguishing LUAD from LUSC. Then we detected the expression of six of these genes (*MLPH*, *TMC5*, *SFTA3*, *DSG3*, *DSC3* and *CALML3*) in lung cancer sections using immunohistochemical staining.

Results: A number of genes were identified as candidate immunohistochemical markers with high sensitivity and specificity in distinguishing LUAD from LUSC. Then the staining results confirmed the potentials of the six genes (*MLPH*, *TMC5*, *SFTA3*, *DSG3*, *DSC3* and *CALML3*) in distinguishing LUAD from LUSC, and their sensitivity and specificity were not less than many commonly used markers.

Conclusions: The results revealed that the six genes (*MLPH*, *TMC5*, *SFTA3*, *DSG3*, *DSC3* and *CALML3*) might be suitable markers in distinguishing LUAD from LUSC, and also validated the feasibility of our methods for identification of candidate markers from high-throughput data.

Keywords: Lung cancer; immunohistochemical marker; receiver operating characteristic (ROC) curve analysis; The Cancer Genome Atlas (TCGA)

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Introduction

As the most frequently diagnosed cancer and the leading cause of tumor death, lung cancer was estimated to account for more than 1.8 million new cases and nearly 1.6 million deaths worldwide in 2012, with a sharp rising from 2008 (1,2).

Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the two major pathologic subtypes of lung cancer, constituting the vast majority of diagnosed lung cancers, but there are a lot of differences in their molecular profiling and characteristics, as well as therapeutic methods (3-5).

Therefore, to accurately distinguish these two subtypes is important for the diagnosis and treatment of lung cancer.

Recently the main method used to distinguish LUAD and LUSC is hematoxylin-eosin (HE) staining of the tumor tissue sections observed under a light microscope. But in tumors with unclear structures caused by low differentiation, necrosis, or serious extrusion, small biopsies or cytologies with a limited number of tumor cells, it is difficult to make a precise diagnosis relying on HE staining alone. At this time, combining immunohistochemical results can refine the diagnosis, thus immunohistochemical staining is now recommended and widely applied in clinical practices (4-6).

At present, there are a number of reliable immunohistochemical markers that have been adopted to distinguish LUAD from LUSC, including *thyroid transcription factor-1 (TTF-1)*, also called *NKX2-1*), *napsin-A (NAPSA)*, *tumor protein p63 (TP63)*, and *cytokeratin (CK) 5/6 (3-5,7-10)*. These markers are highly sensitive, specific, and can be easily detected, the expression is significantly different between LUAD and LUSC. However, due to the lack of a comprehensive analysis of different lung cancer subtypes, there may still be undiscovered markers with higher sensitivity, specificity and application value. In the current study, we systematically analyzed high-throughput data obtained from The Cancer Genome Atlas (TCGA) database. Combining differently expressed gene screening and receiver operating characteristic (ROC) curve analysis, we identified and validated a number of genes which can be used as candidate immunohistochemical markers in distinguishing LUAD from LUSC.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of Zhongshan Hospital, Fudan University, Shanghai, China (Approval No. 2014-101). All work conformed to the provisions of the Declaration of Helsinki. Written informed consent was obtained from all patients participating in this research at the time of hospitalization.

Data acquisition and differently expressed gene screening

Level 3 RNA sequencing (RNA-Seq) V2 data of human LUAD and LUSC samples, which was released by TCGA before April 15, 2014, were obtained from the TCGA

data portal (<https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp>), including 490 LUAD samples and 490 LUSC samples. RNA-Seq by expectation maximization (RSEM) values were used to represent the levels of expression of these genes. The data are presented as means and standard deviations (SD).

All genes recorded in the TCGA data were filtered using the following criteria:

- (I) mean (LUAD) $\geq 1,000$ and mean (LUAD)/mean (LUSC) ≥ 4 ;
- (II) mean (LUSC) $\geq 1,000$ and mean (LUSC)/mean (LUAD) ≥ 4 .

Here, mean (LUAD) and mean (LUSC) denote the mean of the RSEM value of the gene in the LUAD and LUSC samples, respectively. When a gene met one of the two conditions above, it was then entered in the subsequent analyses. Through these criteria, we attempted to identify those genes which were highly elevated and could be easily detected, with tremendous differences between the LUAD and LUSC samples.

Patient selection

Fifty patients with LUAD who underwent curative surgery between Jan 1 and Feb 19, 2014, and 50 other patients with LUSC who underwent curative surgery between Jan 1 and Apr 25, 2014, in the Department of Thoracic Surgery, Zhongshan Hospital, Fudan University, were included in this research. All of the cases were clearly confirmed by pathologic evaluation. Immunohistochemistry results of *TTF1*, *CK7*, *NAPSA*, *surfactant protein A (SPA)*, *TP63*, *HCK proto-oncogene*, *Src family tyrosine kinase (HCK)* and *P40* in the specimens were obtained from the pathologists' original reports. Sections of paraffinembedded tumor tissues were obtained from all cases involved.

