Original Article

Comprehensive analysis of cuproptosis-related lncRNAs in the prognosis and therapy response of patients with bladder cancer

Ding Li[1,2,3#^], Xuan Wu[4#], Xinxin Fan[5#], Cheng Cheng[4], Dongbei Li[4], Wenzhou Zhang[1,2,3]

[1]Department of Pharmacy, The Affiliated Cancer Hospital of Zhengzhou University and Henan Cancer Hospital, Zhengzhou, China; [2]Henan Engineering Research Center for Tumor Precision Medicine and Comprehensive Evaluation, Henan Cancer Hospital, Zhengzhou, China; [3]Henan Provincial Key Laboratory of Anticancer Drug Research, Henan Cancer Hospital, Zhengzhou, China; [4]Department of Internal Medicine, The Affiliated Cancer Hospital of Zhengzhou University and Henan Cancer Hospital, Zhengzhou, China; [5]Department of Hematology, Zhengzhou Third People's Hospital, Zhengzhou, China

Contributions: (I) Conception and design: X Wu, Ding Li, W Zhang; (II) Administrative support: W Zhang; (III) Provision of study materials or patients: X Wu; (IV) Collection and assembly of data: Ding Li, X Wu; (V) Data analysis and interpretation: Ding Li, X Fan, C Cheng, Dongbei Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Wenzhou Zhang. Department of Pharmacy, The Affiliated Cancer Hospital of Zhengzhou University and Henan Cancer Hospital, Zhengzhou 450008, China. Email: hnzzzwzx@sina.com.

Background: Cuproptosis is the recently defined regulatory cell death (RCD) that plays essential roles in tumorigenesis and progression. Long noncoding RNAs (lncRNAs) regulate the gene expression through various means. However, the clinical value of cuproptosis-related lncRNAs in bladder cancer (BLCA) remains poorly described.

Methods: We downloaded the transcriptome sequencing data and clinical information from The Cancer Genome Atlas (TCGA) database. Univariate, multivariate, and lasso Cox regression analyses were performed to construct the prognostic risk signature, the predictive accuracy of which was validated in the subsequent independence and stratification analyses. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were used to explore the underlying molecular mechanisms involved in the signature to explore therapeutic vulnerabilities and potential targets in BLCA. Tumor mutational burden (TMB) and tumor immune dysfunction and exclusion (TIDE) were used to estimate the response to immune checkpoint inhibitors (ICIs). We further explored the potential new drug-target candidates based on the half maximal inhibitory concentration for this patient population.

Results: Fifteen cuproptosis-related lncRNAs significantly associated with survival were identified to construct the risk signature based on the normalized expression level and regression coefficient of each gene. The patients with BLCA and high-risk scores defined by the signature were associated with worse survival outcomes. The differentially expressed genes (DEGs) between the 2 risk groups had different biological activity. Furthermore, the patients in the low-risk group exhibited a higher TMB index and a lower TIDE score. The sensitivity of multiple antitumor drugs was negatively related to risk score, including AR-42, AS605240, FK866, TAK-715, and tubastatin A, while the sensitivity of some antitumor drugs, such as AMG-706, BX-795, and RO-3306, were positively correlated with risk score.

Conclusions: Our study established and verified a novel clinical risk signature with cuproptosis-related lncRNAs that may predict therapy response and prognosis with robust and stable accuracy in patients with BLCA and enhance the personalized management of this patient population.

Keywords: Bladder cancer (BLCA); cuproptosis; long noncoding RNAs (lncRNA); risk signature; drug sensitivity

^ ORCID: 0000-0002-0967-7021.

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Introduction

Bladder cancer (BLCA), with about 81,180 new cases detected in the United States in 2022, is one of the most prevalent types of cancer (1). The main therapeutic strategies for BLCA have traditionally included surgery, radiotherapy, and chemotherapy, with few advances in treatment in BLCA being achieved over the past three decades. However, immunotherapy has brought new hope over the past several years (2). Immunotherapeutic drugs represented by immune checkpoint inhibitors (ICIs) have become the first-line or second-line therapies for BLCA, and have significantly improved the prognosis of patients with BLCA (3). Although new diagnostic techniques and therapeutic strategies are constantly improving, the prognosis for patients with BLCA remains suboptimal (4). Therefore, it is essential to discover more accurate prediction methods for the finer stratification of patients to yield optimal treatment outcomes.

Copper (Cu) is an indispensable trace element existed in all living organisms, which in excessive concentrations produces cytotoxicity and promote tumor proliferation, angiogenesis, and metastasis (5). A previous study showed that Cu-related ambient air pollution was associated with the incidence of BLCA (6). The elevation of plasma Cu levels was also found to promote vascular endothelial growth factor and hypoxia-inducible factor 1 expression in BLCA tissues (7). Moreover, Cu has been reported to be related to the prognosis of BLCA (8). Cuproptosis is a recently discovered regulatory cell death (RCD) different from apoptosis, necroptosis, pyroptosis, and ferroptosis (9). Escaping cell death is one of the hallmarks of cancer (10). Apoptosis has been widely studied over the past three decades due to its roles in cancer defense and has been leveraged in the development of targeted anticancer drugs. However, the therapeutic effect of related drugs has not been ideal because of the endogenous or acquired apoptosis resistance in cancer cells (11). Therefore, it is necessary to explore the nonapoptotic cell death pathway. Cuproptosis is a metal-related RCD like ferroptosis and has the potential to be a promising target in cancer treatment. However, the role of cuproptosis in cancer has not been extensively studied.

Long noncoding RNAs (lncRNAs) with a length of more than 200 nucleotides have no protein coding ability but play major roles in transcriptional regulation, messenger RNA (mRNA) processing regulation, and posttranscriptional regulation, tumorigenesis, and progression (12,13). Accumulating evidence indicates that lncRNAs are involved in immunotherapy response and associated with the prognosis of patients with BLCA (14). However, the clinical value of cuproptosis-related lncRNAs in BLCA remains poorly described, and further in-depth research is needed.

