Cuproptosis-related lncRNAs predict prognosis and immune reponse of lung adenocarcinoma lncRNAs predict lung adenocarcinoma

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Research Article

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Abstract

Background: lung adenocarcinoma（LUAD）accounts for 50% of lung cancers, has a high mortality and a poor prognosis. Long non-coding RNA (lncRNAs) has been suggested to play an important role in the progression of tumors. Cuproptosis is a newly discovered form of cell death that is highly investigated in which excess copper promotes the aggregation of Lipoacylated protein and the destabilisation of Fe-S cluster proteins, leading to proteotoxicity and ultimately to cell death. Therefore, the aim of this study was to explore the role of cuproptosis-related lncRNA signature in clinical prognosis prediction and immunotherapy, and the relationship with drug sensitivity.

Material and Methods: Genomic, clinical, and mutational data for LUAD patients were extracted from The Cancer Genome Atlas (TCGA), cuproptosis-related genes obtained form cuproptosis-related studies. Prognostic signature was constructed by co-expression analysis and Cox regression analysis. Patients were divided into high and low risk groups and then ROC, survival, risk curves, nomogram, C-Index, independent prognostic analysis, and clinical subgroup model validation were performed to observe the value of the signature in prognosis. Subsequently, lncRNAs were analyzed for Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment and immune-related functions, and tumor mutation burden (TMB). Finally, we analyzed the impact of TIDE scores on immune escape and immunotherapy of lncRNAs, identifying potential drugs for LUAD and the sensitivity of the drugs.

Results: A total of 16 cuproptosis-related lncRNAs were obtained (AC016747.2, LINC00205, AC006947.1, LINC00592, AC020634.2, AC026355.2, LINC02848, ZNF571-AS1, CRIM1-DT, SEPSECS-AS1, HIF1A-AS3, AC013267.1, LINC02635, AL162632.3, AC004832.5, AC032011.1.) and developed prognostic signature, we found that high-risk patients had worse overall survival (OS), progression-free survival (PFS) and higher mortality. Independent prognostic analyses, ROC, C-INDEX and nomogram demonstrate that the cuproptosis-related lncRNAs can accurately predict the prognosis of patients. nomogram and heatmap show a clear distribution of high and low risk of cuproptosis-related lncRNAs. Enrichment analysis demonstrated that the biological functions of lncRNAs are associated with the development of tumors. We also found that immune-related functions such as anti-viruses were suppressed in high-risk patients who had mutations in oncogenes, and OS was poorer in patients with high-TMB. Finally, we used TIDE to conclude that high-risk patients have a greater potential for immune escape and less effective immunotherapy, and also to identify a range of drugs that may be effective in the treatment of LUAD.

Conclusion: in conclusion, the 16 cuproptosis-related lncRNAs can accurately predict the prognosis of LUAD patients and may provide new insights into clinical applications and immunotherapy.

Background

Lung adenocarcinoma(LUAD)is a common type of lung cancer and is currently one of the highest incidence and mortality in the world, posing a serious risk to human health[1]. Treatment options for lung cancer include surgery, radiotherapy and chemotherapy[2]. Currently, LUAD is not very well diagnosed,

and most patients are already at an advanced stage when it is detected, losing the opportunity for surgery, and the distant spread of cancer cells can cause serious physical damage. Despite the progress made in diagnosing and treating LUAD, patient survival remains poor. Therefore, it is vital to improve the treatment of LUAD and the quality of life of patients by proposing a rational treatment plan. Nowadays, tumor risk prediction signatures have been constructed to provide a non-invasive understanding of patient survival and to accurately predict prognosis, and are gradually being used in clinical applications[3]. Therefore, there is an urgent need to develop prognostic prediction signature to predict the long-term survival of LUAD patients.

Copper is considered to be an essential cytokine for all organisms and plays a dual, paradoxical role in cells, which maintain intracellular copper concentrations at very low levels through a homeostatic mechanism[4]. Copper-induced cell death is mediated by Iron-sulfur protein, where copper binds to lipid acylation in the TCA cycle, leading to lipid acylated protein aggregation and loss of iron-sulfur cluster proteins, resulting in proteotoxic stress and ultimately cell death, unlike apoptosis and ferroptosis, this unique form of cell death is termed cuproptosis[5]. Copper deficiency has been shown to inhibit tumor angiogenesis and growth in previous animal models and clinical trials[6]. Imbalance of copper can lead to uncontrolled tumor growth7. However, the exact mechanism of cuproptosis is still not fully understood. In Hematopoietic Cancers, a copper-dependent anti-tumor agent has been shown to exert potent anti-tumor effects in vivo and in vitro[8]. In breast cancer, the Endoplasmic Reticulum-Targeting Copper(II) Complex promotes phagocytosis of cancer cells by macrophages[9]. In pancreatic cancer, copper transporter 1 (SLC31A1) and copper chelator tetrathiomolybdate (TM) can promote autophagy and inhibit growth by reducing copper uptake[6]. Thus, exploring the role played by cuproptosis in cancer has great potential for clinical application.

