A novel centrosome-related gene signature for predicting prognosis and treatment effect of lung adenocarcinoma

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Research Article

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Abstract

Background

The structure or function of the centrosome can cause abnormal cell proliferation, leading to tumors. There is increasing evidence that the centrosome is closely associated with the occurrence and development of lung adenocarcinoma (LUAD). We aim to construct a new centrosome-related genes (CRGs) prognostic model in this study.

Methods

The gene expression data of LUAD can be downloaded from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. We used the R to identify differentially expressed genes between normal and malignant lung tissues, constructed a CRGs risk score, evaluated the prognostic value of clinical data in different subgroups with different CRGs risk score signature to construct a CRGs risk model.

Result

A total of 779 CRGs were detected, and three genes related to prognosis were screened, including ID1, LATS2 and PRKCZ, and CRGs. Risk score was constructed based on these three genes, and its accuracy was verified in the GEO dataset. The prognosis is significantly lower in the high-risk group, and this feature can be used as an independent prognostic factor. In addition, the immune and mutation landscape between the different subgroups were found to be significantly different. We found that the Tumor Immune Dysfunction and Exclusion (TIDE) score of the high-risk group was significantly increased, indicating that the low-risk group is more likely to benefit from immunotherapy.

Conclusion

The research results suggest that the CRGs risk model may be a reliable prognostic model for personalized treatment of LUAD patients.

Introduction

It is estimated that about 350 lung cancer people will die in the United States every day in 2023, more than breast, prostate, and pancreatic cancer combined [1]. Non-small cell lung cancer (NSCLC) accounts for about 82% of all lung cancers, and lung adenocarcinoma (LUAD) is the most common type [2]. Although computed tomography (CT) imaging, fiberoptic bronchoscopy, thoracosopic surgery, radiotherapy, and chemotherapy have improved with advances in detection techniques and treatments [3], the prognosis for patients with LUAD remains poor. Therefore, it is necessary to identify more reliable
biomarkers to predict the prognosis of lung adenocarcinoma to develop new diagnostic and treatment strategies.

The centrosome is the primary microtubule organizing center (MTOC) in animal cells and plays an essential role in cell polarity, migration, and cell division [4, 5]. As early as 100 years ago, Boveri pointed out that changes in cell polarity and abnormal chromosome separation in malignant tumors may be due to centrosome dysfunction [6]. Centrosome abnormalities are common in human tumors, including changes in centrosome number, volume, and shape [7]. Gao Meixia et al [8] found that apparent centrosome abnormalities can be found in tumor tissues and sputum samples of non-small cell lung cancer, indicating that centrosome abnormalities may become a biological marker for NSCLC diagnosis. The presence of more than two centrosomes in cells, called centrosome amplification, is a hallmark of human cancer and directly impacts chromosome instability and cancer cell invasion [9, 10]. Studies have shown that centrosome protein CP110 is a key mediator of CDK2 inhibition to induce late-stage disasters in lung cancer, and high expression of CP110 causes centrosome amplification, which is often overexpressed in lung cancer tissues [11, 12]. In summary, significant progress has been made in understanding the role of the centrosome in the development and prognosis of lung cancer. However, no relevant research exists on the CRGs signature model for LUAD.

Therefore, we extracted RNA sequencing data from patients with LUAD from TCGA and GEO databases, and successfully developed a novel CRGs correlation index based on three CRGs (ID1, LATS2, and PRKCZ), and constructed a CRGs risk model, which has significant value in predicting OS prognosis and guiding treatment.

Materials and methods

Data collection

Raw data from LUAD patients were acquired from The Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/tcga), including RNA-seq transcriptome data and relevant clinical and survival information. In addition, we used the GSE50081 cohort from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) as an independent validation cohort. After excluding duplicate and OS time of less than 30 days, 493 lung adenocarcinoma and 59 normal tissue patient data from TCGA were included in this study. Subsequently, single nucleotide mutation (SNV) data were obtained using the TCGA-LUAD cohort. The centrosome-related genes were collected from the literature [13] and the Gene Set Enrichment Analyses (GSEA, http://www.gsea-msigdb.org/gsea/index.jsp) database, and a total of 779 genes were included in this study (Supplementary Table S1).