Immunohistochemistry

Immunohistochemical staining was performed using an EnVision™ HRP-polymer anti-mouse/rabbit IHC Kit (KeyGEN BioTECH, Nanjing, Jiangsu, China) according to the manufacturer's guidelines. Briefly, the primary antibodies specific for *melanophilin (MLPH)*, 1:100 dilution), *transmembrane channel-like 5 (TMC5)*, 1:100 dilution), *surfactant associated 3 (SFTA3)*, 1:100 dilution), *desmoglein 3 (DSG3)*, 1:100 dilution), *desmocollin 3 (DSC3)*, 1:100 dilution) and *calmodulin-like 3 (CALML3)*, 1:100 dilution) were applied to detect the expressions of these genes. Stained specimens were then viewed independently at 100 \times independently by

Table 1 Fifteen genes greatly elevated in LUAD with highest AUC values

Gene	LUAD	LUSC	Fold-change (LUAD/LUSC)	AUC value
<i>MLPH</i>	3,961±3,315	521±769	7.60	0.953
<i>SFTA2</i>	2,833±3,115	161±327	17.59	0.946
<i>TMC5</i>	3,045±2,381	428±646	7.11	0.943
<i>SFTA3</i>	3,073±2,704	271±761	11.33	0.937
<i>DDAH1</i>	2,446±1,405	544±462	4.50	0.934
<i>RORC</i>	1,213±952	130±232	9.31	0.933
<i>TMEM125</i>	1,873±1,362	297±351	6.29	0.931
<i>SMPDL3B</i>	1,482±1,421	238±284	6.22	0.930
<i>ALDH3B1</i>	2,509±2,619	378±646	6.62	0.930
<i>ACSL5</i>	4,050±3,178	604±775	6.70	0.926
<i>NKX2-1</i>	3,246±2,233	309±940	10.50	0.926
<i>ATP11A</i>	7,025±5,571	1,356±1,261	5.18	0.924
<i>CGN</i>	3,626±2,448	796±777	4.55	0.922
<i>FMO5</i>	1,174±1,575	86±136	13.51	0.921
<i>MUC1</i>	22,301±16,816	3,137±3,945	7.11	0.921

LUAD, lung adenocarcinoma; AUC: area under curve; LUSC: lung squamous cell carcinoma.

two investigators. Expression of these genes was determined by semiquantitatively assessing the percentage of marked tumor cells and the staining intensity as previously reported (11,12). Finally, we separated the specimens according to expression in four groups (negative, weak, moderate, and strong).

The primary antibodies [anti-*MLPH* (HPA014685), anti-*TMC5* (HPA042037), anti-*SFTA3* (HPA059427), anti-*DSC3* (HPA049265) and anti-*CALML3* (HPA044999)] were obtained from Sigma-Aldrich (St. Louis, MO, USA). Anti-*DSG3* (ab183743) was obtained from Abcam (Cambridge, MA, USA).

Statistical analysis

Data were analyzed using IBM SPSS for Windows, version 20 (Armonk, NY, USA). ROC curve analysis was used to identify the candidate genes for distinguishing LUAD from LUSC. The Mann-Whitney U test was used to evaluate the differences in genes and markers between LUAD and LUSC samples.

Results

After differently expressed gene screening, 228 genes were filtered out for the next analysis. One hundred and ten genes

were elevated in LUAD compared with LUSC, the other 118 genes were upregulated in LUSC (*Tables S1* and *S2*).

Then, ROC curve analysis was used to evaluate the effectiveness of these 228 genes when applied to distinguish LUAD from LUSC based on the TCGA data (*Tables S1* and *S2*). Part of the genes with the highest area under curve (AUC) values in LUAD and LUSC can be found in *Tables 1* and *2*, respectively. The higher AUC value is indicative of greater sensitivity and specificity. *MLPH*, *SFTA2*, *TMC5*, *SFTA3*, *DSG3*, *KRT5*, *DSC3* and *CALML3* rank highest in these two tables.

Because the appropriate primary antibody of human *SFTA2* could not be obtained when we performed this study, and *KRT5* is one part of *CK5/6* which has been frequently used to distinguish the subtypes of lung cancer, we selected *MLPH*, *TMC5*, *SFTA3*, *DSG3*, *DSC3*, and *CALML3* for the next immunohistochemical staining. As *Figure 1* and *Figure 2* show, the expression distribution profiles of these six genes were quite different in LUAD and LUSC, and the sensitivity and specificity for distinguishing between the two types of lung cancer was high.

As *Figure 3* and *Table 3* show, the results of immunohistochemical staining further confirmed the elevation of *MLPH*, *TMC5*, and *SFTA3* in LUAD, and *DSG3*, *DSC3*, and *CALML3* in LUSC. Then the immunohistochemical results were compared to the markers

Table 2 Fifteen genes greatly elevated in LUSC with highest AUC values

Gene	LUAD	LUSC	Fold-change (LUSC/LUAD)	AUC value
<i>DSG3</i>	88±777	8,728±8,556	98.77	0.973
<i>KRT5</i>	1,227±10,342	116,689±96,742	95.03	0.972
<i>DSC3</i>	128±789	7,515±6,291	58.62	0.970
<i>CALML3</i>	141±1,096	10,039±11,031	71.17	0.964
<i>SERPINB13</i>	22±191	2,166±3,217	95.70	0.956
<i>KRT6B</i>	310±1,208	17,808±27,334	57.45	0.954
<i>KRT6C</i>	136±529	7,372±12,063	54.13	0.954
<i>KRT6A</i>	2,297±8,724	87,096±81,359	37.91	0.951
<i>PVRL1</i>	1,204±1,177	11,200±7,063	9.30	0.950
<i>LOC642587</i>	59±213	1,247±1,247	20.99	0.949
<i>PERP</i>	6,258±4,951	31,500±21,939	5.03	0.947
<i>TP63</i>	325±914	10,976±9,139	33.72	0.946
<i>TRIM29</i>	861±1,930	11,291±7,291	13.10	0.945
<i>ATP1B3</i>	1,866±1,138	9,231±6,592	4.94	0.945
<i>FAT2</i>	125±383	3,737±3,587	29.82	0.943

LUSC: lung squamous cell carcinoma; AUC: area under curve; LUAD, lung adenocarcinoma.