Long-stranded non-coding RNAs (lncRNAs) are a class of RNA molecules with transcripts longer than 200nt that do not encode proteins, but rather regulate gene expression at multiple epigenetic, transcriptional and post-transcriptional levels in the form of RNA[10]. In the past, in-depth studies of lncRNAs have demonstrated that gene expression has reached a new level and that lncRNAs have been shown to play a role in important pathologies and physiologies such as autophagy, development, differentiation, apoptosis, cell cycle[11]. In tumor research, lncRNAs have been shown to be differentially expressed in lung, stomach, liver, colon, breast and pancreatic cancers and to play a role in tumor cells proliferation, migration and invasion[11–15]. Several studies have demonstrated that in Colorectal Cancer, lncRNA SH3PXD2A-AS1 expression is elevated and plays a role in promoting the growth of cancer cells, potentially serving as a diagnostic and therapeutic target[16]. DDX11-AS1 has been identified as a prognostic lncRNA with therapeutic potential in hepatocellular carcinoma patients by analysis of RNA-seq data[17]. In pancreatic cancer, ROR acts as a typical lncRNA with anti-tumor effects in vivo and in vitro, besides, ROR can also inhibit cancer cell growth by activating miR-145[15]. Notably, in endometrial cancer, autophagy-related lncRNAs can be used to predict patients prognosis. However, the role of lncRNAs in LUAD still needs to be further explored.

Cuproptosis-related lncRNAs may have clinical diagnostic and therapeutic implications for LUAD. In this study, we used bioinformatics analysis to obtain cuproptosis-related lncRNAs and to analyze their biological functions and role in predicting prognosis of LUAD patients.

Material And Methods

1. Data processing and Identification of cuproptosis-related lncRNAs

RNA-Seq data of Gene expression of TCGA-LUAD were downloaded from the TCGA database, a total of 54 normal individuals and 501 LUAD individuals, clinical data and mutations of LUAD were obtained from TCGA, according to the gene annotations from the TCGA to distinguish lncRNAs. To identify potential cuproptosis-related lncRNAs, we performed a co-expression correlation analysis of lncRNA and cuproptosis-related gene expression profiles using the limma package at |R|>0.4 and $P < 0.001$.

2. Construction of the prognostic cuproptosis-related lncRNA signature

A list of the cuproptosis-related genes were obtained from cuproptosis-related studies[18–22]. Lasso Cox regression was used to search for cuproptosis-related lncRNAs based on 1000 times tenfold cross-validation, and prognostic lncRNAs were identified based on multivariate Cox regression analysis ($P < 0.05$), followed by calculation of risk scores.

3. Analysis of risk score model

Patients were divided into high and low risk groups based on median values to assess the prognosis of the signature. The survival package calculates overall survival (OS) and progression-free survival (PFS) for LUAD patients and performs univariate and multivariate independent prognostic analyses to assess the independent prognostic value of risk prediction models. The pheatmap package was used to plot patient survival status and lncRNAs expression heatmap according to risk scores. The survivalROC package was used to calculate the 1-, 3-, 5-year AUC of our model and to construct C-Index curves to verify the accuracy of the model in predicting patient survival.

4. Construction of Nomogram and Validation of clinical subgroups

The survival and rms packages constructed nomogram to determine survival rates for 1-, 3- and 5-year patients. The nomogram were constructed for age, gender, stage, T stage, N stage and finally calibration curves were plotted to show the difference between the predicted and actual outcomes of the nomogram.

5. Principal component analysis (PCA) and enrichment functional analysis

PCA was constructed using the limma, scatterplot3d package to explore the distribution of patients with different risk scores. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of cuproptosis-related lncRNAs were performed by the clusterProfiler package and P-value < 0.05 and false discovery rate (FDR) < 0.05 were considered statistically significant.

6. Immune-related functional analysis and Tumor mutation burden analysis

The limma, GSVA package was used to analyze the differences in immune-related functions in LUAD patients and $P < 0.05$ was considered statistically significant, pheatmap packages were used to visualize the results; maftools package was used to compare the relationship between risk score and Tumor mutation burden(TMB). Used the survival package to explore the difference between TMB and patient survival, the $P < 0.05$ was considered statistically significant.

7. Immunotherapy analysis and pharmaceutical screening

We download NSCLC's TIDE from http://tide.dfci.harvard.edu/ and then, using the ggpubr, limma package, analyzed the relationship between risk score and TMB, with $P < 0.05$ considered statistically significant. Screening of therapeutic drugs and observation of drug sensitivity using the pRRophetic, ggplot2, ggpubr packages with pFilter = 0.001 and corPvalue = 0.001.