Identification of differentially expressed genes (DEGs) and Functional enrichment analysis

The “limma” package in R (version 4.3.0) was used to identify CRGs between LUAD and normal tissue in the TCGA dataset (adjusted P < 0.05 and log |fold change (FC)| > 1). The “ggplot2” and “pheatmap”
packages in R were used to construct volcano plots and heat maps for visualizing differentially expressed genes, and Venn diagrams were used to display common genes in the DEGs and CRGs.

To study the biological functions associated with centrosome-related DEGs in LUAD, Genetic Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analytical exploration of centrosome-related DEGs were performed using the “clusterProfiler,” “org.Hs.eg.db” and “enrichplot” package in R.

**Construction and validation of prognostic mitochondrial-related risk score signature**

Perform univariate Cox regression analysis on 779 CRGs. Take the intersection of the resulting 187 genes ($P < 0.05$) related to prognosis and centrosome-related DEGs to obtain 45 genes. Further Lasso regression analysis and multivariate Cox regression analysis were performed to screen for three genes (ID1, LATS2, and PRKCZ) obtained with $P < 0.1$, and a CRGs prognostic risk score was constructed. Divide the samples into high-risk and low-risk groups using the median critical value of the risk score. Kaplan-Meier (K-M) survival analysis was performed using the “survival” and “survminer” packages in R to compare the overall survival (OS) of the two groups.

**Construction and valuation of nomogram**

Univariate Cox regression analysis was used to analyze the CRGs risk score and clinical factors (including age, gender, stage, previous history, and smoking history) were screened for predictive factors related to OS ($P < 0.1$). The relevant factors were further included in the multivariate Cox regression to further screen for independent risk factors for OS ($P < 0.05$) (including previous history, stage, and CRGs risk score). These risk factors were used to construct a comprehensive nomogram for prognostic evaluation of individualized patients. The performance of the nomogram was evaluated using ROC curves and calibration curves.

**Tumor microenvironment**

The “ESTIMATE” package in R is used to calculate the StromalScore, ImmuneScore, ESTIMATE score, and Tumor Purity.

**Calculate the immune infiltration score of 28 types of immune cells.**

The relative infiltration abundance of 28 immune cells (http://cis.hku.hk/TISIDB/index.php) [14] was calculated using the “ssGSEA” package in R, and the relative lot of different immune cells between the high-risk and low-risk groups was compared using the “Wilcox” test.

**Mutation analysis**

Use the R "easyTCGA" package (https://github.com/ayueme/easyTCGA) to acquire the SNV data of TCGA-LUAD. The "maftools" package was then used to analyze mutations in different risk groups of LUAD [15]. The top twenty genes with the highest mutation in each group are shown in a waterfall chart.
Drug susceptibility analysis

The "OncoPredict" package in R was developed by Maeser et al. [16] to predict in vivo drug responses in cancer patients. We calculated susceptibility scores for six drugs commonly used for LUAD using the "OncoPredict" package. The Wilcoxon test is then used to compare differences in sensitivity scores between high- and low-risk groups. In addition, Tumor Immune Dysfunction and Exclusion (TIDE) algorithms [17] are used to predict the sensitivity of immunotherapy.

Result

Identification and functional enrichment analysis of centrosome-related DEGs.

We obtained 493 tumor and 59 normal tissue samples from the TCGA-LUAD cohort, and received 3237 DEGs through differential analysis (Supplementary Table S2). The intersection of 779 CRGs yielded 116 DEGs (Figure 1 B), including 81 UP and 35 DOWN genes (Supplementary Table S3), which were further visualized by volcano plots (Figure 1 A) and heat maps (Figure 1 C).

Then, GO enrichment analysis revealed the important role of centrosome-related DEGs in LUAD. In terms of biological processes (BP), these differentially expressed genes are mainly involved in processes such as cell cycle and microtubule cytoskeleton; in terms of cellular components (CC), they are primarily related to structures such as microtubule cytoskeleton, microtubule organizing center, and spindle (Figure 1 D, Supplementary Table S4). Subsequently, KEGG analysis showed that these genes mainly involve pathways such as cell cycle, cellular senescence, and human T-cell leukemia virus one infection (Figure 1 E, Supplementary Table S5). In summary, functional enrichment analysis showed that these genes are related to the cell cycle and play an important role in cell mitosis.