We thus conducted a comprehensive analysis of cuproptosis-related lncRNAs associated with the prognosis and therapy response of patients with BLCA; following this, we constructed a prognostic risk signature, which might act as an independent prognostic factor and highlight the potential therapeutic targets for this malignancy. Our study further included an investigation of the underlying molecular mechanisms, survival stratification, nomogram construction, identification of somatic mutations, and exploration of potential small-molecule drugs based on the risk characteristics. We expect our findings to offer new insights into the personalized management of patients with BLCA. We present the following article in accordance with

Highlight box

Key findings

- We conducted a comprehensive analysis on the clinical value of cuproptosis-related long noncoding RNAs (lncRNAs) in bladder cancer (BLCA) and developed a risk signature to predict the prognosis and therapy response of BLCA patients.

What is known and what is new?

- Cuproptosis is the recently defined regulatory cell death that plays essential roles in tumorigenesis and progression. LncRNAs regulate the gene expression through various means. However, the clinical value of cuproptosis-related lncRNAs in BLCA remains poorly described. Here, we established and verified a novel clinical risk signature with cuproptosis-related lncRNAs in BLCA.

What is the implication, and what should change now?

- This study indicated the important role of cuproptosis-related lncRNAs in BLCA prognosis and immunity and may provide the potential of cuproptosis-related lncRNA as a therapeutic target for BLCA. However, more biological experiments *in vivo* or *in vitro* and clinical trials are needed to validate the conclusion.

Table 1 The baseline characteristics of patients with BLCA in the training and testing data sets

Covariates	Total, n (%)	Data sets, n (%)		P value
		Testing	Training	
Age (years)				0.854
≤65	158 (40.1)	46 (38.98)	112 (40.58)	
>65	236 (59.9)	72 (61.02)	164 (59.42)	
Gender				0.3606
Female	103 (26.14)	35 (29.66)	68 (24.64)	
Male	291 (73.86)	83 (70.34)	208 (75.36)	
Grade				1
Low	373 (94.67)	112 (94.92)	261 (94.57)	
High	18 (4.57)	5 (4.24)	13 (4.71)	
Unknown	3 (0.76)	1 (0.85)	2 (0.72)	
Tumor stage				0.2126
I	2 (0.51)	0 (0)	2 (0.72)	
II	123 (31.22)	37 (31.36)	86 (31.16)	
III	138 (35.03)	49 (41.53)	89 (32.25)	
IV	129 (32.74)	32 (27.12)	97 (35.14)	
Unknown	2 (0.51)	0 (0)	2 (0.72)	
Stage				
T				0.5853
T0	1 (0.25)	1 (0.85)	0 (0)	
T1	3 (0.76)	1 (0.85)	2 (0.72)	
T2	112 (28.43)	32 (27.12)	80 (28.99)	
T3	190 (48.22)	59 (50.0)	131 (47.46)	
T4	56 (14.21)	15 (12.71)	41 (14.86)	
Unknown	32 (8.12)	10 (8.47)	22 (7.97)	
M				1
M0	188 (47.72)	57 (48.31)	131 (47.46)	
M1	10 (2.54)	3 (2.54)	7 (2.54)	
Unknown	196 (49.75)	58 (49.15)	138 (50.0)	
N				0.4815
N0	228 (57.87)	74 (62.71)	154 (55.8)	
N1	44 (11.17)	11 (9.32)	33 (11.96)	
N2	75 (19.04)	19 (16.1)	56 (20.29)	
N3	6 (1.52)	1 (0.85)	5 (1.81)	
Unknown	41 (10.41)	13 (11.02)	28 (10.14)	

BLCA, bladder cancer.

the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5294/rc).

Methods

Data collection

The transcriptome data and relevant clinical information of patients with BLCA were acquired from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/repository). A total of 394 patients with BLCA and 19 health controls with overall survival (OS) more than 30 days and information on life status (alive or dead) were enrolled through May 16, 2022. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of cuproptosis-related lncRNAs

Nineteen cuproptosis-related genes, including *NFE2L2*, *NLRP3*, *ATP7A*, *ATP7B*, *SLC31A1*, *LIAS*, *FDX1*, *LIPT1*, *LIPT2*, *DLD*, *PDHA1*, *DLAT*, *MTF1*, *PDHB*, *GLS*, *CDKN2A*, *GCSH*, *DBT*, and *DLST*, were extracted from the previously published study (9). Differentially expressed gene (DEG) analysis between the patients with BLCA and healthy controls was carried out with the "limma" package in R (The R Foundation for Statistical Computing, Lanzhou, China). Pearson test was used to evaluate the correlation between differentially expressed lncRNAs and cuproptosis-related DEGs. Pearson correlation coefficient was used to determine the cuproptosis-related lncRNAs (cor >0.4; P<0.05).

Construction and validation of the prognostic risk signature

The included 394 BLCA samples were further randomly assigned into training or testing data sets in a 7:3 ratio. As shown in *Table 1*, the baseline characteristics of patients with BLCA did not indicate any significant differences between the training and testing data sets. Univariate analysis of the training data set was applied to obtain the lncRNAs significantly related to BLCA prognosis (P<0.05) and determine the hazard ratio (HR) value. A total of 95 lncRNAs were identified as being significantly associated with the prognosis of patients with BLCA. Subsequently, lasso Cox regression analysis was conducted with the cuproptosis-related lncRNAs to avoid overfitting, and the signature lncRNAs were further identified through the

"glmnet" R package. The risk score of each patient was then determined according to the normalized expression level of each signature lncRNA and its regression coefficient according to the following formula: risk score = sum (normalized lncRNA expression level × relevant regression coefficient).

Following this, the participants in the training data set were classified into a high-risk group or a low-risk group based on the median value of the risk score. Subsequently, Kaplan-Meier analysis with log-rank test was performed to compare the survival status of the 2 risk groups. The time-dependent receiver operating characteristic (ROC) curves were drawn with the "timeROC" R package to evaluate the predictive power of the risk signature. In addition, the median risk score defined in the training data set was subsequently taken as the cutoff value of the testing data set and that of the entire patient group, and the same prognostic analysis was used to verify the availability of the signature.