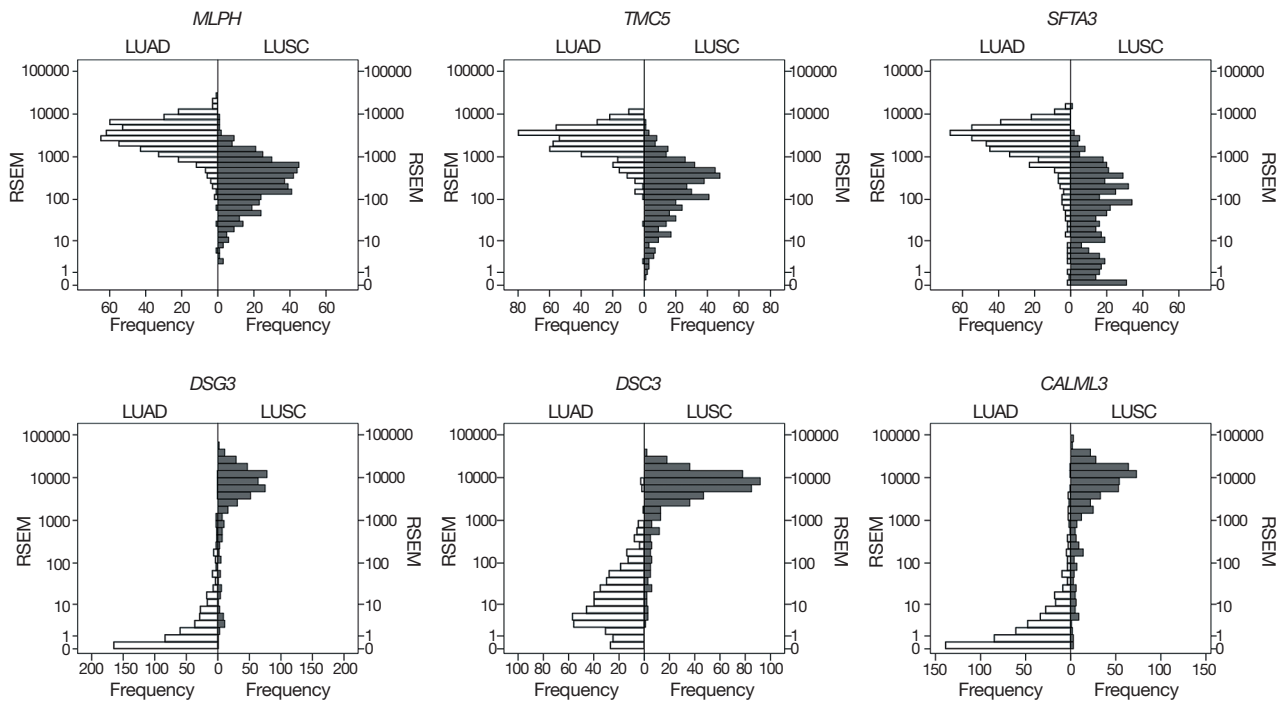


Figure 1 The distribution of expression of the six genes in LUAD and LUSC. LUAD, lung adenocarcinoma; LUSC: lung squamous cell carcinoma.

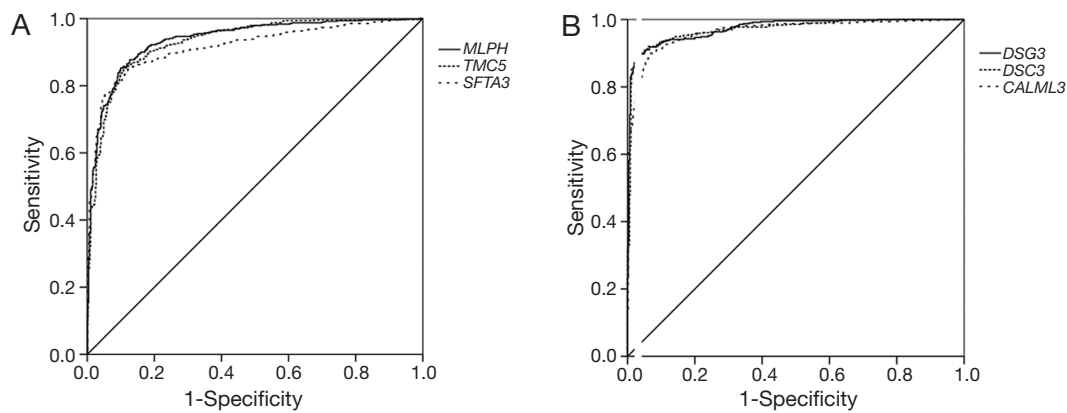


Figure 2 The ROC curves of the six genes when they were used in distinguishing LUAD from LUSC. (A) The ROC curves of *MLPH*, *TMC5*, and *SFTA3*; (B) the ROC curves of *DSG3*, *DSC3*, and *CALML3*. ROC, receiver operating characteristic; LUAD, lung adenocarcinoma; LUSC: lung squamous cell carcinoma.

