Comprehensive Analysis of The Expression and Prognosis For KRTs in LUSC Based on Bioinformatics Analysis

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Abstract

Keratins (KRTs), a family of genes that encode a series of intermediate filament proteins, which expressed in epithelial cells of various tissues and had been identified as being involved in various tumors. Previous evidences indicated that KRTs were implicated in tumorigenesis and development of lung cancer. However, a comprehensive analysis of KRTs in lung squamous cell carcinoma (LUSC) and their roles in the tumorigenesis and progression of this disease is lacking. Therefore, we investigated the transcriptional level, proteomic level, and prognosis of KRTs in patients with LUSC from TCGA, Gene Expression Profiling Interactive Analysis (GEPIA), Human Protein Atlas (HPA), Kaplan-Meier Plotter, and cBioPortal databases. We confirmed that the expression levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 were higher in LUSC tissues than in adjacent normal lung tissues. Survival analyses using Kaplan-Meier Plotter database and TCGA database revealed that the high expression levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 were associated with poor prognosis in patients with LUSC. ROC curves indicated that KRTs had poor roles in predicting the overall survival (OS) of LUSC patients. KRTs and their 50 most frequently altered neighbor genes were mainly enriched in epidermis development, keratinization, keratinocyte differentiation, Rap1 signaling pathway, and transcriptional misregulation in cancer. The results of clinical data analyses indicated that the expression levels of KRT13, KRT16, KRT17, and KRT19 were positively correlated with nodal metastasis, the expression level of KRT15 and KRT17 were positively correlated with primary tumor, and the high expression level of KRT15 was positively correlated with TNM stage. Our study may provide new theoretical basis and research direction for the discovery of potential therapeutic targets and new prognostic biomarkers in patients with LUSC.

Background

Lung cancer is a familiar clinical malignant tumor, which ranks the top among tumors worldwide in terms of morbidity and mortality¹. In January 2021, the International Cancer Research Institute (IARC) published the latest cancer statistics of 36 cancers in 185 countries, and revealed that the new cases of lung cancer reached 2.2 million, ranking second in all new cancer population, and 1.8 million people died of lung cancer, ranking the first in cancer deaths. In China, the new cases and mortality of lung cancer all ranked first (820 thousand and 710 thousand respectively), accounting for 17.9% and 23.8% of new cases and mortality of all cancers².

- Non-small cell lung cancer (NSCLC) approximately accounts for 85% of all lung cancer, and is associated with a poor 5-year overall survival (OS) rate of 19.8%³. The commonest subtypes of lung cancer are lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC)⁴. LUSC is most strongly associated with smoking in a dose-dependent manner, and is more common in men⁵. Generally LUSC grows more fast with larger masses than LUAD of the same stage, and tends to nodal metastasis at the early stages⁶. Pan-cancer studies have shown that the molecular mechanisms of carcinogenesis and progression between LUAD and LUSC are highly heterogeneous^{7,8}, therefore, therapeutic strategies for LUAD are often invalid for LUSC⁹. In recent years, with the continuous development of molecular biology and advocacy of precision medicine, the development of treatment targets and targeted drugs in lung cancer are becoming more and more mature, and the survival time of patients with lung cancer is slightly prolonged. Previous studies have reported that numerous genes were related to LUAD, including EGFR, AKT1, KRAS, TP53, PTEN, and others^{10–13}. However, the research on LUSC lacks valuable gene mutation and the progress is slow, there is no valuable targeted drug for the time being.

LUSC is a product of cumulative genetic and proteomic aberrations. The keratin family was believed to be closely associated with the carcinogenesis and progression of LUSC. Keratins (KRTs) are essential components of intermediate fibrin in the cytoplasm of epithelial cells¹⁴. The expression levels of KRTs in cytoplasm reflects the differentiation of cells, and the abnormal expression of KRTs has a significant effect on the biological behavior of tumor cells¹⁵. Previous studies have discovered that the expression of KRTs was deregulated in mutiple human malignancies, including lung cancer¹⁶, breast

cancer¹⁷, and colon cancer¹⁸. In addition, the expression levels of KRTs are distinct in different subtypes of lung cancer. For example, compared with normal lung tissues, the expression level of KRT5 was significantly upregulated in LUSC tissues, but not in LUAD tissues and small cell lung cancer (SCLC) tissues^{19,20}.

The abnormal expressions of KRTs and their relationship with clinicopathological features and prognosis in patients with LUSC have been partly reported. However, the specific roles of KRTs in LUSC are not completely clear, and there is no systematic evaluation to study the expression levels of KRTs in LUSC, or to assess whether their expression levels are related to the clinical characteristics and progression of this cancer. Previous studies have generated a large volume of differentially expressed genes (DEGs) and microarray data, which makes it possible to search for gene function prediction²¹, disease-associated biomarkers²², and alternative splicing²³ by analyzing these data. In present study, we analyzed the expression levels of KRTs in patients with LUSC in detail to determine the expression pattern, potential function, and diagnostic and prognostic value of KRTs in LUSC.

Materials And Methods

Data collection and processing. High-throughput gene expression data of LUSC tissues and paracancerous lung tissues were downloaded from the Cancer Genome Atlas (TCGA) data portal (<https://tcga-data.nci.nih.gov/tcga>). These RNA-seq data (HTSeq-count) from Illumina HiSeq RNASeq platform consisted of 502 LUSC samples and 49 adjacent normal lung samples, and were achieved from the publicly available Genomic Data Commons (GDC) data portal (<https://portal.gdc.cancer.gov/>).

Exploration of the aberrantly expressed KRTs in LUSC. In order to identify the aberrantly expressed KRTs, the 'limma' package in RStudio (version 1.4.1106) was applied to compare the expression profiles of LUSC tissues with those of adjacent lung tissues²⁴. The $|\log_2(\text{fold change [FC]})| > 2$, $P\text{value} < 0.01$, and false discovery rate (FDR) < 0.01 were considered as the threshold values for DEGs identification. We selected the KRTs for our study, and used 'gplots' and 'ggplot2' packages of RStudio software to produce the heatmap and volcano plot of the differentially expressed KRTs.