Construction and evaluation of prognostic nomogram

The correlation between prognosis and risk score as well as with traditional clinical variables was assessed by univariate analysis. Risk factors for prognosis were further evaluated by multivariate Cox regression analysis. To further validate the clinical value of the risk signature and conduct a visual quantitative analysis, we constructed a prognostic nomogram by using the "RMS" R package based on the age, clinical stage, grade, T stage, gender, and risk score. ROC curves were drawn to assess the utility of the prognostic nomogram with the "ROC survival" R package.

Biological process and pathway analysis

To explore the potential drug targets, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were used to evaluate the underlying molecular mechanisms based on the DEGs between the 2 risk groups through use of the "clusterProfiler" R package.

Mutation characteristic analysis

Based on somatic mutation data downloaded from TCGA database, a waterfall map was used to present the mutation landscapes in the 2 risk groups with the "maftools" R package. We performed the mutation characteristic analysis with tumor mutational burden (TMB) determined as the quantity of mutations per megabase of DNA. The "limma" R package was used to evaluate the mutation characteristics, and the "ggpubr" R package was used to perform survival analysis to compare the survival difference involved in the TMB score and risk score.

Screening of potential drugs

We evaluated the response of the small-molecule drugs for patients with BLCA based on the half maximal inhibitory concentration (IC_{50}) calculated by using the "pRRophetic" R package based on the Genomics of Drug Sensitivity in Cancer (GDSC) database. The "pRRophetic" R package is a drug sensitivity prediction tool that has been validated in multiple independent clinical trials (15).

Statistical analysis

We performed all the statistical analysis in R version 4.1.3. Survival analysis was conducted by Kaplan-Meier curves analysis. The prognostic independence was assessed by univariate and multivariate Cox regression analyses. ROC curve analysis was implemented to evaluate the sensitivity and reliability of the risk signature. A $P<0.05$ value indicated the statistical significance.

Results

Construction of the prognostic cuproptosis-related lncRNA signature

The flowchart of this study is displayed in *Figure 1*. The expression data of 19 cuproptosis-related mRNAs and 16,876 lncRNAs were obtained from the RNA-sequencing (RNA-seq) profiles of the patients with BLCA. A total of 956 cuproptosis-related lncRNAs were identified based on the coexpression correlation analysis (cor >0.4; $P<0.05$). A Sankey diagram indicated the close relationship between cuproptosis-related genes and lncRNAs. Among the 19 cuproptosis-related genes, *ATP7A* was associated with the largest number of lncRNAs (*Figure 2A*). Based on the lasso COX regression analysis, our prognosis-related signature containing 15 cuproptosis-related lncRNAs were finally identified (*Figure 2B,2C*). Among them, AL031429.2, AL390236.1, AC007365.1, LINC01184, LINC02159, RAP2C-AS1, and AL731537.1 appear to be detrimental factors, with HRs >1; meanwhile, MIR181A2HG, AC010186.3, LINC-PINT, BDNF-AS, LINC02443,

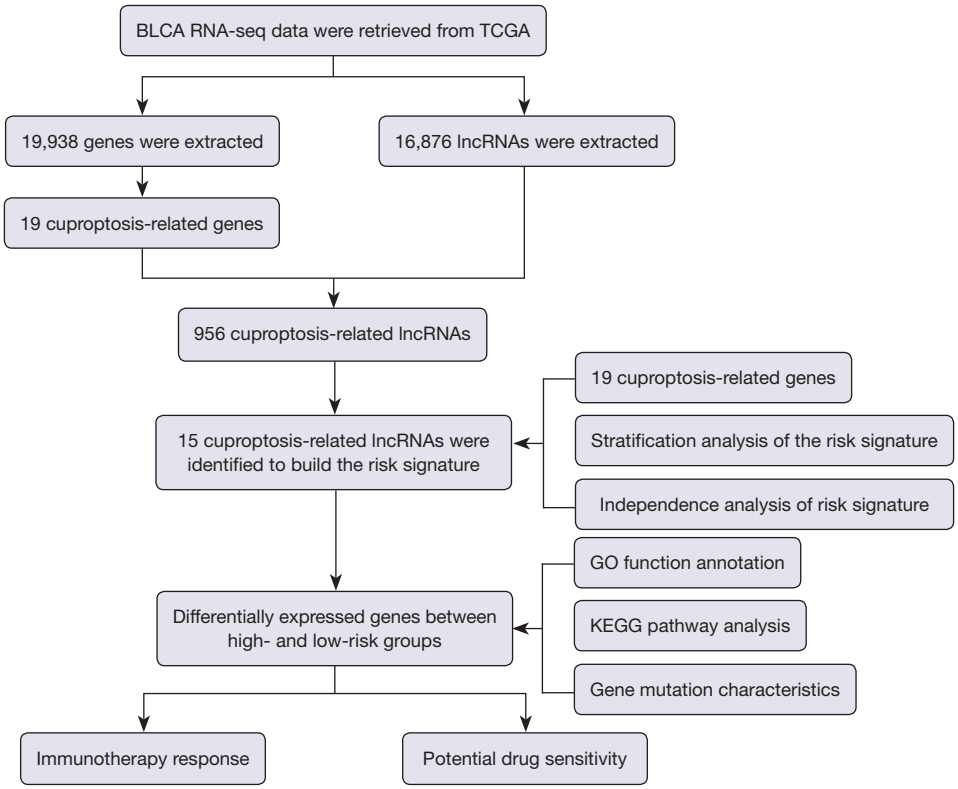

Figure 1 The flowchart of this study. BLCA, bladder cancer; TCGA, The Cancer Genome Atlas; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

AC006160.1, AC005954.1, and AC090948.1 appear to be protective factors, with HRs <1 (*Figure 2D*).