Results

1. Identification of cuproptosis-related lncRNAs and construction of prognostic signature

By |R|>0.4 and $P < 0.001$ criteria, 2244 cuproptosis -related lncRNAs were identified from 16,876 incRNAs and 19 cuproptosis-related genes, co-expression relationships with cuproptosis-related genes and cuproptosis-related lncRNAs were visualised using Sankey diagram (Fig. 1). In the training group, LASSO COX regression analysis was used to identify cuproptosis-related lncRNAs, univariate Cox regression analysis identified 37 lncRNAs and then, multivariate COX analysis identified 16 lncRNAs as independent prognostic factors, The risk score for each sample was then calculated based on the expression levels of the 16 lncRNAs(Fig. 2a-2c) risk score =(-0.79670054952258*AC016747.2)+ (0.454977942296554*LINC00205)+(0.971038537693743*AC006947.1)+ (0.466282143262505*LINC00592)+(0.580816870667386*AC020634.2)+ (-0.364069056069612*AC026355.2)+(-2.94320726773469*LINC02848)+(-0.760200155891508*ZNF571-AS1)+(0.429955081610087*CRIM1-DT)+(-1.0655839243752*SEPSECS-AS1)+ (0.272457956361295*HIF1A-AS3)+(1.56215847973464*AC013267.1)+ (-0.364308918322871*LINC02635)+(1.84725474576194*AL162632.3)+ (0.646354490290833*AC004832.5)+(1.019129061711 61*AC032011.1). The correlation heatmap also shows the relationship between cuproptosis-related genes and lncRNAs (Fig. 2d).

2. Survival analysis of the signature

According to the median value of the risk scores as the cutoff value, the patients were divided into a low risk group and a high risk group. We found that Overall survival (OS) and Progression-free survival (PFS) were significantly shorter in the high-risk group than in the low-risk group in both the training, testing groups, and all groups, respectively (Fig. 3). As shown in the Fig. 4, the risk curves reflect the relationship between riskscore and survival status in LUAD patients, and we found that mortality was higher in high-

risk patients than in low-risk patients. Heatmap shows high and low risk levels for 16 lncRNAs, for example, LINC00205, LINC00592, AL162632.3S are high risk lncRNAs, AC026355.2, LINC02848, ZNF571-AS1 are low risk lncRNAs.

3. Independent analysis of prognostic factors

Univariate Cox regression and multivariate Cox regression analyses were used to determine whether the signature we constructed could be used as independent prognostic factors, independent of other clinical characteristics. Multivariate Cox regression results showed that stage (HR = 1.553, 1.342–1.798, $P < 0.05$) and risk score (HR = 1.028, 1.016–1.040, $P < 0.05$) were independently associated with OS, indicating that prognostic signature is an independent prognostic factor for patients with LUAD (Fig. 5a-5b). Next, we used receiver operating characteristic (ROC) curves to assess the predictive accuracy of the risk score. As shown in fig, the area under the ROC curve (AUC) for the riskscore was 0.756, which was better than age (0.536), gender (0.596), and stage (0.712). Similarly, the area under the ROC curve (AUC) for 1, 3 and 5 years were 0.756, 0.739 and 0.759 respectively (Fig. 5c), suggesting that the prognostic signature has good diagnostic significance.

4. Construction of a predictive nomogram and principal component analysis

We constructed a nomogram using the Age, Gender, Stage, T, risk score, N from the signature, and the nomogram can reliably predict the 1-, 3- and 5-years survival of patients(Fig. 6a-6b). Next, we constructed C-Index curves to compare the consistency indices of risk score with other clinical characteristics (Age, Gender, Stage), and we also explored whether there were differences in patient survival over time(Fig. 6c). This suggests that the signature we constructed not only has high predictive accuracy, but can also be used to compare the survival of patients across different periods. Finally, we did principal components analysis (PCA) to observe the distribution of patients for all genes, cuproptosis-related genes, cuproptosis–related lncRNAs, and risk lncRNAs, and the results showed a clear distribution of risk lncRNAs, demonstrating that these lncRNAs can be reliably used to construct the signature(Fig. 7). As shown in the figure, we found that the C-index values of risk scores were higher than those of other clinical characteristics, and the overall survival of patients in the low-risk group in stages I-II and III-IV was significantly better than that of patients in the high-risk group(Fig. 6d-6e).

5. Functional enrichment analysis and immune-related functional analysis

GO results showed that the cuproptosis-related lncRNAs enriched in negative regulation of proteolysis, regulation of pepridase activity, negative regulation of hydrolase activity (Fig. 8a). KEGG analysis showed that these lncRNAs may be related to the Cytokine – cytokine receptor interaction, Neutrophil extracellular trap formation, and MAPK signaling pathways, suggesting these incrnas are involved in the process of tumor development (Fig. 8b). Furthermore, We preformed immune-related functions to analysis the immune status of low-risk group and high-risk group, the results showed that type-III-IFN-response was significantly more active in the low-risk group than in the high-risk group, with no significant differences in other immune functions (Fig. 8c).