The GEPIA database. To determine the difference of the transcriptional level of KRTs between LUSC tissues and normal lung tissues, we used the "Expression analysis-Profile" and "Expression analysis-Box Plots" modules of the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) web server (<http://gepia2.cancer-pku.cn/#analysis>) to obtain scatter diagrams and box plots of the transcriptional level between the LUSC tissues and the adjacent normal lung tissues, under the settings of P -value cutoff = 0.01, $|\log_2\text{FC}| = 1$, and "Match TCGA normal and GTEx data"²⁵.

The HPA database. The Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>) is a database of immunohistochemistry (IHC) - based protein expression profiles in cancer tissues, normal tissues, and cell lines²⁶. The KRTs protein expression IHC images in clinical specimens of LUSC patients were obtained from this database.

Survival analysis. Firstly, the Kaplan-Meier plotter database (<http://kmplot.com>) was used to evaluate the survival effects of KRTs, which was able to assess the impact of 54,000 genes on the prognosis of 21 cancers, including breast cancer, ovarian cancer, lung cancer, and gastric cancer²⁷. In addition, LUSC patients obtained from TCGA database were divided into the high expression group and the low expression group based on the median expression of KRTs, and the survival curves were produced with 'hash' and 'survival' packages of RStudio.

Diagnostic role of the KRTs in LUSC. The receiver operating characteristic (ROC) curve was performed to assess the diagnostic roles of KRTs in patients with LUSC. ROC curve analysis was performed in RStudio using procedures from the 'pROC' package²⁸.

Potential molecular mechanism of KRTs in LUSC. It is speculated that the abnormal expression of KRTs in LUSC may be closely related to genetic alterations, including amplification, deletion, or point mutations. Therefore, cBioPortal was used to summarize the possible genetic changes of KRTs in LUSC²⁹.

Co-expression and network of KRTs in LUSC. Gene expression data of LUSC tissues and adjacent normal lung tissues were extracted from the TCGA data portal. We selected the gene expression data of KRTs to study. The co-expression analysis of KRTs was performed in RStudio using procedures from the 'pheatmap' package. Relative coefficient > 0.3 was considered statistically significant.

PPI network and functional enrichment analysis of KRTs and altered neighbor genes. Based on the GEPIA database (<http://gepia2.cancer-pku.cn/#analysis>), we constructed a network of KRTs and 50 most frequently changed neighboring genes. The selected genes were input into the online search tool for the retrieval of interacting genes (STRING, version 11.0) database to evaluate the interaction among the genes³⁰. Interactions with a combined score > 0.4 were defined as statistically significant. Cytoscape software (version 3.8.2) was used to visualize the integrated regulatory networks and to mark with different colors according to the degree calculated by CytoHubba (version 0.1) plugin³¹. To identify the pathways which had the most significant involvement with the KRTs, all genes were input into the Database for Annotation Visualization and Integrated Discovery (DAVID)³² for gene ontology (GO) analysis, which included the biological process (BP), cellular component (CC), and molecular function (MF)³³, and Kyoto Encyclopedia of Genes and Genomes (KEGG)³⁰ pathway analysis enrichment, and then visualized in bubble chart by 'ggplot2' package of RStudio. $P < 0.05$ and gene count ≥ 5 were considered to indicate a significant difference in the functional enrichment analysis. In addition, the molecular complex detection (MCODE, version 2.0.0) plugin of Cytoscape software was used to identify the important molecules in PPI networks³⁴. The GO analysis of KRTs was analyzed and plotted using ClueGO (version 2.5.7) and CluePedia (version 1.5.7)³⁵.

Relationship between KRTs and clinicopathological features in LUSC. The clinical data of LUSC was extracted from the TCGA data portal. Gene expression data and clinical data were combined by 'hash' package of RStudio, and the clinical data was divided into high expression group and low expression group according to the median expression. The relationships between the expression levels of KRTs and clinical characteristics were analyzed by statistical analysis of high expression group and low expression group, and were visualized in forestplot by 'forestplot' package of RStudio. Statistical analyses were performed using Pearson's chi-squared test in SPSS (version 22.0). The odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated for each clinical parameter by STATA14. The cutoffs of P value was defined as 0.05.

Results

Aberrantly expressed KRTs based on TCGA database in LUSC. The expression levels of each gene transformed with log2 were calculated by DESeq. Following the calculating criteria, we achieved 6,703 aberrantly expressed genes between LUSC tissues and normal lung tissues, including 4,983 highly and 1,720 lowly expressed genes. Then we listed the 57 KRTs according to the value of $|\log_2 FC|$ (Table 1), which demonstrated that KRTs were upregulated in LUSC. The 'gplots' and 'ggplot2' packages of RStudio were used to produce heatmap and volcanoplot of the 57 KRTs (Fig. 1).

Table 1
The aberrantly expressed KRTs in LUSC based on TCGA database

Gene symbol	LogFC	Pvalue	FDR
KRT1	3.150	4.04E-09	1.07E-08
KRT10	2.627	4.42E-19	2.32E-18
KRT13	7.657	1.82E-32	1.93E-31
KRT14	8.642	1.65E-33	1.83E-32
KRT15	6.278	2.61E-45	4.72E-44
KRT16	8.019	7.89E-48	1.57E-46
KRT16P4	5.377	4.46E-21	2.64E-20
KRT16P5	5.269	2.86E-18	1.43E-17
KRT16P6	6.226	1.33E-20	7.66E-20
KRT17	6.686	2.07E-58	5.65E-57
KRT17P1	5.444	1.31E-40	1.99E-39
KRT17P2	4.824	1.20E-35	1.48E-34
KRT17P3	6.956	3.81E-31	3.79E-30
KRT17P6	5.916	5.57E-45	9.93E-44
KRT19	2.283	9.96E-26	7.58E-25
KRT19P1	2.816	7.94E-23	5.21E-22
KRT2	3.249	4.70E-10	1.34E-09
KRT20	5.796	2.16E-13	7.91E-13
KRT23	3.906	3.80E-16	1.67E-15
KRT24	6.596	3.38E-13	1.22E-12
KRT25	4.112	1.55E-07	3.56E-07
KRT28	2.792	0.001	0.002270644
KRT3	5.407	4.07E-29	3.69E-28
KRT31	9.879	1.56E-34	1.82E-33
KRT32	4.445	7.49E-26	5.74E-25
KRT33A	8.790	5.43E-29	4.89E-28
KRT33B	5.453	1.58E-18	8.07E-18
KRT34	6.314	7.04E-34	7.96E-33
KRT35	4.117	6.51E-10	1.84E-09
KRT36	4.168	1.56E-14	6.17E-14
<i>Note:</i> $P < 0.05$ was considered significant.			
Abbreviations: FDR, false discovery rate; KRTs, keratins; LUSC, lung squamous cell carcinoma; TCGA, the cancer genome atlas.			