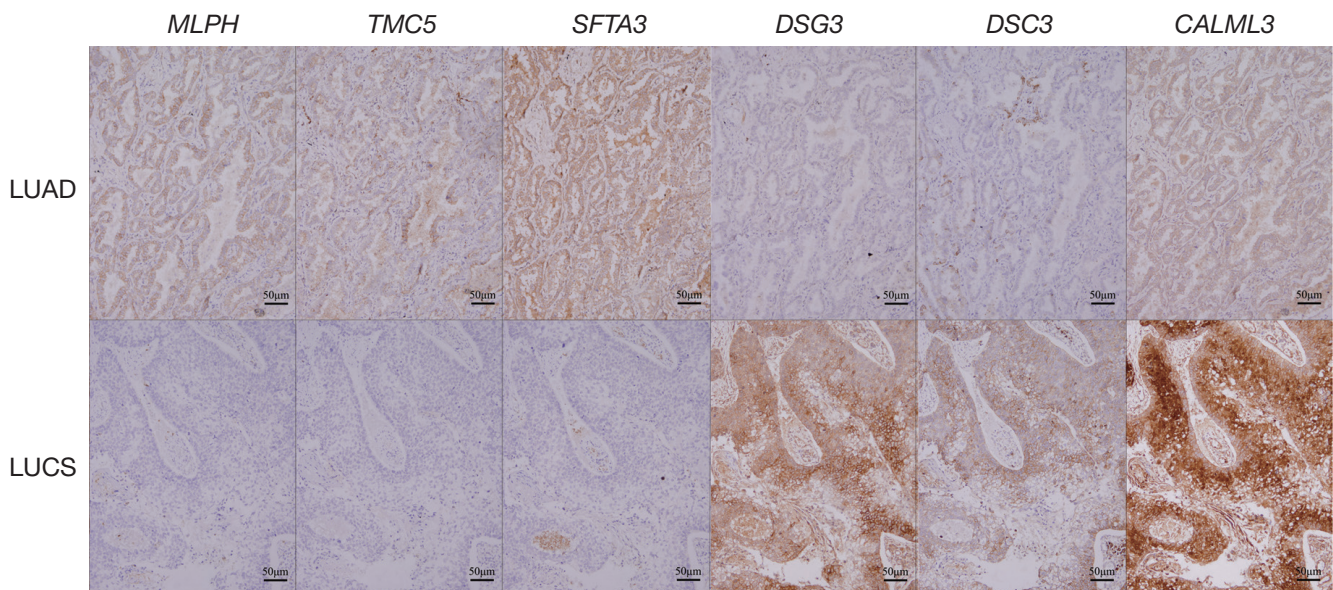


Figure 3 The immunohistochemical staining results of the six genes in LUAD and LUSC. Scale bar: 50 μ m. LUAD, lung adenocarcinoma; LUSC: lung squamous cell carcinoma.

used in our hospital clinic; the staining scores were obtained from the pathologists' original reports. As *Table 3* shows, the sensitivity and specificity of the six genes could be more than 80% and higher than some markers frequently used.

Discussion

Combining differently expressed gene screening and ROC curve analysis, we identified the differently expressed genes

with the highest AUC values based on TCGA data, which might be suitable to be applied as markers in distinguishing LUAD from LUSC. To validate our analyses, the expression of six candidate genes was detected in lung cancer samples by immunohistochemical staining. The staining results confirmed the potentials of these six genes in distinguishing LUAD from LUSC, and also validated the feasibility of our methods for identification of candidate markers from high-throughput data.

Table 3 The immunohistochemical staining results.

Gene and markers	LUAD				LUSC				P value	Threshold (LUAD/LUSC)	Sensitivity (%)	Specificity (%)
	Negative	Weak	Moderate	Strong	Negative	Weak	Moderate	Strong				
LUAD												
<i>MLPH</i>	1	20	23	6	44	5	1	0	<0.001	weak/negative	98	88
<i>TMC5</i>	2	17	31	0	43	7	0	0	<0.001	weak/negative	96	86
<i>SFTA3</i>	0	6	39	5	38	12	0	0	<0.001	weak/negative	88	100
<i>TTF1</i>	0	24	21	5	44	6	0	0	<0.001	weak/negative	100	88
<i>CK7</i>	0	11	28	11	42	5	3	0	<0.001	weak/negative	100	84
<i>NAPSA</i>	3	39	5	3	47	3	0	0	<0.001	weak/negative	94	94
<i>SPA</i>	24	26	0	0	47	3	0	0	<0.001	weak/negative	52	94
LUSC												
<i>DSG3</i>	40	10	0	0	5	11	29	5	<0.001	negative/weak	90	98
<i>DSC3</i>	35	12	3	0	5	9	24	12	<0.001	negative/weak	90	97
<i>CALML3</i>	38	11	1	0	0	5	17	28	<0.001	weak/moderate	90	98
<i>TP63</i>	41	9	0	0	3	24	20	3	<0.001	negative/weak	94	86
<i>HCK</i>	3	37	10	0	0	7	13	30	<0.001	weak/moderate	86	80
<i>P40</i>	50	0	0	0	17	33	0	0	<0.001	negative/weak	66	100

The staining scores of *TTF1*, *CK7*, *NAPSA*, *SPA*, *TP63*, *HCK* and *P40* were obtained from the pathologists' original reports. The threshold indicates the criteria to distinguish LUAD from LUSC when the sum of the sensitivity and specificity reaches a peak. e.g., "weak/negative" means if the sample's staining score ranks from weak to strong it will be identified as LUAD, and negative as LUSC. LUAD, lung adenocarcinoma; LUSC: lung squamous cell carcinoma.