Construction and validation of the CRGs risk score

Based on the collected 779 CRGs, 186 prognosis-related genes (P<0.05) were screened by univariate Cox regression analysis (Supplementary Table S6). By intersecting with centrosome-related DEGs, 45 genes were further reduced to 11 (Figure 3 A), and the number of genes was further narrowed to 3 (P<0.1) by multivariate Cox regression analysis (Figure 3 C). Finally, three CRGs, including ID1, LATS2 and PRKCZ, were used to construct a prognostic risk score for LUAD patients. ID1, LATS2, and PRKCZ are poorly expressed in LUAD tumor tissues compared to normal tissues (Figure 3 D E G).

The risk score for each patient in the training and validation cohorts is then calculated by the following formula (Supplementary Table S7). All genes are calculated using log2(gene expression+1).

Risk score = h0(t)*exp(β1X1+β2X2+...+βnXn)

Patients were divided into high and low risk subgroups based on the median risk score. Both groups of OS were assessed using the K-M survival curve, with the lower risk group having worse OS than the high-
risk group (Figure 3 A-D). The relationship between risk score and survival status, OS, risk subgroup, and heat maps of the expression of the three genes are shown in Figure 3. These expressions prove that our risk model has good performance.

Construction of nomogram

To evaluate the prognostic value of risk scores, we identified previous history, stage, and CRGs risk score as independent prognostic factors for LUAD through univariate and multivariate COX regression analysis using univariate and multivariate Cox regression analysis (Table 1). The correlation nomogram was constructed based on multivariate Cox regression analysis structure. The ROC curves of 1, 3, and 5 years show AUC values of 0.768, 0.747, and 0.712, respectively (Figure 4 A). The calibration curve shows that the actual survival probability of 1, 3, and 5 years is in good agreement with the survival probability predicted by the nomogram model (Figure 4 B).

Table 1. Univariate and Multivariate Cox Proportional Hazards Model Results of OS.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Univariate Cox Analysis</th>
<th>Multivariate Cox Analysis</th>
</tr>
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<tr>
<td></td>
<td>P</td>
<td>HR(95%CI)</td>
</tr>
<tr>
<td>Age</td>
<td>0.575</td>
<td>1.00(0.99-1.02)</td>
</tr>
<tr>
<td>Gender</td>
<td>0.444</td>
<td>1.12(0.83-1.52)</td>
</tr>
<tr>
<td>Stage</td>
<td>Stage</td>
<td>Stage</td>
</tr>
<tr>
<td>Stage I</td>
<td>&lt;0.001</td>
<td>2.32(1.6-3.37)</td>
</tr>
<tr>
<td>Stage II</td>
<td>&lt;0.001</td>
<td>3.34(2.26-4.93)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>&lt;0.001</td>
<td>3.72(2.14-6.47)</td>
</tr>
<tr>
<td>History</td>
<td>0.062</td>
<td>1.47(0.98-2.2)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.734</td>
<td>0.95(0.96-1.3)</td>
</tr>
<tr>
<td>Risk</td>
<td>&lt;0.001</td>
<td>0.58(0.43-0.79)</td>
</tr>
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</table>

Centrosome-related risk score was associated with tumor microenvironment

We calculated the correlation between risk scores and tumor immune signatures using the "ESTIMATE" algorithm. We calculated the Stromal Score, Immune Score, ESTIMATE score, and Tumor Purity of LUAD patients. The results showed no significant difference in the immune score between the high and low risk groups (Figure 5), but the trend of risk score and immune score (Figure 5 C) was basically the same. The ESTIMATE analysis indicated that tumor purity was higher in the high risk group (Figure 5 F), whereas the stromal score (Figure 5 E) and immune score (Figure 5 D) were lower in the high risk group.
To further assess the diverse immune microenvironment, we explored the relative proportion of 28 immune cells in LUAD patients in the TIME using the "ssGSEA" algorithm (Figure 5 B, Supplementary Table S8). The results show significant differences in immune infiltration between the two different subgroups of 16 immune cells, with the high risk group being higher than the low risk group (Figure 5 A). These results suggest that high and low-risk patients have different immune cell subsets and potentially different anti-tumor abilities. The study suggests that immune infiltration may play an essential role in the prognosis of patients with LUAD.