Gene symbol	LogFC	Pvalue	FDR
KRT37	5.510	3.10E-20	1.75E-19
KRT38	5.168	1.38E-13	5.12E-13
KRT40	3.182	1.89E-07	4.32E-07
KRT41P	5.153	4.56E-18	2.26E-17
KRT42P	4.480	1.67E-22	1.07E-21
KRT43P	4.956	3.46E-12	1.16E-11
KRT5	7.382	4.95E-65	1.64E-63
KRT6A	8.174	5.28E-67	1.85E-65
KRT6B	8.109	2.00E-47	3.92E-46
KRT6C	8.138	1.53E-35	1.87E-34
KRT71	2.183	3.68E-10	1.06E-09
KRT74	6.886	9.26E-52	2.09E-50
KRT75	7.961	3.93E-30	3.74E-29
KRT76	5.765	1.67E-15	7.03E-15
KRT77	8.284	6.97E-29	6.22E-28
KRT78	5.904	1.19E-33	1.33E-32
KRT80	2.049	8.44E-16	3.62E-15
KRT81	5.901	8.82E-19	4.56E-18
KRT82	3.436	8.75E-15	3.51E-14
KRT83	4.985	3.68E-18	1.83E-17
KRT84	6.829	7.24E-22	4.49E-21
KRT85	3.122	2.25E-08	5.58E-08
KRT86	2.280	2.40E-09	6.48E-09
KRT8P30	2.394	2.14E-11	6.79E-11
KRT8P35	2.663	1.70E-19	9.17E-19
KRT8P38	2.898	8.09E-09	2.09E-08
KRT9	7.137	4.56E-37	5.94E-36
<i>Note:</i> $P < 0.05$ was considered significant.			
Abbreviations: FDR, false discovery rate; KRTs, keratins; LUSC, lung squamous cell carcinoma; TCGA, the cancer genome atlas.			

Transcriptional level of KRTs in patients with LUSC. Using the GEPIA database, we compared the transcriptional levels of KRTs between LUSC tissues and normal lung tissues. The results indicated that the transcriptional levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 were higher in LUSC tissues than in normal lung tissues (Fig. 2).

Protein expression level of KRTs in patients with LUSC. To determine the difference of KRTs protein expression between LUSC tissues with normal lung tissues, we obtained IHC staining images of KRTs from HPA database (Fig. 3). Consistent with the above results of KRTs mRNA expression, the results showed that the protein levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 were higher in LUSC tissues than in normal lung tissues.

Association of the KRTs with the prognosis of patients with LUSC. The prognosis of LUSC patients was analyzed using the Kaplan-Meier survival plotter database. The results indicated that low expression levels of KRT5 [HR = 1.27 (1.12–1.44), $P = 0.00018$], KRT6A [HR = 1.39 (1.23–1.58), $P = 2.7 \times 10^{-7}$], KRT6B [HR = 1.39 (1.22–1.58), $P = 3.4 \times 10^{-7}$], KRT6C [HR = 1.43 (1.26–1.63), $P = 2.3 \times 10^{-8}$], KRT13 [HR = 1.17 (1.03–1.32), $P = 0.016$], KRT14 [HR = 1.16 (1.02–1.32), $P = 0.022$], KRT15 [HR = 1.15 (1.01–1.30), $P = 0.036$], KRT16 [HR = 1.55 (1.36–1.76), $P = 1.5 \times 10^{-11}$], KRT17 [HR = 1.44 (1.27–1.64), $P = 1.3 \times 10^{-8}$], KRT19 [HR = 1.30 (1.15–1.48), $P = 4 \times 10^{-5}$], and KRT23 [HR = 1.20 (1.06–1.37), $P = 0.0039$] were correlated with significantly better overall survival (OS) in LUSC patients (Fig. 4A), low expression levels of KRT5 [HR = 1.25 (1.03–1.51), $P = 0.024$], KRT6A [HR = 1.37 (1.13–1.66), $P = 0.0012$], KRT6B [HR = 1.42 (1.18–1.72), $P = 0.00029$], KRT6C [HR = 1.58 (1.30–1.91), $P = 2.8 \times 10^{-6}$], KRT13 [HR = 1.34 (1.11–1.62), $P = 0.0027$], KRT14 [HR = 1.85 (1.52–2.25), $P = 3.6 \times 10^{-10}$], KRT15 [HR = 1.96 (1.61–2.39), $P = 7.7 \times 10^{-12}$], KRT16 [HR = 1.34 (1.10–1.62), $P = 0.0029$], KRT17 [HR = 1.35 (1.12–1.64), $P = 0.0019$], KRT19 [HR = 1.44 (1.18–1.74), $P = 0.00022$], and KRT23 [HR = 1.33 (1.10–1.61), $P = 0.0036$] were correlated with significantly better progression-free survival (PFS) in LUSC patients (Fig. 4B).

In addition, we downloaded the survival data of patients with LUSC from TCGA database, combined the survival data and gene expression data, and divided them into high expression group and low expression group to study the effect of KRTs on the prognosis of patients with LUSC, the results showed that low expression levels of KRT5 ($P = 0.00493$), KRT6A ($P = 0.00163$), KRT6B ($P = 2 \times 10^{-5}$), KRT6C ($P = 0.00026$), KRT13 ($P = 0.01032$), KRT14 ($P = 0.00121$), KRT15 ($P = 0.00074$), KRT16 ($P = 0.02083$), KRT17 ($P = 9 \times 10^{-4}$), KRT19 ($P = 0.00027$), and KRT23 ($P = 0.00111$) were predicted to have significantly better OS in LUSC patients (Fig. 4C), which were consistent with the results of Kaplan-Meier survival plotter database.