6. Tumor mutation burden analysis and drug sensitivity analysis

We used the maftools algorithm to observe mutations in the high and low risk groups and showed that for most genes, the frequency of mutations was higher in the high risk group than in the low risk group (TP53: low risk,43%;high risk 52%. TTN: low risk, 44%; high risk, 47%. MUC16: low risk, 38% ; high risk,42%) (Fig. 9a). Furthermore, we explored whether there was a difference in tumor mutation burden between the high and low risk groups, however, no significant difference was observed ($P = 0.84$) (Fig. 9b), the reasons for which need to be further explored, we then investigated whether there was a difference in survival between patients with high and low TMB. As shown in the Fig. 9c, OS was significantly better in the low TMB than in the high TMB group (P 0.05). In addition, the difference in sensitivity to immunotherapy between patients in the high-risk and low-risk groups was further investigated using the TIDE algorithm. We found higher TIDE in the low risk group compared to the high risk group (Fig. 10a), suggesting that patients in the low risk group had a lower potential for immune escape and that immunotherapy was better. Finally, we used the pRRophetic packages to screen potentially effective anti-tumor drugs, including Masitinib, Tipifarnib, Bexarotene, 5-Fluorouracil, Midostaurin, Vinorelbine, Etoposide, Doxorubicin, and the indication results are shown in the Table 1. We then further analyzed the sensitivity of these drugs and we found that patients in the high risk group had lower IC50 values, representing a higher sensitivity of the drugs in the high risk patients (Fig. 10b-10i).

Table 1
Anti-tumour drugs and indications

Anti-tumors drugs	Indications
Masitinib	Melanoma.
Tipifarnib	lung cancer, lymphoma, pancreatic cancer.
Bexarotene	cutaneous T-cell lymphoma.
5-Fluorouracil	liver cancer, stomach cancer.
Midostaurin	Lung cancer, Haematological tumours.
Vinorelbine	Non-small cell lung cancer, breast cancer, malignant lymphoma.
Etoposide	lung cancer, malignant lymphoma, malignant germ cell tumour.
Doxorubicin	Cholangiocarcinoma.

Disscussion

Lung cancer has the highest incidence and mortality rate among malignant tumors in China, and LUAD, as a type of lung cancer, has a very poor prognosis[23]. Currently available lung cancer screening tools,

such as Low-dose computed tomography (LDCT), are effective in reducing lung cancer mortality, but have a high false positive rate. Therefore, the construction of a reliable lung cancer risk model to accurately determine the prognosis and survival of lung cancer patients is of great significance in the prevention and control of lung cancer[24]. lncRNAs are a class of non-protein-coding RNAs > 200nt in length, accounting for more than 80% of non-coding RNAs[25]. In recent years, it has been shown that lncRNAs play an important regulatory role in lung cancer. A study found that lncRNA KTN1-AS1 acts as a pro-oncogene in NSCLC and can affect the NSCLC cell cycle by regulating CDK1, suggesting that lncRNAs may be a novel lung cancer biomarker and therapeutic target[26]. Cuproptosis as a unique form of cell death has only recently been identified[18–22]. This copper-dependent cell death occurs through the direct binding of copper to lipid acylated components of the tricarboxylic acid cycle (TCA cycle) in mitochondrial respiration, leading to the aggregation of acylated protein and the subsequent downregulation of Iron-sulfur protein, resulting in proteotoxicity and ultimately cell death[27]. Although clinical trials have been conducted with the copper ionophore micromolecule anticancer drug Elesclomol[28], the results have been unsatisfactory. However, few studies have examined the co-regulatory role of cuproptosis and lncRNAs in lung cancer.

In our study, cuproptosis-related lncRNAs were obtained using co-expression of lncRNAs and cuproptosis-related genes, and 16 prognosis cuproptosis-related lncRNAs, including AC016747.2, LINC00205, AC006947.1, LINC00592, AC020634.2, AC026355.2, LINC02848, ZNF571-AS1, CRIM1-DT, SEPSECS-AS1, HIF1A-AS3, AC013267.1, LINC02635, AL162632.3, AC004832.5, AC032011.1 were obtained by univariate and multivariate Cox regression analysis and prognostic signature were constructed. The results of ROC, survival, nomogram and pheatmap showed that the prognostic features of the 16 cuproptosis-related lncRNAs accurately distinguished between high- and low-risk and early- and late-stage patients, and reliably predicted outcomes in LUAD patients as prognostic factors independent of other common clinical characteristics. Of the 16 cuproptosis-related lncRNAs in the LUAD signature, only LINC00205 has been shown to function in cancer, LINC00205 was shown to be an oncogene overexpressed and involved in tumors progression in lung, liver, retinal neuroblastoma and gastric cancers[29–33,34(p185-)]. In hepatocellular carcinoma, LINC00592 was found to be associated with patient prognosis and LINC00592 could target CSDE1, CDK6, miR-122-5p and EPHX1 to promote the proliferation of hepatocellular carcinoma cells[29(p1),30(p6),31,32], Li et al found that LINC00205 targets the oncogene miR-185-5p as a potential therapeutic target in lung cancer, in gastric cancer, LINC00205 promoted the proliferation and migration of gastric cancer cells by inhibiting miR-26a[35], Zhang et al suggested that LINC00205 targeting HMGB1 promoted the proliferation of neuroblastoma and correlated with the overall survival of patients[33], the role of other 15 lncRNAs was first investigated in cancers. Then, the GO enrichment analysis shows the cuproptosis-related lncRNAs were enriched in negative regulation of proteolysis, regulation of pepridase activity, negative regulation of hydrolase activity, KEGG pathway analysis revealed that lncRNAs were mainly enriched in Cytokine – cytokine receptor interaction, Neutrophil extracellular trap formation, and MAPK signaling pathway, this suggests that remodeling of the extracellular environment and accumulation of harmful substances, promotes the growth and expansion

of tumor cells, facilitates the spread of tumor cells to distant tissues and prevents the penetration of immune cells and drugs into tumor cells.