**Mutation status of LUAD patients in high-risk and low-risk groups**

Tumor mutation patterns play an essential role in tumor development. Therefore, we plotted the mutation waterfall charts of the high and low risk subgroups and analyzed the mutation status between the two groups. As shown in Figure 6, we analyzed and compared the mutation landscape of the top 20 high-frequency mutated genes between different risk groups. TP53, TTN, CSMD3, RYR2, MUC16, ZFHX4, XIRP2, USH2A, and SPTA1 are common high-frequency mutated genes between the two groups. Among the top 4 mutated genes, the mutation rates of TP53 and MUC16 were significantly different between the two subgroups (Figure 6 C, D, E, F).

**Risk score predicts therapeutic benefits in LUAD**

Cisplatin, paclitaxel, docetaxel, gemcitabine, gefitinib, and osimertinib are commonly used drugs in the treatment of LUAD. We analyzed the drug sensitivity scores of several drugs using the R package “OncoPredict” and found significant differences between the high and low risk subgroups (Figure 6).

Immunotherapy has brought significant improvements to the prognosis of cancer patients, and immunotherapy resistance is a major obstacle to the use of immune checkpoint inhibitors in LUAD. We further evaluated the effectiveness of the central body-related gene risk score in immunotherapy using the TIDE algorithm (Supplementary Table S9). We found that TIDE scores was significantly different between different risk score subgroups, with significantly higher scores in the high-risk group. Immune infiltration analysis shows that the high-risk group has a higher tumor purity (Figure 4 F). The higher the TIDE prediction score (Supplementary Figure 1), the higher the likelihood of immune evasion, indicating that patients are less likely to benefit from ICI treatment [18]. Therefore, these results suggest that the benefits of immunotherapy may be reduced in high risk group patients.

**Discussion**

Personalized treatment of LUAD poses huge challenges due to tumor heterogeneity. Lung cancer remains one of the tumors with the highest morbidity and mortality in the world [2]. Chromosomal instability (CIN) can lead to tumor heterogeneity, leading to different responses to treatments among different individuals, resulting in unsatisfactory treatment effects and the inability to customize specific individualized treatment plans for patients [19]. Centrosome amplification is one of the leading causes of CIN [20], excessive centrosomes lead to the nucleation of excessive microtubules, destroying tissue polarity and
cellular structure, thus leading to genomic instability [21]. Therefore, studying the biological mechanisms and prognostic biomarkers of LUAD and CRGs may provide opportunities to improve precision treatment of LUAD.

This study is the first prognostic model for LUAD based on CRGs. In this study, we constructed a prognosis-related risk score based on 3 CRGs (ID1, LATS2, and PRKCZ), through limma, LASSO, univariate and multivariate regression analysis. Studies have shown that ID1 and LATS2 are localized to centrosomes, PRKCZ can regulate centrosome localization, and three genes play an important role in tumor development [22–24]. These three characteristic genes have been studied to a certain extent in the development and treatment of LUAD.