Our analyses revealed that the expression distribution profiles of *MLPH*, *TMC5*, *SFTA3*, *DSG3*, *DSC3*, and *CALML3* were markedly different between LUAD and LUSC, and their sensitivity and specificity were not less than many commonly used markers. And we believed that the sensitivity and specificity would be improved after wide use in clinical practices. *DSG3* and *DSC3* are both transmembrane glycoproteins that belong to calcium-dependent cell adhesion molecules, and their diagnostic values in distinguishing LUSC from LUSC have been frequently reported (13-18). *DSG3* and *DSC3* are also greatly elevated in other squamous tumors and reduced in many other adenocarcinomas (19-21). The downregulation of *DSG3* and *DSC3* is in part due to DNA methylation and associated with poor prognosis in tumors (13,15,22-24). Although our results showed the potential diagnostic abilities of *MLPH*, *TMC5*, *SFTA3*, and *CALML3*, their

expressions and functions in lung cancer have received little attention and remain unclear.

Most of the genes recommended as markers in distinguishing LUAD from LUSC also ranked tops in our tables according to the order of the AUC values, such as *TTF-1* (*NKX2-1*), *NAPSA*, *TP63* and *S100 calcium binding protein A7* (*S100A7*) (Tables 1, 2, S1, and S2) (4-6). Another commonly used marker, *CK5/6*, detects the proteins coded by *keratin* (*KRT*) 5, *KRT6A*, and *KRT6B*, all three genes ranked high in Table 2 (4-6). Many other genes ranked high in our tables such as *mucin 1* (*MUC1*), *carcinoembryonic antigen-related cell adhesion molecule 6* (*CEACAM6*), *tripartite motif containing 29* (*TRIM29*) and *S100 calcium binding protein A2* (*S100A2*), were also reported that they could be used in distinguishing LUAD from LUSC (17,25,26).

With the rapid development of microarrays and RNA-Seq in recent years, more and more high-throughput data have

been accumulated. How to effectively identify suitable biomarkers from these data for disease diagnosis and sub-classification is now receiving a lot of attention. Therefore, we hope our method to investigate candidate markers by combing differently expressed gene screening and ROC curve analysis, will be widely applied and further improved in the future.

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Footnote

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

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Supplementary

Table S1 The ROC curve analyze results of genes greatly elevated in LUAD

Gene	LUAD	LUSC	Fold-change (LUAD/LUSC)	AUC value
<i>MLPH</i>	3,961±3,315	521±769	7.60	0.953
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<i>MUC1</i>	22,301±16,816	3,137±3,945	7.11	0.921
<i>KCNK5</i>	1,458±1,260	212±262	6.86	0.921
<i>PRR15L</i>	1,306±1,207	187±334	6.96	0.915
<i>SLC44A4</i>	2,905±2,552	387±636	7.50	0.907
<i>CLDN3</i>	2,127±2,016	356±930	5.97	0.907
<i>ST3GAL5</i>	1,751±1,535	318±304	5.49	0.906
<i>CD55</i>	9,112±9,307	2,068±2,001	4.41	0.898
<i>LPCAT1</i>	17,427±17,015	3,703±5,206	4.71	0.895
<i>CEACAM6</i>	41,068±39,526	4,992±11,717	8.23	0.889
<i>SELENBP1</i>	4,213±4,536	697±820	6.04	0.889
<i>GPR116</i>	5,436±5,921	842±1,175	6.46	0.887
<i>SLC34A2</i>	42,409±40,305	5,358±10,219	7.91	0.886
<i>HPN</i>	1,351±1,788	219±406	6.16	0.885
<i>TESC</i>	1,759±3,143	126±754	13.92	0.882
<i>PLEKHA6</i>	1,199±943	269±402	4.45	0.882
<i>FOLR1</i>	3,586±4,963	305±641	11.76	0.881
<i>NAPSA</i>	35,629±37,838	3,240±6,098	11.00	0.879
<i>LMO3</i>	2,516±2,520	318±722	7.91	0.878
<i>STEAP4</i>	4,339±4,707	753±1,528	5.76	0.877
<i>B3GNT7</i>	2,440±3,524	421±761	5.79	0.875
<i>VSTM2L</i>	1,714±2,342	213±496	8.03	0.874
<i>MUC21</i>	2,461±4,873	103±613	23.87	0.873
<i>RHOBTB2</i>	3,058±3,121	731±806	4.18	0.873
<i>DPP4</i>	3,010±3,391	389±1,004	7.74	0.872
<i>MACC1</i>	1,519±1,287	369±402	4.12	0.872

Table S1 (continued)

Table S1 (continued)