We then analyzed the relationship between immune-related function, tumor mutation burden (TMB) and risk scores in LUAD patients. We found that type-III-IFN-reponse was inhibited in high-risk patients, It is known that IFN is a substance that interferes with virus replication in vitro, and III-IFNs is an essential component of antiviral immunity[36], it is suggested that inhibition of III-IFN-response may be one of the main causes of immune escape and that its activation is essential to maintain immune potency. TMB is often used as a predictive biomarker for immune checkpoint blockade (ICB) in melanoma, lung, and bladder cancers[37–39]. We found a significant decrease in survival in patients with high TMB ($P < 0.05$) and increased TP53, TTN expression in high-risk patients. Studies reported that TP53 is a frequently mutated oncogene in human cancers, affecting the development of breast, lung, bladder, esophageal, prostate, pancreatic and colorectal cancers, and is involved in the normal physiology and metabolism of diabetes, liver and cardiovascular diseases[40–44], and is associated with the survival of patients with many cancers[45]. Besides, an increasing research has demonstrated that TTN participate in the development of multiple cancer types, shen et al found that TTN can target the miR-376a-3p/PUM2 axis and promote the growth of endometrial cancer cells, suggesting that TTN may be a therapeutic target for endometrial cancer[46], fu et al found that TTN acts as a pro-oncogene in osteosarcoma by targeting miR-134-5p and promoting the expression of malignant brain tumor domain containing 1 (MBTD1), which ultimately promotes the growth of osteosarcoma cells[47], xiao et al found that in bladder cancer, reducing TTN expression inhibited the proliferative capacity of BC cells[47]. Our results are consistent with those of previous studies, suggesting that TP53, TTN maybe a target for cancer immunotherapy. Then, using the TIDE algorithm, we found that immunotherapy was less effective in high-risk patients, which is consistent with our previous findings of lower overall survival, higher mortality and suppressed immune function in high-risk patients. Recently, immunotherapy has been influenced by multiple molecular mechanisms as a prevalent tumor treatment, including PD-L1/PD-1, CD24/Siglec-10, EMT signaling, hypoxia-/HIF-1α drivers and C/EBPβ transcription factors[48,49(p1),50–52]. Notably, the role of non-coding RNAs in immunotherapy has been widely demonstrated, zhou et al found that miR-1468-5p could suppress the immune system by targeting lymphatic PD-L1, causing cervical cancer cells to evade treatment[53], in neuroblastoma, the oncogenic factor miR-186 attenuates the efficacy of immunotherapy by inhibiting natural killer (NK) cells[54], zhang et al Identified LncRNA GATA3-AS1 as a potential therapeutic target for breast cancer, promoting immune escape by targeting GATA3[55(p1)]. However, the relationship between lncRNAs and immune-related functions is still not fully understood, and in the future we will evaluate the prognosis of lung cancer patients in terms of immune cell infiltration and explore the role of immune cells in targeting therapy for LUAD patients. Finally, we used the pRRophetic algorithm to screen for effective drugs for tumor immunotherapy and explored the sensitivity of these drugs, which have been used in lung, lymphoma, pancreatic, breast, kidney and bileduct cancers, followed by the observation that high-risk patients are more sensitive to anti-cancer drugs, which is consistent with studies of drugs. However, the drug mechanisms and their impact on LUAD progression need further study.

Conclusion

In our study, we constructed a cuproptosis-related lncRNAs signature for LUAD and analyzed the relationship between risks score-based groups and tumor mutational burden, immunotherapy, and drug sensitivity. Our study provides new insights into the prediction of survival in LUAD patients and the clinical treatment of patients.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data supporting the findings of this study are available from the respective authors upon reasonable request.

Competing interests

The authors declare that the study was conducted without any financial relationships that could be considered as potential conflicts of interest.

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Authors' contributions

WF Wang and CQ Li designed the implementation of the reaserch,drafted preliminary papers,and participated in investigations. WF Wang, QS Su, and LH Sheng participated in research design and implementation,manuscript revision, manuscript submission, and fund acquisition.All authors read and approved the final manuscript.

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Figures

Figure 1

Sankey diagram showed the results of cuproptosis-related genes and cuproptosis-related lncRNA co-expression

Figure 2

Identification of the cuproptosis-related lncRNAs.

(a)LASSO regression of prognostic cuproptosis-related lncRNAs.