ROC of the KRTs in patients with LUSC. We obtained the gene expression data and clinical data from TCGA database. ROC curve analysis was performed in RStudio using procedures from the 'pROC' package. The results indicated that the KRTs had different specificity and sensitivity in predicting the prognosis of LUSC patients (Fig. 5). The area under receiver operating characteristic (AUC) of KRT5 was 60.753, which was the biggest of all KRTs. The sensitivity and specificity of KRT13 and KRT23 were 74.194 and 86.31, respectively, which were the biggest of all KRTs.

Potential molecular mechanism of the KRTs in patients with LUSC. We used the cBioPortal online tool for TCGA LUSC cohort to analyze the alterations of KRTs. The mutation, which can change the function of protein by changing gene sequence, is the most common gene alteration in KRTs (Fig. 6A). In the current study, the results showed that 13% (59/469) cases had genetic alterations, including inframe mutation, missense mutation, splice mutation, truncating mutation, amplification, and deep deletion (Fig. 6B). The KRT23 (2.8%) was the most common mutational gene among the KRTs, including missense mutation, splice mutation, amplification, and deep deletion.

The co-expression of KRTs in patients with LUSC. TCGA database was used to analyze the expression data of KRTs, and RStudio software was used to calculate the correlation between them, and Pearson's correction was performed. The heatmap was shown in Fig. 7. The results indicated that there were significant and positive correlations between the following KRTs: KRT5 with KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, and KRT19; KRT6A with KRT6B, KRT6C, KRT14, KRT16, KRT17, and KRT19; KRT6B with KRT6C, KRT14, KRT16, and KRT17; KRT6C with KRT14, KRT16, and KRT17; KRT13 with KRT15 and KRT19; KRT14 with KRT16 and KRT17; KRT15 with KRT19; and KRT16 with KRT17.

GO function and KEGG pathway analyses for KRTs and their related genes. We constructed the network for KRTs and their 50 most frequently altered neighbor genes, and found that there were 262 nodes and 1,121 edges in this network (Fig. 8A). The results of functions of 262 genes were shown in Table 2. GO enrichment analysis predicted the functional roles of 262 genes on the basis of three aspects, including biological process (BP) terms, cell component (BP) terms, and molecular function (BP) terms. We found that the 262 genes were mainly enriched in epidermis development, keratinization, and keratinocyte differentiation in BP terms (Fig. 8B), enriched in cornified envelope, extracellular exosome, and desmosome in CC terms (Fig. 8B), and enriched in structural molecule activity, structural constituent of cytoskeleton, and cadherin binding involved in cell-cell adhesion in MF terms (Fig. 8B). KEGG pathway analysis demonstrated that the 262 genes were enriched in P53 signaling pathway, drug metabolism-cytochrome P450, Rap1 signaling pathway, and transcriptional misregulation in cancer (Fig. 8C).

Table 2
GO analysis and KEGG pathway analysis of the KRTs and their neighbor genes associated with LUSC

Category	GO ID	Term	Count	Pvalue
BP	GO:0008544	epidermis development	23	1.56E-22
BP	GO:0031424	keratinization	14	4.53E-14
BP	GO:0030216	keratinocyte differentiation	16	7.93E-14
BP	GO:0018149	peptide cross-linking	12	5.08E-11
BP	GO:0016337	single organismal cell-cell adhesion	10	9.14E-06
BP	GO:0031581	hemidesmosome assembly	5	1.48E-05
BP	GO:0007155	cell adhesion	18	1.76E-04
BP	GO:0098609	cell-cell adhesion	13	3.30E-04
BP	GO:0042060	wound healing	7	7.46E-04
BP	GO:0043588	skin development	5	0.001
BP	GO:0010951	negative regulation of endopeptidase activity	8	0.001
BP	GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	9	0.001
BP	GO:0007010	cytoskeleton organization	8	0.006
BP	GO:0050680	negative regulation of epithelial cell proliferation	5	0.007
BP	GO:0007605	sensory perception of sound	7	0.009
BP	GO:0006508	proteolysis	14	0.019
BP	GO:0060070	canonical Wnt signaling pathway	5	0.026
BP	GO:0030036	actin cytoskeleton organization	6	0.032
BP	GO:0016055	Wnt signaling pathway	7	0.042
BP	GO:0006810	transport	10	0.047
CC	GO:0001533	cornified envelope	14	1.99E-14
CC	GO:0070062	extracellular exosome	86	2.49E-14
CC	GO:0030057	desmosome	10	1.22E-11
CC	GO:0005882	intermediate filament	13	3.46E-08
CC	GO:0005913	cell-cell adherens junction	18	1.60E-06
CC	GO:0005911	cell-cell junction	13	3.32E-06
CC	GO:0005886	plasma membrane	85	1.08E-05
CC	GO:0016324	apical plasma membrane	13	5.38E-04
CC	GO:0005576	extracellular region	38	7.04E-04
CC	GO:0005737	cytoplasm	92	0.002
<i>Note: P < 0.05 was considered significant.</i>				
Abbreviations: BP, biological process; CC, cellular component; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; KRTs, keratins; LUSC, lung squamous cell carcinoma; MF, molecular function.				

Category	GO ID	Term	Count	Pvalue
CC	GO:0005887	integral component of plasma membrane	33	0.002
CC	GO:0005856	cytoskeleton	13	0.004
CC	GO:0043005	neuron projection	10	0.005
CC	GO:0009986	cell surface	16	0.006
CC	GO:0005615	extracellular space	30	0.007
CC	GO:0045095	keratin filament	6	0.011
CC	GO:0031225	anchored component of membrane	6	0.017
CC	GO:0030018	Z disc	6	0.021
CC	GO:0015629	actin cytoskeleton	8	0.027
CC	GO:0001726	ruffle	5	0.032
CC	GO:0016323	basolateral plasma membrane	7	0.033
CC	GO:0005925	focal adhesion	11	0.036
CC	GO:0030054	cell junction	12	0.043
CC	GO:0042470	melanosome	5	0.046
MF	GO:0005198	structural molecule activity	26	6.41E-15
MF	GO:0005200	structural constituent of cytoskeleton	12	3.37E-07
MF	GO:0098641	cadherin binding involved in cell-cell adhesion	15	5.06E-05
MF	GO:0004252	serine-type endopeptidase activity	12	8.28E-04
MF	GO:0005509	calcium ion binding	20	0.005
MF	GO:0004867	serine-type endopeptidase inhibitor activity	6	0.011
MF	GO:0008236	serine-type peptidase activity	5	0.011
MF	GO:0003924	GTPase activity	9	0.016
MF	GO:0030674	protein binding, bridging	5	0.022
KEGG	hsa04115	p53 signaling pathway	6	0.001
KEGG	hsa00982	Drug metabolism-cytochrome P450	5	0.010
KEGG	hsa04015	Rap1 signaling pathway	8	0.016
KEGG	hsa05202	Transcriptional misregulation in cancer	7	0.018

Note: $P < 0.05$ was considered significant.