Gene	LUAD	LUSC	Fold-change (LUAD/LUSC)	AUC value
<i>ABCC3</i>	5,208±3,908	1,169±1,428	4.45	0.869
<i>FGL1</i>	1,227±4,239	50±553	24.17	0.868
<i>SPINK1</i>	3,748±10,070	134±1,321	27.86	0.868
<i>C16orf89</i>	5,412±8,524	326±626	16.60	0.866
<i>ATP8A1</i>	1,186±1,289	289±329	4.10	0.863
<i>AHCYL2</i>	3,891±4,065	782±626	4.97	0.861
<i>CYP2B7P1</i>	3,261±9,555	259±714	12.58	0.856
<i>PON3</i>	1,042±1,294	235±662	4.43	0.855
<i>TMPRSS2</i>	2,486±2,505	565±827	4.40	0.853
<i>AGR2</i>	11,318±15,822	1,998±3,064	5.66	0.852
<i>C1orf116</i>	5,471±5,568	931±814	5.88	0.850
<i>C4orf31</i>	1,549±1,809	301±725	5.13	0.850
<i>RNASE1</i>	13,190±15,196	2,749±2,810	4.80	0.846
<i>ALPK3</i>	1,139±1,068	224±372	5.08	0.846
<i>HOPX</i>	7,935±12,980	1,136±1,974	6.98	0.845
<i>DPCR1</i>	1,687±14,092	17±40	99.16	0.835
<i>C5orf4</i>	1,037±1,551	230±450	4.51	0.834
<i>XAGE1D</i>	3,375±4,395	413±1,514	8.16	0.817
<i>SLC26A9</i>	1,281±2,386	116±229	10.99	0.816
<i>TREM1</i>	1,139±1,735	248±357	4.58	0.807
<i>C4BPA</i>	5,525±10,596	733±1,371	7.53	0.807
<i>CLIC6</i>	3,400±3,554	658±1,120	5.16	0.806
<i>RASD1</i>	2,210±3,304	393±728	5.62	0.800
<i>SFTPB</i>	195,735±252,122	29,275±45,424	6.69	0.799
<i>TSPAN8</i>	2,050±5,256	220±659	9.32	0.799
<i>AGR3</i>	1,328±1,793	205±376	6.47	0.799
<i>SUSD2</i>	4,164±7,302	600±1,568	6.93	0.790
<i>MFSD4</i>	1,158±1,461	172±214	6.72	0.790
<i>PIGR</i>	20,188±41,363	1,719±3,039	11.74	0.788
<i>HPGD</i>	2,926±6,201	489±1,115	5.98	0.788
<i>FGB</i>	5,412±24,204	312±3,894	17.32	0.788
<i>MSLN</i>	10,685±21,563	1,039±6,275	10.28	0.785
<i>SERPINA1</i>	24,209±47,249	5,747±8,054	4.21	0.781
<i>GCNT3</i>	1,071±2,121	195±496	5.47	0.777
<i>MUC5B</i>	22,738±53,189	1,754±8,646	12.96	0.775
<i>FGA</i>	8,319±35,185	500±4,168	16.61	0.772
<i>TFPI2</i>	3,447±14,530	525±4,287	6.56	0.764
<i>ALOX15B</i>	1,444±2,164	327±623	4.41	0.763
<i>AMY1A</i>	1,596±6,215	220±473	7.25	0.754
<i>HLA-DQB2</i>	1,216±3,432	259±462	4.70	0.751
<i>CLDN2</i>	1,224±4,183	63±287	19.17	0.748

Table S1 (continued)

Table S1 (continued)

Gene	LUAD	LUSC	Fold-change (LUAD/LUSC)	AUC value
<i>PGC</i>	33,835±138,066	389±1,462	86.86	0.748
<i>PPP1R1B</i>	1,452±2,143	299±789	4.85	0.747
<i>CACNA2D2</i>	1,313±2,012	221±376	5.93	0.746
<i>AQP5</i>	1,562±3,262	125±322	12.49	0.745
<i>FGG</i>	10,438±37,227	1,092±7,279	9.55	0.739
<i>PAEP</i>	1,822±6,186	78±975	23.27	0.738
<i>CTSE</i>	6,809±12,689	1,058±1,650	6.44	0.735
<i>MUC13</i>	1,434±3,740	175±955	8.16	0.731
<i>AZGP1</i>	2,531±6,377	583±3891	4.34	0.730
<i>CEACAM5</i>	20,407±34,340	4,095±12,219	4.98	0.723
<i>SLC7A2</i>	2,658±4,735	515±909	5.16	0.723
<i>CYP4B1</i>	2,242±4,144	444±875	5.05	0.721
<i>LGALS4</i>	1,133±4,373	17±96	64.50	0.715
<i>TFF3</i>	3,040±8,131	457±1,565	6.65	0.713
<i>VSIG1</i>	1,259±4,284	73±352	17.04	0.712
<i>SCGB3A1</i>	10,328±58,644	585±1,433	17.63	0.711
<i>CRLF1</i>	2,809±6,631	319±1,329	8.80	0.695
<i>S100P</i>	5,442±10,667	1,111±3,795	4.90	0.693
<i>GPR110</i>	1,332±1,797	306±564	4.34	0.688
<i>PLUNC</i>	10,603±42,374	851±3,069	12.46	0.683
<i>MUC6</i>	1,217±8,355	75±611	16.22	0.681
<i>CALCA</i>	3,578±19,341	224±3,022	15.96	0.679
<i>SCGB3A2</i>	8,546±23,575	1,224±2,096	6.98	0.670
<i>CLDN18</i>	2,013±7,033	307±823	6.55	0.653
<i>TFF1</i>	1,249±5,541	34±230	36.46	0.647
<i>CPS1</i>	5,079±15,544	436±3515	11.63	0.593
<i>HP</i>	4,502±22,250	1,056±2,141	4.26	0.591
<i>PCSK2</i>	1,817±10,039	100±397	18.01	0.568
<i>MSMB</i>	1,343±7,980	175±874	7.67	0.560
<i>PCSK1</i>	1,049±6,553	142±1,047	7.36	0.340

ROC, receiver operating characteristic; LUAD, lung adenocarcinoma; LUSC: lung squamous cell carcinoma; AUC: area under curve.