Validation of the prognostic cuproptosis-related lncRNA signature

The samples with risk scores calculated by the above-mentioned formula were assigned into a high-risk group or low-risk group based on median risk score in the training, testing, and total data sets. In the training data set, the patients with high-risk scores had a higher mortality rate than the patients with low-risk scores (*Figure 3A,3B*). Moreover, the detrimental factors, AL031429.2, AL390236.1, AC007365.1, LINC01184, LINC02159, RAP2C-AS1, and AL731537.1, had a higher expression level in the high-risk group, whereas the protective factors, MIR181A2HG, AC010186.3, LINC-PINT, BDNF-AS, LINC02443, AC006160.1, AC005954.1, and AC090948.1, showed lower expression levels (*Figure 3C*). In addition, Kaplan-Meier curves also demonstrated that the high-risk group had significantly worse outcomes when compared to the low-risk group (*Figure 3D*). The area under the curves (AUCs) of the time-dependent ROC in predicting the 1-, 3-, and 5-year survival rates of patients with BLCA were 0.787, 0.801, and 0.797, respectively (*Figure 3E*). Additionally, the results in the testing and total data sets were consistent with those in the training data set (*Figure 4A-4E*). Taken together, the signature displayed a robust and stable prognostic ability.

Independent prognostic and clinicopathological correlation analyses of the cuproptosis-related lncRNA signature

Univariate (*Figure 5A*) and multivariate Cox (*Figure 5B*) regression analyses showed that risk score, age, and tumor stage were the independent prognostic factors. We subsequently performed prognostic analysis in early-stage (stage I–II) and advanced-stage (stage III–IV) BLCA patients to verify whether the predictive performance of the signature is affected by the stage. The results revealed that the high-risk group had worse outcomes at both the early and advanced stage (Figure S1A,S1B), which suggested that

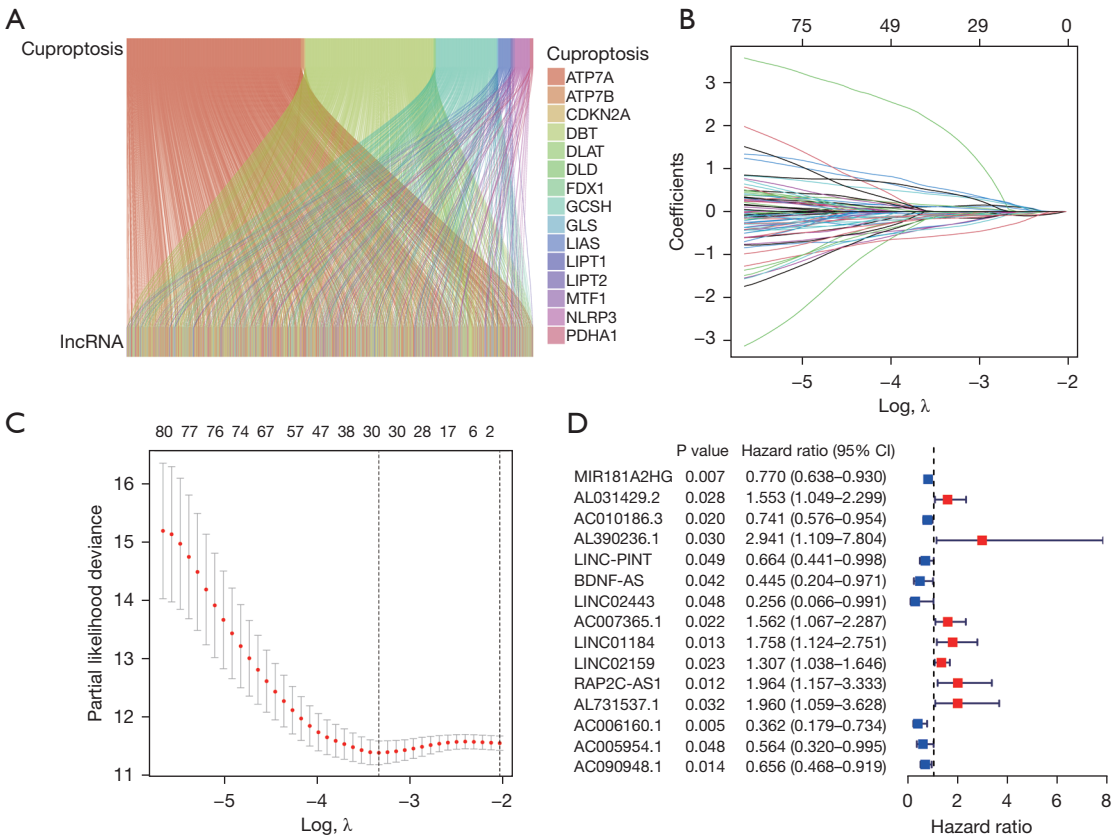

Figure 2 Construction of the cuproptosis-related lncRNA signature. (A) The correlation between cuproptosis-related DEGs and differentially expressed lncRNAs. (B,C) Lasso regression was performed to calculate the (B) coefficients and (C) minimum criteria. (D) Forest plot of the univariate Cox regression analysis for the 15 lncRNAs. DEG, differentially expressed gene.

the signature possessed a stable predictive ability in variable BLCA subgroups.

Furthermore, to quantitatively evaluate the survival probability, we constructed a nomogram with traditional clinical variables and the risk score. Calibration curves of 1-, 3-, and 5-year survival probabilities were used to verify the predictive probability of the nomogram, which was consistent with the actual one (*Figure 5C,5D*). The proposed nomogram demonstrated a better prognostic performance than did the other existing clinical variables.

The AUC value for predicting the prognosis of patients with BLCA was 0.757, which was higher than that of tumor stage (AUC =0.637) and age (AUC =0.667; *Figure 5E*). The concordance index (C-index) analysis supported the accuracy of the signature (*Figure 5F*). Our findings collectively indicate that this signature has a favorable performance in predicting the prognosis of BLCA.