(b)LASSO regression screened of cuproptosis-related lncRNAs at the minimum point of cross-validation.

(c) Forest plot showed different lncRNAs for high and low risk, with red representing high risk lncRNAs and green representing low risk lncRNAs.

(d)Correlation heatmap showed the relationship between cuproptosis-related lncRNAs and cuproptosis-related genes for the signature. Red represents positive correlations, blue represents negative correlations.

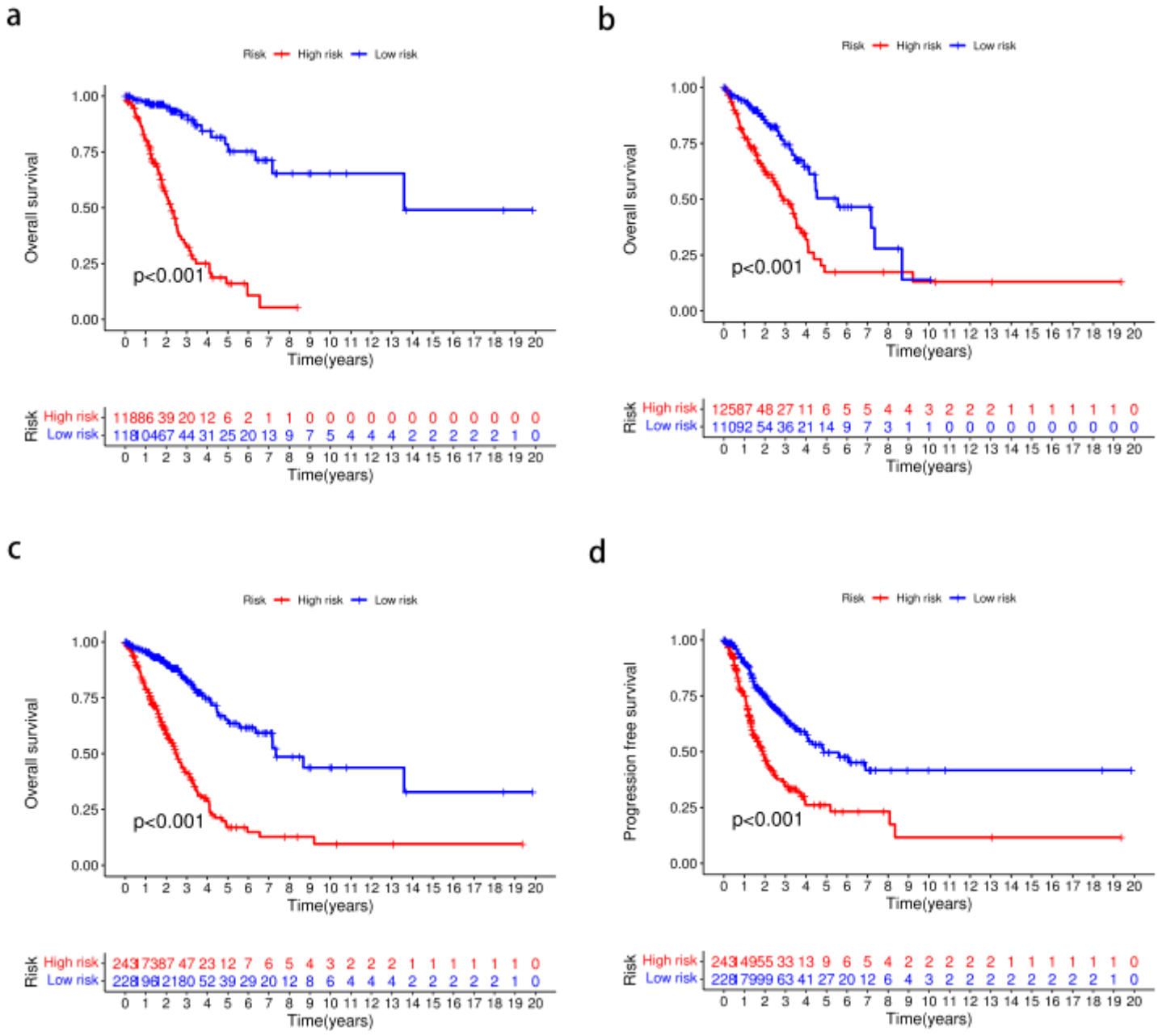

Figure 3

Kaplan–Meier survival analyses of patients.

OS of patients in (a)high-risk, (b) low-risk, (c)all groups, respectively.

(d)PFS of patiens in all groups .