Table S2 The ROC curve analyze results of genes greatly elevated in LUSC

Gene	LUAD	LUSC	Fold-change (LUSC/LUAD)	AUC value
<i>DSG3</i>	88±777	8,728±8,556	98.77	0.973
<i>KRT5</i>	1,227±10,342	116,689±96,742	95.03	0.972
<i>DSC3</i>	128±789	7,515±6,291	58.62	0.970
<i>CALML3</i>	141±1,096	10,039±11,031	71.17	0.964
<i>SERPINB13</i>	22±191	2,166±3,217	95.70	0.956
<i>KRT6B</i>	310±1,208	17,808±27,334	57.45	0.954
<i>KRT6C</i>	136±529	7,372±12,063	54.13	0.954
<i>KRT6A</i>	2,297±8,724	87,096±81,359	37.91	0.951
<i>PVRL1</i>	1,204±1,177	11,200±7,063	9.30	0.950
<i>LOC642587</i>	59±213	1,247±1,247	20.99	0.949
<i>PERP</i>	6,258±4,951	31,500±21,939	5.03	0.947
<i>TP63</i>	325±914	10,976±9,139	33.72	0.946
<i>TRIM29</i>	861±1,930	11,291±7,291	13.10	0.945
<i>ATP1B3</i>	1,866±1,138	9,231±6,592	4.94	0.945
<i>FAT2</i>	125±383	3,737±3,587	29.82	0.943
<i>CLCA2</i>	87±691	6,787±7,536	77.23	0.943
<i>SPRR2A</i>	43±546	4,036±8,211	93.51	0.940
<i>JAG1</i>	1,118±1,157	7,365±7,830	6.58	0.939
<i>KRT14</i>	315±3,191	26,428±57,383	83.77	0.939
<i>SERPINB5</i>	358±904	4,421±3,570	12.32	0.937
<i>KRT13</i>	225±2,423	18,866±41,338	83.76	0.934
<i>CSTA</i>	190±403	4,222±5,543	22.20	0.934
<i>PKP1</i>	882±2,176	19,788±16,151	22.42	0.934
<i>DAPL1</i>	15±102	1,098±1,932	69.02	0.933
<i>IRF6</i>	647±369	3,108±1,757	4.80	0.932
<i>KRT16</i>	310±1,070	17,386±35,463	56.03	0.932
<i>SLC6A8</i>	965±1,028	7,254±5,830	7.52	0.929
<i>SPRR2E</i>	13±179	1,158±3,196	84.41	0.929
<i>A2ML1</i>	106±1,345	1,717±3,166	16.10	0.929
<i>GPC1</i>	1,375±1,171	9,223±8,003	6.71	0.926
<i>HR</i>	60±115	1,104±1,530	18.30	0.923
<i>KRT17</i>	2,926±8,839	62,551±69,399	21.37	0.921
<i>COL7A1</i>	442±945	5,390±5,665	12.17	0.919
<i>SLC2A1</i>	4,007±4,652	23,021±18,217	5.74	0.918
<i>ANXA8</i>	240±740	3,194±3,237	13.30	0.916
<i>PTHLH</i>	149±307	3,642±5,287	24.41	0.914
<i>GBP6</i>	71±203	2,247±2,528	31.33	0.913
<i>ABCC5</i>	1,037±1,012	7,355±7,806	7.09	0.912
<i>SPRR1A</i>	36±250	2,333±4,852	63.44	0.912
<i>SNAI2</i>	255±444	1,149±731	4.49	0.911

Table S2 (continued)

Table S2 (continued)