Enrichment analysis of DEGs in the low-risk group and high-risk group

To explore the biological pathways involved in the risk signature, we performed GO enrichment and KEGG pathway analyses with the DEGs between the 2 risk groups. The main biological processes (BPs) involved in response to muscle system process, muscle contraction, epidermis development, connective tissue development, external encapsulating structure organization, extracellular matrix organization, cartilage development, and skin development (*Figure 6A*). The most abundant cellular component (CC) terms were the collagen-containing extracellular matrix, intermediate filament cytoskeleton, contractile fiber, myofibril, cornified envelope, and anchored component of membrane (*Figure 6B*). The most abundant molecule function (MF) terms were receptor ligand activity, sulfur

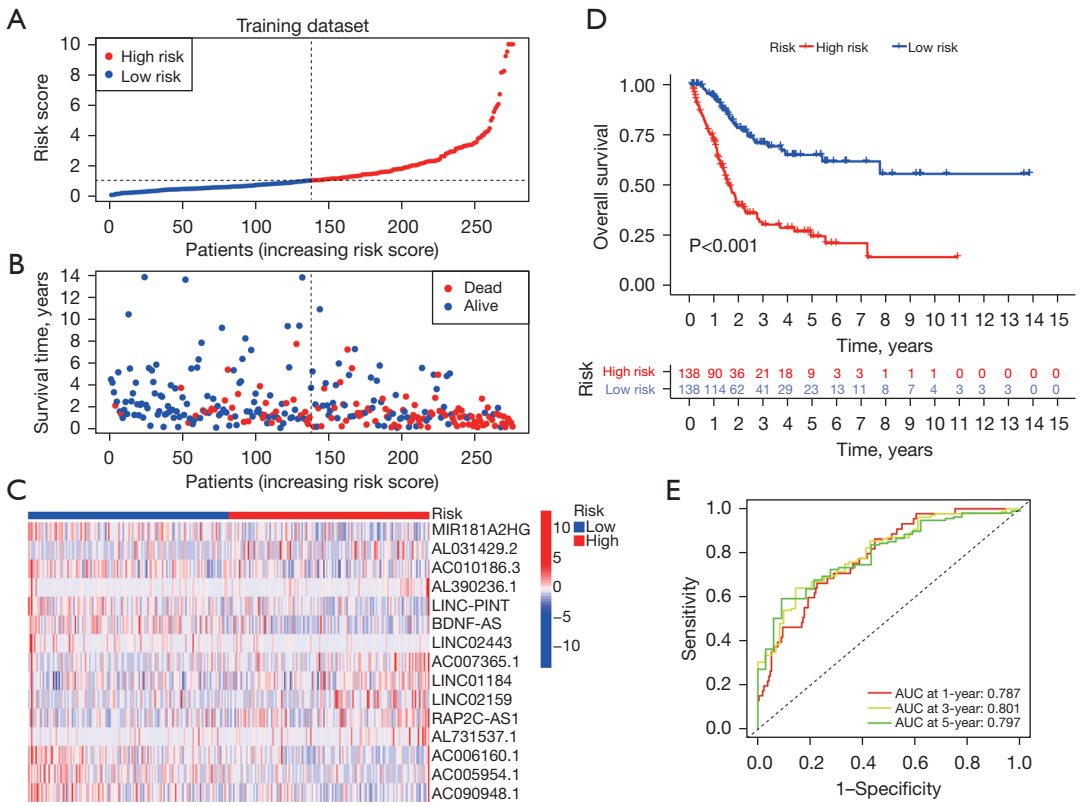

Figure 3 Validation of the prognostic cuproptosis-related lncRNA signature. (A) Distribution of the risk score in the training data set. (B) Survival status of patients in the 2 risk groups. (C) The 15-lncRNA expression heatmap in the 2 risk groups. (D) Kaplan-Meier survival analysis between the 2 risk groups in the training data set. (E) The AUC values for 1-, 3-, and 5-year overall survival prediction in the training data set. AUC, area under the curve.

compound binding, glycosaminoglycan binding, heparin binding, structural constituent of cytoskeleton, and serine-type peptidase activity (*Figure 6C*). KEGG analysis showed that the DEGs were involved in various signaling pathways, most commonly in pathways of the muscle system and epidermis development (*Figure 6D*).

Comparison of mutation characteristics related to the signature

A high TMB score predicts stronger tumorigenesis and more positive immunotherapy response (16). We studied the genomic mutations status between the 2 risk groups based on single-nucleotide variation data downloaded from TCGA. The waterfall map showed that the high-risk (*Figure 7A*) and low-risk (*Figure 7B*) groups had different mutation characteristics. The TMB score was then calculated, and the result showed that the TMB score in the low-risk group was significantly higher than that in the high-risk group (*Figure 7C*). Kaplan-Meier curve analyses revealed there to be no significant difference in the survival time between the high-TMB score and low-TMB score groups. However, after the TMB score was combined with our signature, the patients in the high-risk and low-TMB group had significantly worse outcomes than did those in the low-risk and high-TMB group. Combining TMB level and risk score may more accurately forecast the prognosis of patients with BLCA (*Figure 7D*,*7E*). Most notably, the TMB and risk score of the signature demonstrated good predictive power.

Evaluation of immune microenvironment based on the signature

Considering the clinical application and benefits of ICIs, we next analyzed the immune cells infiltration and immune checkpoints. The results demonstrated the infiltration of