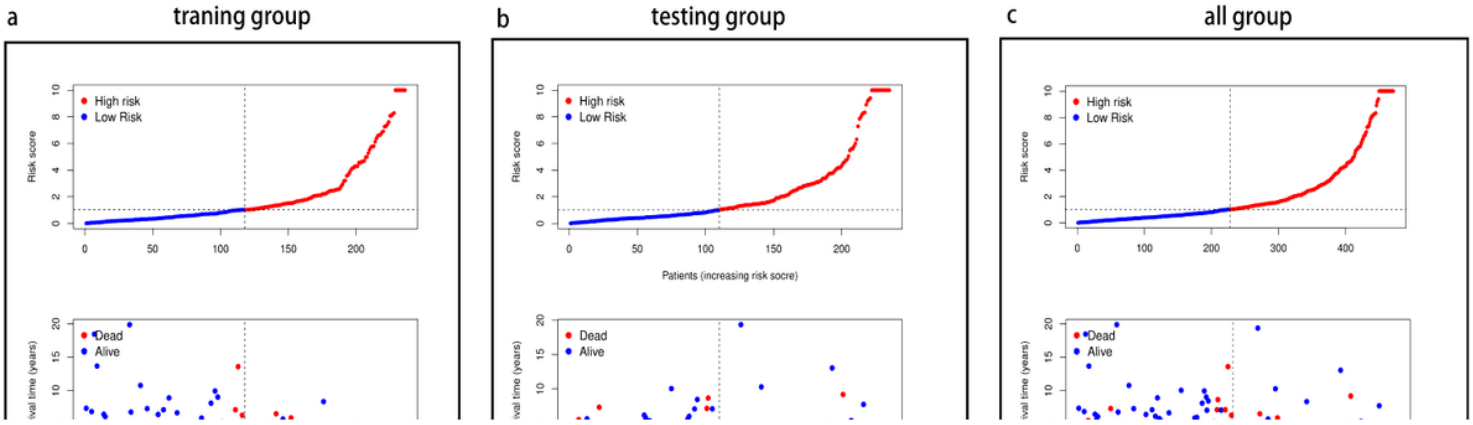

Figure 4

Predicting the performance of characteristics.

Risk curves represented the distributed survival status of LUAD patients with different risk scores; heatmap represented the characteristics of lncRNAs in the (a) training group. (b) testing group. (c) all group.