Abbreviations: BP, biological process; CC, cellular component; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; KRTs, keratins; LUSC, lung squamous cell carcinoma; MF, molecular function.

Category	GO ID	Term	Count	Pvalue
KEGG	hsa04974	Protein digestion and absorption	5	0.024
KEGG	hsa04540	Gap junction	5	0.024
KEGG	hsa05200	Pathways in cancer	11	0.024
KEGG	hsa05146	Amoebiasis	5	0.043
<i>Note:</i> $P < 0.05$ was considered significant.				
Abbreviations: BP, biological process; CC, cellular component; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes; KRTs, keratins; LUSC, lung squamous cell carcinoma; MF, molecular function.				

In addition, we use the MCODE plugin in Cytoscape to construct the module with the highest MCODE score, which contains 41 nodes and 320 edges (Fig. 8D). Subsequent GO function analysis revealed that the 41 genes were mainly enriched in epidermis development, keratinization, keratinocyte differentiation, intermediate filament, desmosome, cornified envelope, structural molecule activity, structural constituent of cytoskeleton, and calcium ion binding (Table 3, Fig. 8E).

Table 3
GO analysis of 41 genes in the most significantly enriched module

Category	GO ID	Term	Count	P value
BP	GO:0008544	epidermis development	15	3.19E-23
BP	GO:0031424	keratinization	10	1.14E-15
BP	GO:0030216	keratinocyte differentiation	10	9.28E-14
BP	GO:0018149	peptide cross-linking	8	1.92E-11
BP	GO:0031581	hemidesmosome assembly	5	1.21E-08
BP	GO:0016337	single organismal cell-cell adhesion	7	1.13E-07
BP	GO:0007155	cell adhesion	8	7.85E-05
BP	GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	5	4.80E-04
BP	GO:0007010	cytoskeleton organization	5	5.16E-04
CC	GO:0005882	intermediate filament	12	4.53E-16
CC	GO:0030057	desmosome	8	3.92E-14
CC	GO:0001533	cornified envelope	9	5.03E-14
CC	GO:0070062	extracellular exosome	26	7.55E-12
CC	GO:0045095	keratin filament	6	2.23E-06
CC	GO:0005911	cell-cell junction	5	4.87E-04
CC	GO:0005913	cell-cell adherens junction	5	0.005
MF	GO:0005737	cytoplasm	18	0.033
MF	GO:0005198	structural molecule activity	18	1.40E-21
MF	GO:0005200	structural constituent of cytoskeleton	9	1.31E-10
<i>Note:</i> $P < 0.05$ was considered significant.				
Abbreviations: BP, biological process; CC, cellular component; GO, gene ontology; MF, molecular function.				

Finally, we conducted the pie charts of 11 KRTs with ClueGO plugin of Cytoscape. The detailed functional notes and classification pie charts were listed in Fig. 8F, 44.44% terms belong to cornification, 22.22% to staphylococcus aureus infection, 11.11% to structural constituent of cytoskeleton, 11.11% to keratin filament, and 11.11% to intermediate filament cytoskeleton.

Relationship between the KRTs and clinical features in patients with LUSC. Pearson's chi-squared test, which based on the TCGA database, indicated that the expression level of KRT13 was positively correlated with nodal metastasis [OR = 1.56 (1.07–2.27), $P = 0.019$], the expression level of KRT15 was positively correlated with primary tumor [OR = 33.78 (17.51–65.19), $P = 0.000$] and TNM stage [OR = 1.63 (1.03–2.60), $P = 0.038$], the expression level of KRT16 was positively correlated with nodal metastasis [OR = 56.67 (17.71–181.29), $P = 0.000$], the expression level of KRT17 was positively correlated with primary tumor [OR = 1.09 (1.07–2.67), $P = 0.023$] and nodal metastasis [OR = 38.24 (18.48–79.12), $P = 0.000$], and the expression level of KRT19 was positively correlated with nodal metastasis [OR = 1.58 (1.08–2.29), $P = 0.017$] (Table 4, Fig. 9).

Table 4
Correlation between KRTs expression and the clinicopathologic features of LUSC patients

Clinical characteristics	Total (N)	OR in KRT5 expression	P value	OR in KRT6A expression	P value	OR in KRT6B expression	P value	OR in KRT6C expression	P value
Primary tumor (T1–2 vs. T3–4)	501	0.95 (0.60–1.48)	0.813	1.29 (0.82–2.03)	0.261	1.23 (0.78–1.93)	0.371	1.36 (0.87–2.14)	0.176
Lymph nodes (N0 vs. N1–3)	485	1.26 (0.87–1.82)	0.231	1.09 (0.75–1.58)	0.660	1.01 (0.70–1.47)	0.951	1.05 (0.72–1.52)	0.802
Distant metastasis (M0 vs. M1)	410	1.34 (0.30–6.06)	0.703	0.75 (0.16–3.38)	0.703	1.34 (0.30–6.06)	0.703	0.75 (0.16–3.38)	0.703
Stage (I–II vs. III–IV)	487	1.17 (0.74–1.85)	0.497	1.05 (0.66–1.66)	0.832	0.99 (0.63–1.57)	0.983	1.17 (0.74–1.85)	0.497
		OR in KRT13 expression	P value	OR in KRT14 expression	P value	OR in KRT15 expression	P value	OR in KRT16 expression	P value
Primary tumor (T1–2 vs. T3–4)	501	1.05 (0.67–1.64)	0.836	1.23 (0.78–1.93)	0.731	33.78 (17.51–65.19)	0.000	1.29 (0.82–2.03)	0.261
Lymph nodes (N0 vs. N1–3)	485	1.56 (1.07–2.27)	0.019	0.94 (0.65–1.37)	0.750	1.13 (0.78–1.65)	0.529	56.67 (17.71–181.29)	0.000
Distant metastasis (M0 vs. M1)	410	0.75 (0.16–3.38)	0.703	0.75 (0.16–3.38)	0.703	2.54 (0.49–13.23)	0.253	0.39 (0.08–2.05)	0.253
Stage (I–II vs. III–IV)	487	1.17 (0.74–1.85)	0.497	1.11 (0.70–1.75)	0.656	1.63 (1.03–2.60)	0.038	1.11 (0.70–1.75)	0.656
		OR in KRT17 expression	P value	OR in KRT19 expression	P value	OR in KRT23 expression	P value		
Primary tumor (T1–2 vs. T3–4)	501	1.09 (1.07–2.67)	0.023	1.52 (0.96–2.39)	0.070	1.16 (0.74–1.83)	0.506		
Lymph nodes (N0 vs. N1–3)	485	38.24 (18.48–79.12)	0.000	1.58 (1.08–2.29)	0.017	0.88 (0.60–1.27)	0.486		
Distant metastasis (M0 vs. M1)	410	1.34 (0.30–6.06)	0.703	0.75 (0.16–3.38)	0.703	2.54 (0.49–13.23)	0.253		
Stage (I–II vs. III–IV)	487	1.38 (0.87–2.19)	0.168	1.54 (0.97–2.46)	0.065	0.99 (0.63–1.57)	0.983		
<i>Note:</i> $P < 0.05$ was considered significant.									
Abbreviations: KRTs, keratins; LUSC, lung squamous cell carcinoma; OR, odd ratio.									