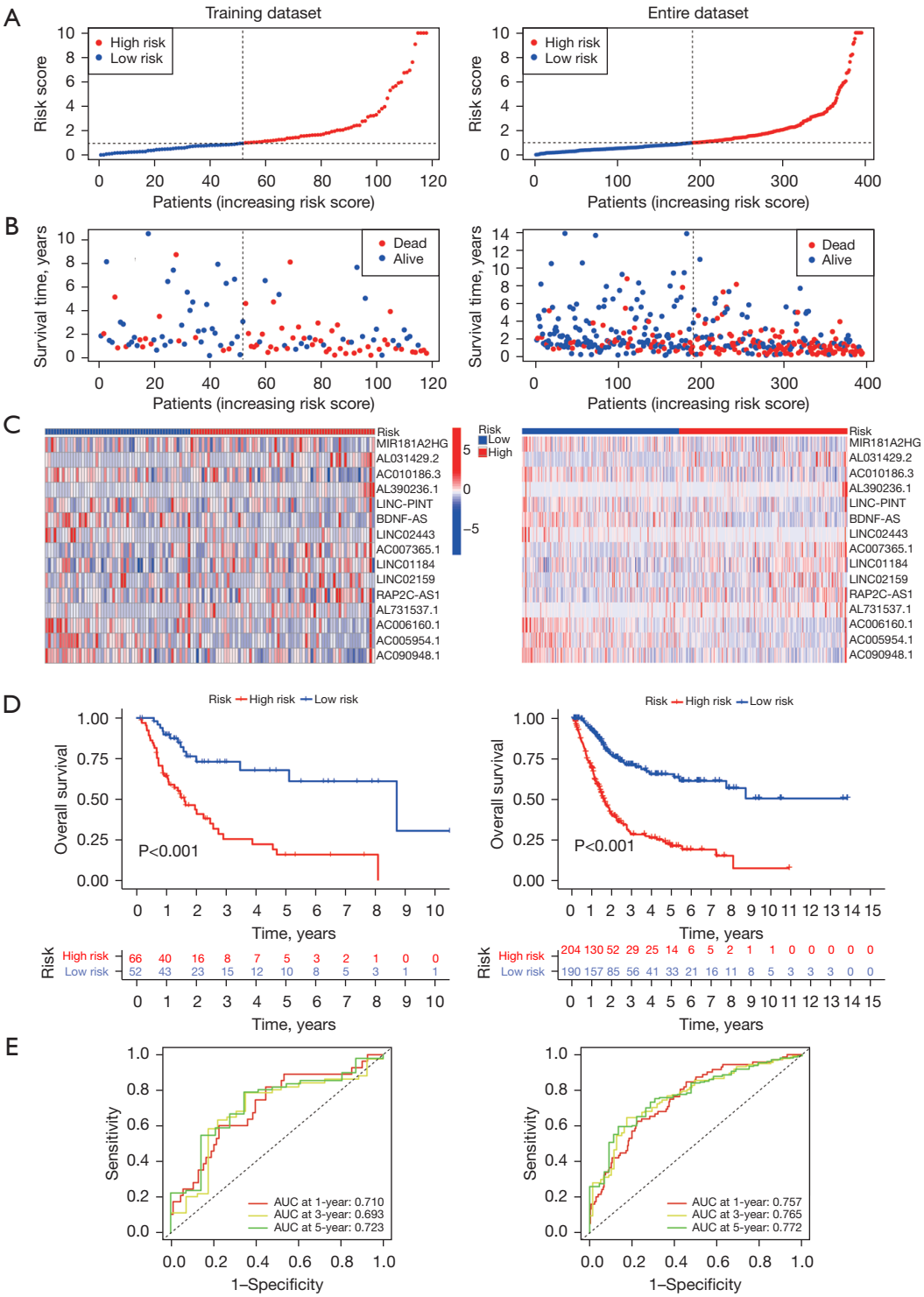

Figure 4 Validation of the prognostic cuproptosis-related lncRNA signature in the testing and total data sets. (A) Distribution of the risk score in the testing (left) and total (right) data sets. (B) Survival status of the patients in the testing (left) and total (right) data sets. (C) The 15-lncRNA expression heatmap in the 2 risk groups in the testing (left) and total (right) data sets. (D) Survival analysis for the signature in the testing (left) and total (right) data sets. (E) AUC values of 1-, 3-, and 5-year overall survival prediction in the testing (left) and total (right) data sets. AUC, area under the curve.