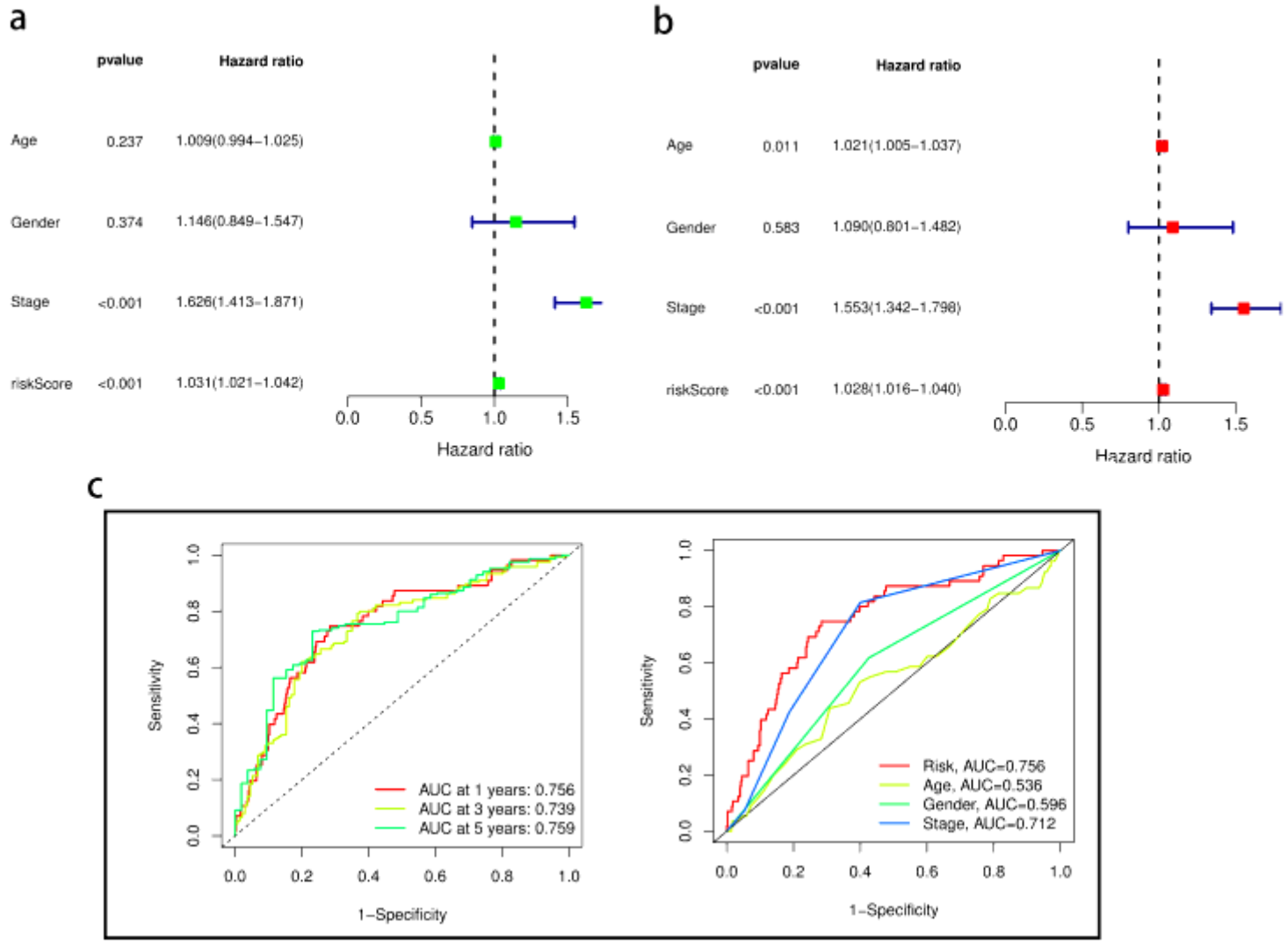

Figure 5

The prognostic value of the signature for LUAD.

(a) Univariate (b) multivariate independent prognostic analysis to analyze that the risk score was independently associated with OS.

(c) ROC curve analysis was performed on the accuracy of the model predictions.

Figure 6

Nomogram and clinical subgroups for predicting LUAD outcomes.

(a) Prognostic nomogram to predict the OS in LUAD.

(b) Calibration curves for 1-, 3-, 5-year.

(c) C-Index curve analyzed the concordance index of the risk score.

Patients were grouped to see if the model was applicable to LUAD patients at (d) Stage I and II (e) Stage III and IV stages.

Figure 7

PCA analysis.

PCA analysis observed the distribution of patients according to (a) all genes. (b) Genes associated with cupulocytosis. (c) lncRNAs associated with cupulosis. (d) Risk lncRNAs.

Figure 8

Functional enrichment analysis and immune-related functional analysis.

(a)GO enrichment analyses of the 16 cuproptosis-related lncRNAs.

(b)KEGG enrichment analyses of the 16 cuproptosis-related lncRNAs. (c)Immune-related functions of the 16 cuproptosis-related lncRNAs.

Figure 9

The relationship between TMB and the signature.

(a) Waterfall plot revealed the top 15 mutation genes in LUAD for the high-risk, low-risk groups.

(b) Differential TMB in high-risk and low-risk groups in LUAD.

(c) Survival curves for the high TMB and low TMB groups in LUAD and a combined TMB-risk survival curve.

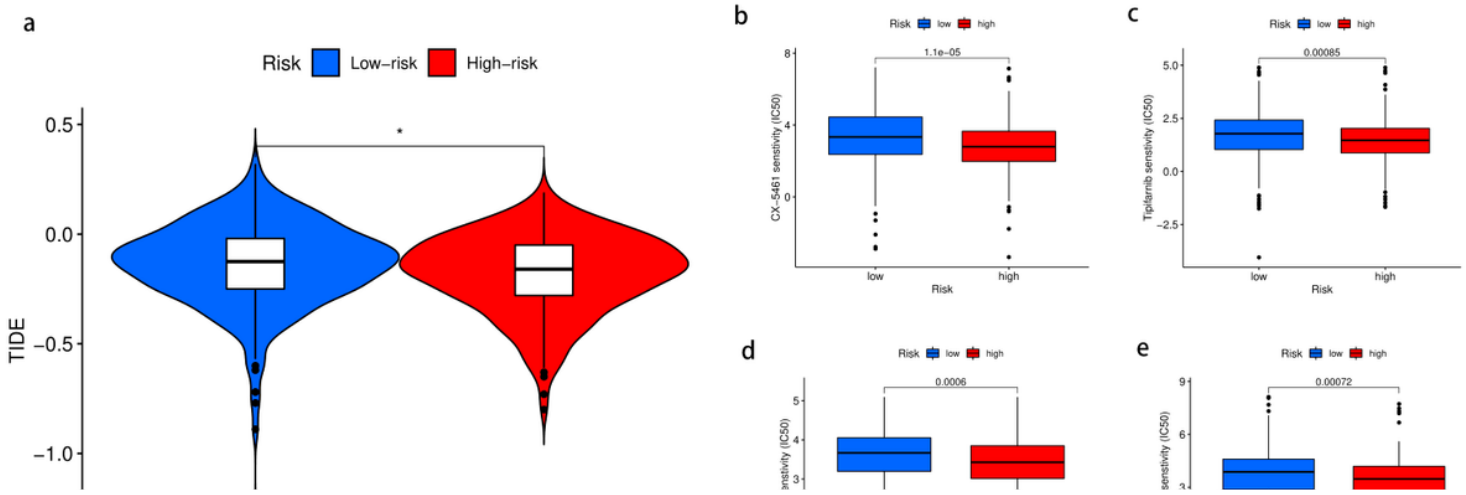

Figure 10

Immunotherapy and drug sensitivity.

(a)Different TIDEs in the high-risk and low-risk groups, *represents statistically significant differences.

Observed the drug sensitivity of (b) CX-5461 (c) Tipifarnib (d) Bexarotene (e) 5-Fluorouracil (f) Midostaurin (g) Vinorelbine (h) Etoposide (i) Doxorubicin.