Overexpression of ID1 can inhibit cell differentiation, enhance proliferation and invasion, and has been identified as a biomarker and therapeutic target for a variety of malignant tumors, and is an independent prognostic factor for LUAD [25–27]. Studies have shown that ID1 is overexpressed in human LUAD, and ID1 is involved in mediating lung cancer cell proliferation and tumor growth through the Akt and Src pathways [28, 29]. However, high expression of ID1 has also been associated with good prognosis in postoperative patients treated with adjuvant paclitaxel combined with cisplatin [30]. Our gene expression profile analysis showed that ID1 is lowly expressed in LUAD, and similar results were obtained by calculating the expression of ID1 through the online database GEPIA (Supplementary Fig. 2) [31]. Therefore, the role of ID1 in lung cancer remains uncertain. As a potential tumor suppressor gene, LATS2 shows down-regulation of LATS2 expression in many cancers and maybe a new regulator of cellular homeostasis [32, 37]. The expression of LATS2 is down-regulated in lung cancer. Silencing LATS2 induces p53 accumulation, related to drug sensitivity by regulating p53-mediated processes. A low LATS2 to LATS1 ratio sensitizes cancer cells to cisplatin administration [33–35]. Therefore, LATS2 gene expression in LUAD patients can be used as a potential biomarker of their survival status. Members of the protein kinase C (PKC) family are critical regulators of many cellular functions and play an essential role in tumor suppression or promotion [37, 38]. PKCζ, the product of the PKC family member gene PRKCZ, is both upregulated and upregulated in human cancers, leading to confusion about its role in cancer [39, 40]. There is evidence that it can act as a tumor suppressor in LUAD, and downregulation of its expression can induce epithelial-to-mesenchymal transition and invasion of LUAD cells [41, 42]. The role and mechanism of the PRKCZ gene in LUAD are very complex, and more experiments are needed to demonstrate its potential treatment strategies.

Targeted therapy has become an important treatment method for LUAD. However, even for sensitive patients, acquired resistance will appear sooner or later, and overcoming resistance has become an important bottleneck in the field of targeted drugs. Although ID1 overexpression is a poor prognostic factor in LUAD, ID1 overexpression in gefitinib-treated NSCLC cells maintained Akt activity and upregulated FLICE like inhibitory proteins to dissociate the caspase-8-RIP1 complex Binding of RIP1 and RIP3 further activates necroptosis-like cell death in gefitinib-treated NSCLC and induces cellular sensitivity to epidermal growth factor receptor tyrosine kinase inhibitors independent of mutational status of NSCLC influence [43]. This provides a new idea for EGFR-TKI resistance. In addition, the LAST2 gene and ID1
gene also play an essential role in chemotherapy sensitivity. Studies have shown that reducing the expression of LATS2 can make lung adenocarcinoma cells sensitive to cisplatin exposure [30, 44]. In our risk score, LUAD patients in the high risk group had significant differences in drug sensitivity to gefitinib and osimertinib. Combined with the previous experiments, the application of our risk model in drug sensitivity requires further verification through a large number of experiments.

Studies have reported that centrosome abnormalities contribute to tumor immune escape and distant metastasis. Centrosomes mediate tumor cells to release tumor immunosuppression and invasion-related inflammatory factors such as IL-8, angiopoietin-like protein 4, and growth and differentiation 15 through ECASP, and participate in the formation of the tumor microenvironment. Aneuploidy is an important reason for tumor immune escape [45]. Centrosome abnormalities lead to aneuploid tumor cells inhibiting MHC class I molecular antigen presentation, reducing CD8+ T lymphocyte infiltration, and escaping the body’s immune surveillance. At the same time, aneuploidy can recruit Th2 cells and M2 macrophages to generate an immunosuppressive microenvironment by activating the intracellular cGAS-STING pathway and Rho-GTPases pathway. The centrosome is an intracellular MTOC, and its structural and functional changes lead to cytoskeleton rearrangement, metabolic changes and enhanced cell motility in tumor cells, making invasion and distant metastasis more likely to occur [45, 46]. The centrosome plays a very important role in cancer, so a broader understanding of the relationship between cancer, the centrosome and the immune microenvironment is very important. Some experimental results have confirmed that knocking out or inhibiting the expression of centrosome proteins can effectively inhibit the proliferation of various tumor cells, suggesting that the treatment of centrosomes may be a potential direction for future tumor treatment [47].

Therefore, our study has obvious clinical significance. On the one hand, we have observed that the survival time of patients in the low risk group is significantly prolonged, and the clinical detection and corresponding treatment measures for patients in the high risk group should be strengthened to prevent the recurrence of clinical diseases. On the other hand, through drug sensitivity analysis, patients with high risk scores have significant differences in the sensitivity of conventional chemotherapy and targeted drugs compared with patients with low scores; at the same time, the TIDE algorithm found that risk scores are significantly positively correlated with TIDE scores, which is helpful for us to know the clinical treatment. The developed centrosome-associated signature can be used not only as a prognostic tool but also as a guide for individualized therapy.