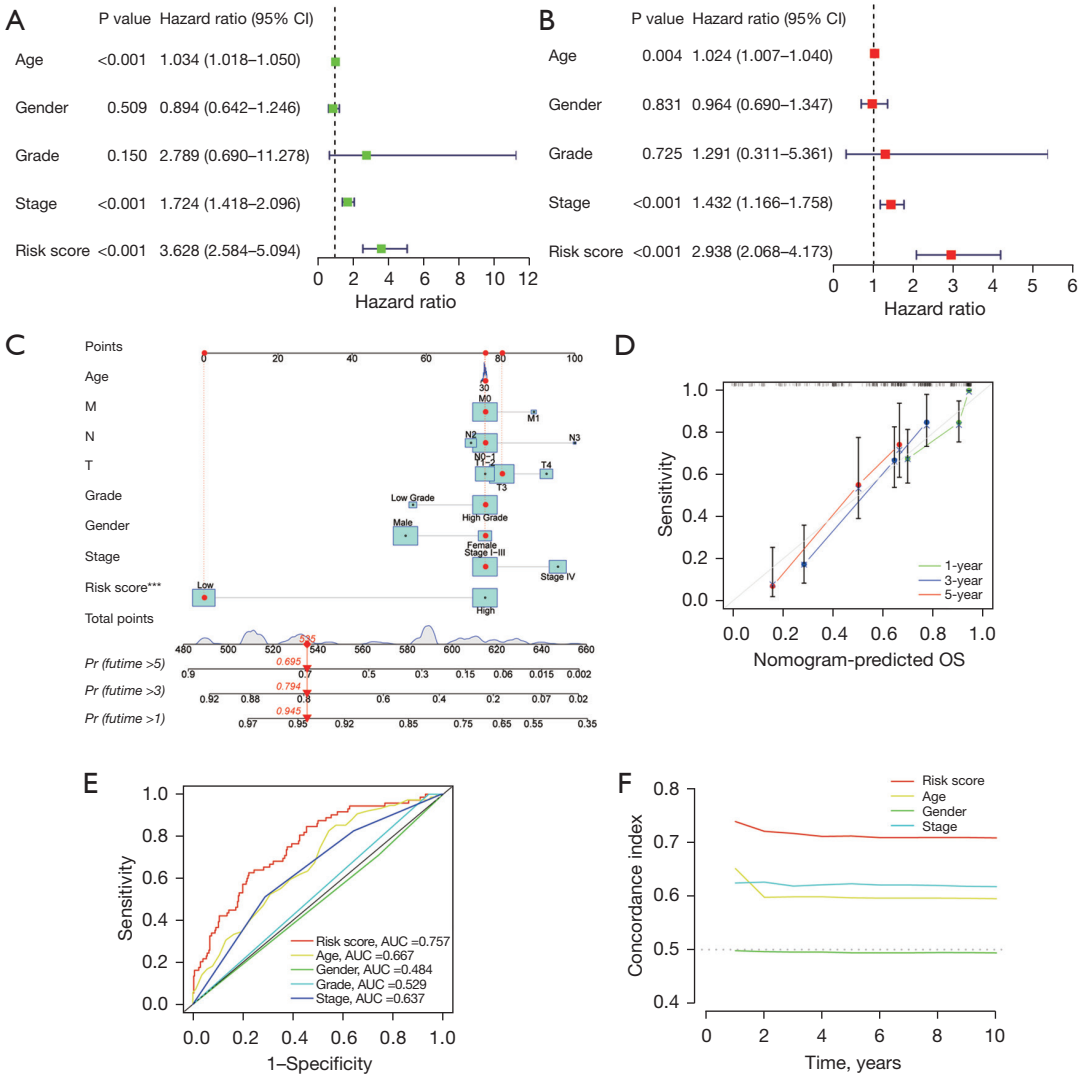

Figure 5 Independent prognostic and clinicopathological correlation analyses of the cuproptosis-related lncRNA signature. (A,B) Univariate (A) and multivariate (B) Cox regression analysis of the risk score and clinical variables. (C) A nomogram for survival prediction based on traditional clinical variables and risk score. (D) Calibration curves for the validation of nomogram. (E) AUC values of the risk score and clinical variables. (F) C-index analysis indicated accuracy of the risk score and traditional clinical variables. ***, P<0.001. OS, overall survival; AUC, area under the curve; Pr, probability.

the T cell regulatory (Tregs) significantly elevated in the low-risk group (*Figure 8A*). Besides, immune checkpoints such as TNFRSF9, PDCD1LG2 (PD-L2), CD276, CD44, and NRP1 were positively associated with the risk scores. Meanwhile, LGALS9, TNFRSF14, TNFRSF15, TNFRSF25, TMIGD2, and CD160 were negatively associated with the risk scores (*Figure 8B*). We further scored the tumor immune dysfunction and exclusion (TIDE), and the results indicated that TIDE score was higher in the high-risk group (*Figure 8C*), which means that the high-risk group had a stronger immune escape ability.

Evaluation of antitumor drug sensitivity based on the signature

Current drugs are not ideal for the treatment of patients with BLCA. To explore potentially effective drugs, we evaluated the response of the small-molecule drugs for