Gene	LUAD	LUSC	Fold-change (LUSC/LUAD)	AUC value
<i>SLC16A1</i>	597±1,019	2,486±1,753	4.16	0.910
<i>TFRC</i>	3,415±3,639	18,175±19,185	5.32	0.910
<i>FOXE1</i>	80±276	1,593±1,939	19.72	0.908
<i>BMP7</i>	172±530	1,843±1,470	10.70	0.907
<i>ITGA6</i>	1,937±3,063	8,650±7,228	4.46	0.906
<i>NTRK2</i>	173±794	7,764±9,701	44.79	0.905
<i>ST6GALNAC2</i>	287±316	1,438±978	5.00	0.904
<i>CELSR2</i>	487±386	2,204±1,814	4.53	0.904
<i>ODZ2</i>	29±146	1,147±1,729	38.99	0.904
<i>ADAM23</i>	26±90	1,535±2,091	57.10	0.902
<i>GJB6</i>	96±265	2,657±4,069	27.65	0.899
<i>ANXA8L2</i>	133±347	1,201±1,194	8.99	0.897
<i>LGALS7</i>	33±147	1,397±3,297	41.66	0.897
<i>S100A7</i>	79±824	2,320±11,972	29.29	0.896
<i>RHCG</i>	62±554	2,294±5,834	36.71	0.894
<i>NRARP</i>	217±196	1,068±1,082	4.92	0.894
<i>S100A2</i>	1,037±4,073	14,533±20,550	14.01	0.890
<i>ADH7</i>	71±513	2,704±3,930	37.83	0.887
<i>LYPD3</i>	428±839	3,478±4,530	8.12	0.886
<i>SPRR3</i>	75±497	4,179±9,702	55.54	0.884
<i>COL4A5</i>	312±414	1,956±2,391	6.26	0.884
<i>CXCR7</i>	609±1,045	4,107±4,471	6.74	0.883
<i>C3orf58</i>	458±333	1,881±1,718	4.10	0.883
<i>PTPRZ1</i>	222±538	2,422±2,239	10.88	0.882
<i>GPR87</i>	239±399	1,358±1,159	5.68	0.881
<i>RAPGEFL1</i>	302±456	1,882±1,782	6.22	0.880
<i>UGT1A7</i>	8±77	1,054±2,247	128.92	0.880
<i>SPRR2D</i>	87±428	2,165±4,477	24.63	0.878
<i>SPRR1B</i>	178±777	3,747±6,231	20.96	0.878
<i>KRT15</i>	1,280±4,508	20,918±28,994	16.33	0.878
<i>PI3</i>	352±4431	5,523±12,731	15.67	0.876
<i>SFN</i>	3,844±3,146	17,013±14,551	4.43	0.876
<i>FABP5</i>	157±305	1,443±2,707	9.15	0.876
<i>RBP1</i>	360±732	2,217±3,706	6.15	0.873
<i>DST</i>	2,550±2,332	10,378±8,529	4.07	0.873
<i>PITX1</i>	329±586	2,003±2,523	6.08	0.870
<i>FAM84A</i>	302±428	1,341±1,198	4.44	0.865
<i>UPK1B</i>	266±1,452	2,995±5,424	11.24	0.864
<i>ADM</i>	503±728	2,123±2,249	4.22	0.862
<i>SOX2</i>	479±830	43,21±4,483	9.02	0.862
<i>CLDN1</i>	2,085±3,554	15,300±19,672	7.34	0.861

Table S2 (continued)

Table S2 (continued)

Gene	LUAD	LUSC	Fold-change (LUSC/LUAD)	AUC value
<i>MAGEA4</i>	323±2,589	2,327±4,114	7.19	0.860
<i>NDUFA4L2</i>	632±1,412	4,587±5,094	7.25	0.860
<i>SERPINB4</i>	78±380	1,223±3,002	15.63	0.853
<i>FGFBP1</i>	236±491	2,053±2,791	8.70	0.851
<i>SERPINB3</i>	344±1,591	3,359±6,296	9.75	0.848
<i>NTS</i>	1,909±15,405	8,452±21,005	4.43	0.846
<i>FGFR2</i>	547±653	2,244±2,092	4.10	0.845
<i>RGMA</i>	233±383	1,250±1,463	5.35	0.841
<i>ALDH3B2</i>	288±450	1,176±1,362	4.08	0.838
<i>CYP2S1</i>	568±775	3,034±2,938	5.33	0.833
<i>GPNMB</i>	6,752±7,084	30,334±47,047	4.49	0.831
<i>NDRG4</i>	172±226	1,102±1,372	6.39	0.825
<i>GJB2</i>	862±1,422	6,171±10,796	7.15	0.820
<i>ABCA13</i>	257±471	1,296±1,327	5.04	0.812
<i>FBN2</i>	154±1,446	1,750±3,324	11.34	0.812
<i>CRYAB</i>	187±291	1,272±4,611	6.80	0.811
<i>MMP10</i>	194±1,193	3,002±7,273	15.47	0.808
<i>NRCAM</i>	221±609	1,241±1,578	5.61	0.806
<i>HAS3</i>	1,028±1,839	4,158±4,225	4.04	0.804
<i>IL1RN</i>	449±537	2,017±2,468	4.49	0.804
<i>S100A8</i>	1,344±8,937	1,1440±28,668	8.51	0.802
<i>CNTNAP2</i>	164±561	1,116±1,722	6.78	0.798
<i>COL17A1</i>	1,339±3,023	6,832±10,661	5.10	0.797
<i>AKR1B10</i>	2,145±7,972	9,111±13,901	4.25	0.794
<i>WNT5A</i>	633±563	2,606±2,816	4.12	0.789
<i>CYP4F3</i>	141±485	1,153±1,964	8.14	0.773
<i>LY6D</i>	214±729	3,033±6,896	14.13	0.765
<i>ALDH3A1</i>	1,848±7,693	8,124±17,776	4.40	0.759
<i>IVL</i>	207±501	1,093±2,097	5.26	0.758
<i>CYP4F11</i>	271±579	2,195±3,752	8.09	0.725
<i>GSTM2</i>	458±496	2,044±3,044	4.46	0.703
<i>GSTM3</i>	609±941	2,866±4,641	4.70	0.696
<i>GPC3</i>	540±1,255	2,291±3,642	4.24	0.684
<i>KRT4</i>	228±1,156	2,160±9,487	9.45	0.644
<i>OLFM1</i>	248±296	1,325±2,310	5.33	0.642
<i>GSTM1</i>	257±559	1,626±4,391	6.32	0.557
<i>C4orf7</i>	87±314	1,896±12,269	21.63	0.530

ROC, receiver operating characteristic; LUSC: lung squamous cell carcinoma; LUAD, lung adenocarcinoma; AUC: area under curve.