Discussion

Numerous studies have reported that the expression levels of KRTs in many tumors were dysregulated^{16–20}, and the roles of KRTs in occurrence, progression, and metastasis of multiple cancers has been partially confirmed^{36–39}. However, systematic bioinformatics analyses about the function of KRTs in LUSC are limited and have yet to be performed. This is the first time to systematically study the expression of KRTs in LUSC and their prognostic values. We hope our findings will help to provide new therapeutic measures and improve the prognosis of patients with LUSC.

- KRTs family is a group of intermediate filament proteins which express in various types of epithelial cells^{40,41}. Previous studies have confirmed that KRTs played significant roles in maintaining the structural stability of epithelial cells and were involved in numerous biological activities, including epithelial cell signal transduction, stress response, tumor cell apoptosis, and tumor cell proliferation^{42,43}. Moreover, Nazarian et al.⁴⁴ reported that the KRTs were dysregulated in a variety of tumor tissues, and played vital roles in invasion and metastasis of tumors. In summary, this kind of proteins has great potential in diagnosis, typing, and prognosis prediction of tumors. Therefore, the roles of KRTs as multifunctional regulators of epithelial tumorigenesis are worthy of further exploration. We used TCGA database to screen abnormally expressed KRTs between LUSC samples and adjacent normal lung samples, and found that 57 KRTs met our screening criteria. Subsequently, we used GEPIA database and HPA database to observe the transcriptional levels and protein levels of 57 KRTs. The results showed that the expression levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 in LUSC tissues were significantly higher than in adjacent normal lung tissues. Survival analyses showed that the high expression levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 were closely related to the poor OS and PFS of patients with LUSC. ROC curves indicated that the KRT5, KRT13, and KRT23 had potential diagnostic value in patients with LUSC.

KRT5 is usually specifically expressed in the basal layer of the epidermis, and its normal expression is essential for the protection of epithelial cells⁴⁵. Moll et al.¹⁹ reported that KRT5 was specifically expressed in squamous cell carcinoma of multiple tissues. For lung cancer, the expression level of KRT5 in LUSC tissues was significantly higher than in adjacent normal lung tissues^{20,46,47}, which was helpful for the differential diagnosis of LUSC and LUAD⁴⁷. In our study, TCGA database revealed that the expression level of KRT5 was higher in LUSC tissues than in adjacent normal lung tissues. By analyzing the Kaplan-Meier plotter database and TCGA database, we determined the prognostic value of KRT5 in patients with LUSC, and the results indicated that the high KRT5 expression was significantly associated with poor OS and PFS in patients with LUSC followed up for 200 months. In addition, the AUC of KRT5 was 60.753, which had a certain predictive effect on the prognosis of LUSC.

KRT6 is a group of keratins associated with stratified epithelium, including KRT6A, KRT6B, and KRT6C. Studies have reported that KRT6A, KRT6B, and KRT6C were involved in the epidermization of squamous epithelium, so their expression in LUSC increases sharply^{46–49}. The KRT6A is an important part of type II keratin family, and plays a considerable role in the epidermization of squamous epithelium^{50,51}. The overexpression of KRT6A in LUSC and LUAD can be used as a prognostic marker of lung cancer^{47,52,53}. In addition, studies have reported that KRT6A and KRT6B were related to the progression of renal carcinoma and breast cancer^{54,55}. The expression levels of KRT6A, KRT6B, and KRT6C in squamous cell carcinoma-like (SCCL) subtype urothelial carcinoma were significantly increased, and were closely related to poor prognosis⁵⁶. In our report, the expression levels of KRT6A, KRT6B, and KRT6C in LUSC tissues were higher than in normal lung tissues, which was consistent with the reported in the literature. However, there is no previous report on the relationship between KRT6 expression and tumor prognosis. Our research found that the high expression levels of KRT6A, KRT6B, and KRT6C were significantly associated with poor OS and PFS in patients with LUSC.

KRT13 encodes an intermediate cytoskeletal protein, which is usually expressed in the basal compartments of stratified epithelium⁵⁷. Previous studies have reported that KRT13 was expressed at different levels in different tumors, low KRT13 expression was found in oral squamous carcinomas⁵⁸, esophageal squamous cell carcinoma⁵⁹, bladder cancer⁶⁰, lymph node-positive uterine cervix cancer⁶¹, and head and neck squamous cell carcinoma cell lines⁶², by contrast, high KRT13

expression was detected in LUSC⁴⁷, colorectal cancer⁶³, gastric cancer⁶⁴, and tongue squamous cell carcinoma⁶⁵. The overexpression of KRT13 was associated with the squamous cell metaplasia of the upper airway tract epithelium^{66,67}, and was prominent among the gene expression markers for LUSC⁴⁶. Yang et al.⁶⁸ found that the HOXC6 promotes lung cancer progression by upregulating KRT13 expression. In addition, KRT13 was implicated in urothelial and stem cell differentiation⁶⁹. In our report, we demonstrated that the expression level of KRT13 in LUSC tissues was higher than in normal lung tissues, the high KRT13 expression was correlated with poor prognosis in patients with LUSC, and the sensitivity of KRT13 was highest, which seemed consistent with the role of KRT13 as an oncogene and a diagnosis factor of LUSC.