There are several limitations in our study. First, our study has only been developed and validated with public datasets, and thus requires external validation in a multicenter cohort. Second, prospective clinical trials are necessary to verify the applicability of our findings in treated LUAD patients. Finally, fundamental experiments must be used to elucidate centrosome-associated genes further and target therapeutic mechanisms for further interpretation.

Conclusion
our study identified and validated a prognostic model based on three centrosome-related genes with independent prognostic significance for LUAD patients.

**Declarations**

**Acknowledgements**

Not applicable.

**Fundings**

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**Availability of data and materials**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ Supplementary Material.

**Authors' contributions**

RD and DG completed the main design and conception of the study. RD, KL, QC and CS complete the collection data. RD, KL, CS and DG analyze and interpret the data. RD, CS and HZ processes the data graphics. RD and KL drafted the first draft of the article, and DG made final revisions to the article. All authors have read and approved the final manuscript.

**References**


Figures
Figure 1

Identification of DEGs related to mitochondrion and functional analysis. A. Volcano plot of 3237 DEGs in LUAD tumor and normal groups. B. Venn diagram showed that the overlap of 3237 DEGs and 729 CRGs led to 116 hub genes being identified. C. Heatmaps of 116 centrosome-related DEGs. D. GO enrichment of centrosome-related DEGs, including biological process (BP), cellular component (CC), and molecular function (MF). E. KEGG pathways of centrosome-related DEGs.
Figure 2

Construction of centrosome-related prognostic risk score using TCGA-LUAD cohort. A, B. LASSO regression of the 44 OS-related genes. Cross-validation in the LASSO regression model to select the tuning parameter. The abscissa shows the log ($\lambda$) value, and the ordinate shows partial likelihood deviance. C. Multivariable Cox regression analysis revealed 3 genes were associated with prognosis of patients with LUAD ($P<0.1$). D. Gene expressions of the 3 prognosis-related genes in TCGA-LUAD. *$P<0.05$, **$P<0.01$, ***$P<0.001$. 

<table>
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<td>FPRCZ</td>
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<td>AURKA</td>
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<td>CDC63</td>
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<tr>
<td>PAX6</td>
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Figure 3

Stability validation of centrosome-related prognostic risk model in the TCGA-LUAD and GEO50081 cohort. A, D. Kaplan–Meier curves of the OS of patients in the high and low risk groups in the TCGA-LUAD cohort (A), and GEO50081 cohort (D). B, E ROC curves for predicting 1, 3, and 5-year OS in the TCGA-LUAD cohort (B), and GEO50081 cohort (E). C, F. Distribution of CRGs risk score, survival status and the gene
expression of 3 model genes between the high and low risk groups for TCGA-LUAD cohort (C), and GEO50081 cohort (F).

Figure 4

Construction of Nomogram. A, B. Calibration plots and ROC curves for 1-, 3-, and 5-year survival projections. C. The nomogram for the prediction of 1-, 3-, and 5-year OS in LUAD patients.
Figure 5

Relationship between the molecular subtypes and tumor immune microenvironment. A. Differences in the infiltration level of immune cells between low and high risk groups. B. Heat map showing the difference in Immune infiltration score between the two Subgroups. C, D, E, F. Comparison of estimate scores, immune scores, stromal scores and tumor purity in two Subgroups. G, F, I, J. Spearman correlation between
estimate scores, immune scores, stromal scores and tumor purity in two Subgroups. (**P<0.0001, ***P < 0.001, **P < 0.01, *P < 0.05).

Figure 6

Mutation status in the low- and high-risk groups in LUAD. A, B. The top 20 genes according to mutation frequency in low- and high-risk groups. C, D, E, F. Mutation rate of the top 4 mutant genes in low-risk and high-risk groups.
Figure 7

A–F Comparison of Drug sensitivity score of six chemotherapy and target drugs in two subtypes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• Supplementarytable.xlsx
• sf.1.tif
• sf2.tif