KRT14 is an intermediate filament protein, which is usually expressed by basal epithelial progenitor cells located in epithelial niches of healthy adult tissues. Previous study indicated that KRT14 was not detected in normal tissues, was significantly increased in many tumor tissues, and was expressed in squamous cell carcinoma of different origins and degrees of differentiation⁷⁰. According to the IHC analysis of surgical specimens, the expression level of KRT14 in lung cancer tissues was significantly increased⁷¹, especially in LUSC tissues^{46,47}, which indicated that KRT14 played an important role in the occurrence and development of LUSC. Chen et al.⁴⁷ found that the high KRT14 expression could be helpful in the differential diagnosis of LUAD and LUSC. In addition, Huang et al.⁷² reported that KRT14 was not expressed in normal cervical tissue, but with the increase of the grade of cervical epithelial neoplasia, the expression level of KRT14 gradually increased, which indicated that KRT14 could be used as one of the indicators for the diagnosis of early cervical cancer. In our study, we demonstrated that the expression level of KRT14 in LUSC tissues was higher than in cancer-free lung tissues, and high KRT14 expression was related to poor OS and PFS in patients with LUSC.

KRT15 is a type I keratin, which is mainly expressed in basal keratinocytes of stratified epithelium and plays a significant role in maintaining cytoplasmic stability^{73,74}. Previous studies conformed that the overexpression of KRT15 was involved in tumor formation and progression, including NSCLC^{75,76}, breast cancer^{77,78}, oral squamous neoplasms⁷⁹, urothelial cell carcinomas⁸⁰, and hepatocellular carcinoma⁸¹. Sanchez-Palencia et al.⁸² reported that KRT15 expression level was higher in LUSC tissues than in cancer-free lung tissues, and may be a marker of LUSC. In addition, the high expression KRT15 was associated with poor prognosis of colorectal cancer⁸³ and gastric cancer⁸⁴. In the present study, KRT15 was significantly overexpressed in LUSC tissues, and its high expression level was related to the poor prognosis in patients with LUSC.

KRT16 is an important part of type I cytoskeleton. KRT16 was reported to play a role in LUAD tumorigenicity via EMT, and increased KRT16 expression was associated with poor outcomes of tumors, including LUAD⁸⁵, oral squamous cell carcinoma³⁶, and metastatic breast cancer⁸⁶, which indicated KRT16 had an oncogenic role in tumors. In our report, we demonstrated that the expression level of KRT16 was higher in LUSC tissues, which was significantly related to poor OS and PFS in patients with LUSC.

KRT17 is a 48 kDa type I keratin²⁰. Extensive tissue screening results showed that KRT17 was low expressed in mature epithelial tissues, but regenerated and highly expressed in cancer tissues⁸⁷, and high expression of KRT17 indicated poor prognosis in NSCLC⁸⁸, which indicated that KRT17 can be a biomarker in predicting progression and poor prognosis in patients with LUSC^{87,88}. Chen et al.⁴⁷ reported that KRT17 was highly expressed in LUSC, and in LUSC was significantly higher than in LUAD, suggesting that KRT17 may be a tumor marker for differentiating LUSC and LUAD subtypes of lung cancer. In addition, KRT17 was also highly expressed in breast cancer^{89,90}, cutaneous squamous cell carcinoma⁹¹, cervical carcinoma⁹², gastric carcinoma⁹³, and oral sprays⁹⁴, which was associated with poor prognosis of these tumors⁸⁹⁻⁹⁴. In this report, we found that KRT17 was highly expression in LUSC tissues, which was correlated with poor OS and PFS of the patients with LUSC.

KRT19 is a member of the keratin family, which is functionally related to maintaining the structural integrity of epithelial cells, including bronchial epithelial cells⁹⁵. Yuan et al.⁹⁶ discovered that compared with LUAD, KRT19 was upregulated in LUSC, and the high expression level of KRT19 was closely related to poor prognosis of LUSC. In addition, KRT19 has been

identified as a tumor-associated biomarker for a variety of tumors, including esophageal squamous cell carcinoma⁹⁷, cervical carcinoma⁹⁸, breast cancer⁹⁹, colorectal cancer¹⁰⁰, and hepatocellular carcinoma¹⁰¹. Moreover, the overexpression of KRT19 was associated with poor prognosis of hepatocellular carcinoma¹⁰² and breast cancer¹⁰³. In the present study, KRT19 was significantly overexpressed in LUSC tissues, which was related to poor OS and PFS in LUSC patients.

KRT23 is an important member of the KRT gene family, and locates on human chromosome 17q21.2. Previous studies have shown that KRT23 is abnormally expressed in a variety of tumor tissues, including pancreatic carcinoma¹⁰⁴, colorectal carcinoma¹⁰⁵, and hepatocellular carcinoma¹⁰⁶. In our report, we demonstrated that the expression level of KRT23 was higher in LUSC tissues than in normal lung tissues, and the high KRT23 expression was significantly correlated with poor prognosis in patients with LUSC. ROC curve analysis found that the specificity of KRT23 was 86.31, which had a certain predictive effect on the prognosis of LUSC.

In our study, the analysis of co-expression of KRTs found that KRTs were co-expressed in different degrees in LUSC, which was consistent with the finding of Travis, who found that KRT5, KRT6, KRT13, KRT14, KRT16, KRT17, and KRT19 were the gene expression markers for LUSC⁴⁶.

Subsequently, we used cBioPortal to detect the possible genetic alterations of KRTs in LUSC. The result indicated that mutation was the commonest gene alteration in KRTs. Previous studies have shown that the mutations of transcription factors were related to the occurrence and development of cancer, the abnormal expression of oncogenic transcription factors and chromatin regulatory factors were the internal reason for the tumorigenesis of cancer cells¹⁰⁷. The abnormal function of these regulatory factors provided some clues for the mechanisms of abnormal gene expression^{108,109}.

GO function and KEGG pathway analyses found that the KRTs and their neighbor genes were mainly enriched in epidermis development, keratinization, keratinocyte differentiation, and Rap1 signaling pathway. This was consistent with the knowledge that cytokeratin was abundant and stable in epithelial cells, and it had high specificity in tissues and cells, so it could be used as an ideal biological indicator to identify the origin of tumor and predict prognosis¹¹⁰. Some studies have reported that keratinization of tumors was closely related to the poor prognosis of tumor patients^{111,112}, suggesting the importance of keratinization in neoplastic prognosis¹¹¹. Studies on Rap1 signaling pathway have reported that abnormality of Rap1 signaling pathway was highly associated with the occurrence and development of some tumors, including pancreatic cancer, melanoma, and human chronic myelogenous leukemia^{113–115}.

Finally, we analyzed the relationship between the expression level of KRTs and the clinical characteristics of patients with LUSC. It turned out that the expression levels of KRT13, KRT16, KRT17, and KRT19 were positively correlated with nodal metastasis, the expression levels of KRT15 and KRT17 were positively related to primary tumor, and the expression level of KRT15 was positively correlated with TNM stage. There were some studies on the relationship between KRTs and clinical features of tumors. Liu et al.⁸⁵ reported that KRT16 was upregulated in LUAD and the highly KRT16 expression was related to nodal metastasis. Huang et al.³⁶ suggested that high expression of KRT16 indicates the poorer pathological differentiation, advanced stages, and increased nodal metastasis of OSCC patients. In addition, the expression level of KRT16 was significantly increased in the process of nodal metastasis in primary breast cancer¹⁷. Wang et al.⁸⁸ found that the highly expressed KRT17 was associated with poor differentiation and nodal metastasis in patients with NSCLC. Hu et al.¹¹⁶ found that the expression level of KRT17 was significantly associated with the tumor size, depth of invasion, nodal metastasis, and TNM stage in patients with gastric cancer. Nordgard et al.¹¹⁷ confirmed that KRT19 can be used as a molecular biomarker of disseminated tumor cells in regional lymph nodes from NSCLC patients.

In this study, we comprehensively analyzed the effects of KRTs on LUSC, and provided a thorough understanding of the heterogeneity and complexity of the molecular mechanisms of LUSC. Our results indicated that the increased expression of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 in LUSC tissues might play an important role in LUSC oncogenesis and progression, and could also serve as molecular markers to identify high-risk group

of LUSC patients. In addition, our findings suggested that KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 were potential therapeutic targets for LUSC, and were potential prognostic markers for patients with LUSC.

Conclusions

In brief, our study is the first to demonstrate that the expression levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 are elevated in LUSC tissues compared with normal lung tissues, and the high expression levels of KRT5, KRT6A, KRT6B, KRT6C, KRT13, KRT14, KRT15, KRT16, KRT17, KRT19, and KRT23 predict poor prognosis. These novel findings not only shed light on the molecular alterations in LUSC but also provide the foundation for further research in this area.

Declarations

Data Availability Statement

High-throughput gene expression data of LUSC tissues and normal lung tissues were extracted from the Cancer Genome Atlas (TCGA) data portal (<https://tcga-data.nci.nih.gov/tcga>). These RNA-seq data (HTSeq-count) from Illumina HiSeq RNASeq platform consisted of 502 LUSC samples and 49 adjacent non-cancerous lung samples, and were achieved from the publicly available Genomic Data Commons (GDC) data portal (<https://portal.gdc.cancer.gov/>).

Author Contribution Statement

Qiangqiang Zheng designed this study, Qiangqiang Zheng analyzed the data. Qiangqiang Zheng wrote the manuscript. All authors reviewed the manuscript.

Conflict of Interest Statement

The authors declare no competing financial interests and competing non-financial interest.

Ethics Statement

Not applicable.

Acknowledgements

Not applicable.

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Figures

Figure 1

Heatmap and Volcanoplot of the distribution of KRTs between LUSC tissues and normal lung tissues. (A) Heatmap of 57 KRTs between LUSC tissues and normal lung tissues. The upregulated and downregulated genes were shown in red and green, respectively. (B) Volcanoplot of 57 KRTs between LUSC tissues and normal lung tissues. The upregulated genes were shown in red.

Figure 2

Transcriptional expression of distinct KRTs family members. (A) scatter diagram. (B) box plot (*P < 0.05).

Figure 3

The IHC - based protein expression of KRTs in LUSC tissues and normal lung tissues. All the IHC staining images were obtained from the HPA database.

Figure 4

The OS of LUSC patients with high or low expression of KRTs. The prognostic significance of the KRTs in patients with LUSC, according to the (A) Kaplan-Meier plotter database and (B) TCGA database. The red curve represents the survival curve of LUSC patients with high gene expression, the black curve represents the survival curve of LUSC patients with low gene expression.

Figure 5

ROC curves for the KRTs in LUSC. Red represents sensitive curve, blue indicates identity line. The X axis shows false positive rate, presented as “100-Specificity (%)”. The Y axis indicates true positive rate, shown as “Sensitivity (%)”. These curves were provided by ‘pROC’ package of RStudio.

Figure 6

The genetic alterations of KRTs in LUSC. (A) The alteration frequency of KRTs. (B) Mutations of every KRT gene. Brown represents inframe mutation, green represents missense mutation, orange represents splice mutation, dark grey represents truncating mutation, red represents amplification, and blue represents deep deletion.

Figure 7

Co-expression of different KRTs in LUSC (RStudio).

Figure 8

The PPI network and enrichment analyses of KRTs and genes significantly associated with KRT alterations. (A) PPI network of KRTs and genes significantly associated with KRT alterations. (B) GO analysis of KRTs and genes significantly associated with KRT alterations. (C) KEGG functional enrichment analysis of KRTs and genes significantly associated with KRT alterations. (D) MCODE identification of the most significantly enriched module. The circle represents upregulated genes, and the triangle represents downregulated genes. (E) GO analysis of the most significantly enriched module. (F) ClueGO analysis of 11 KRTs.

Figure 9

Forest plots of OR for the association between KRTs expression and clinicopathological features in patients with LUSC.