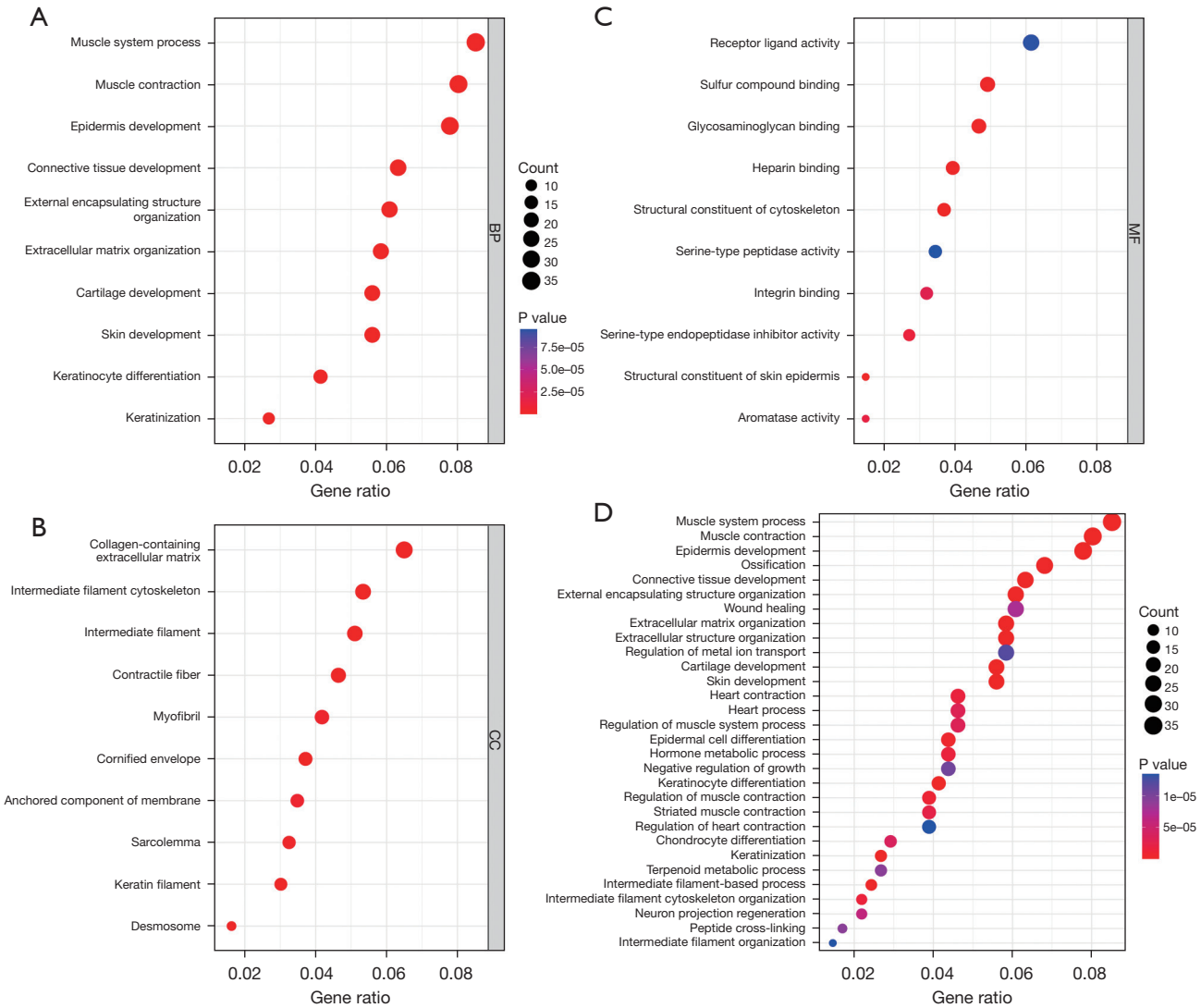

Figure 6 Enrichment analysis of DEGs in the low-risk group and high-risk group. (A) Top 10 most-enriched biological processes. (B) Top 10 most-enriched cellular components. (C) Top 10 most-enriched molecular functions. (D) Top 30 KEGG pathways. BP, biological process; MF, molecule function; CC, cellular component; DEG, differentially expressed gene; KEGG, Kyoto Encyclopedia of Genes and Genomes.

patients with BLCA based on IC_{50} calculated by using the "pRRophetic" R package through the GDSC database. The IC_{50} is the concentration of drug required for 50% inhibition, which is an indicator of drug sensitivity. The evaluation of IC_{50} in multiple antitumor drugs yielded a risk score that was correlated with the IC_{50} value of multiple drugs, with positive correlations being found with AR-42, AS605240, FK866, TAK-715, and tubastatin A, and negative correlations with AMG-706, BX-795, and RO-3306 (*Figure 9A-9H*).

Discussion

Although most of the conventional anticancer drugs target key aspects of cells that are often shared by all rapidly proliferating cells, traditional cancer therapies often have serious off-target effects. Therefore, a newly developed therapeutic agents should focus on improving selectivity to thus both reduce side effects and overcome drug resistance. Some Cu ionophores have shown promising potential in anticancer activity due to the inherent selectivity in inducing cuproptosis preferentially in cancer cells to normal cells (17).

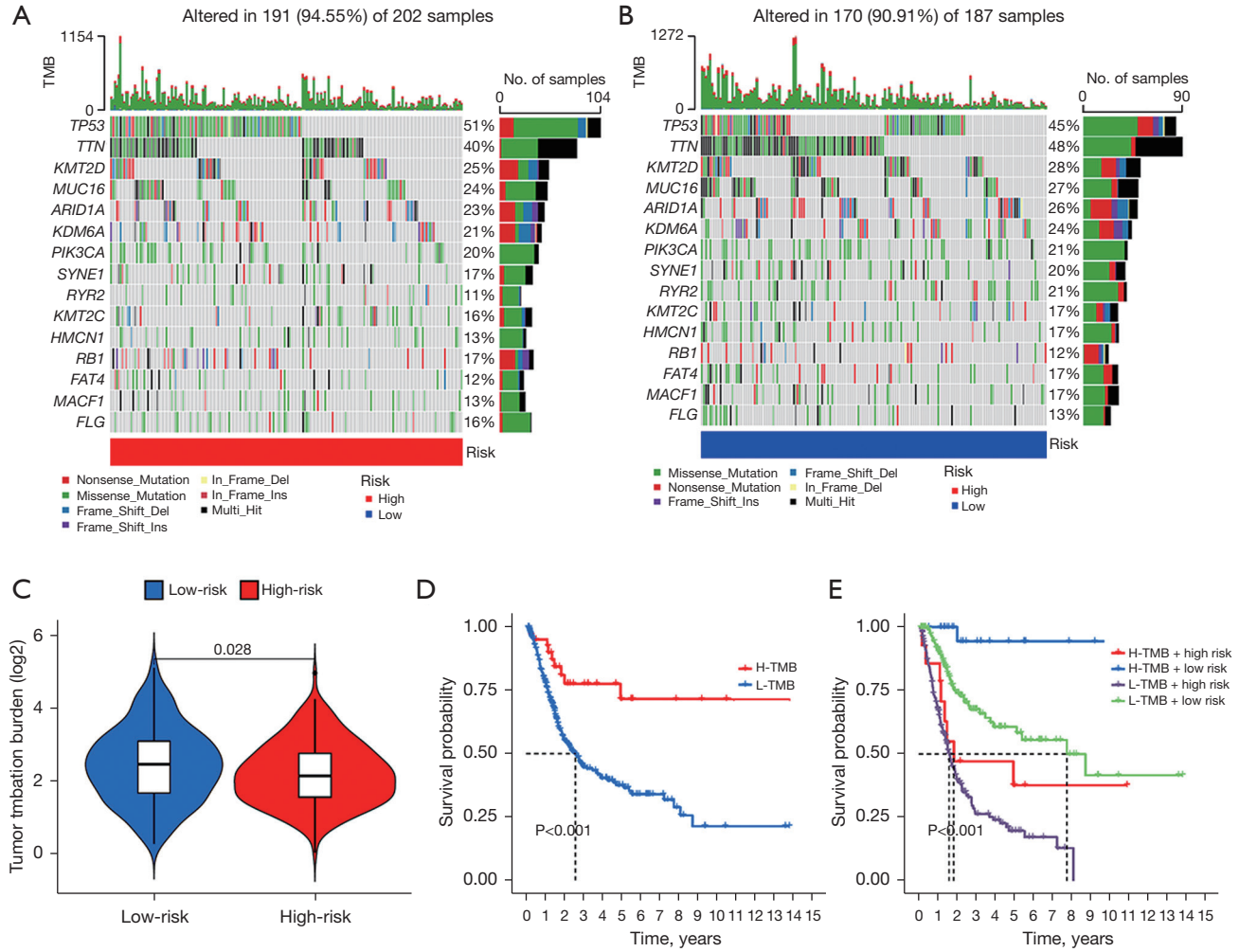

Figure 7 Comparison of mutation characteristics and immunotherapy response related to the signature. (A,B) The mutation landscapes in the (A) high- and (B) low-risk groups. (C) TMB score in the 2 risk groups. (D) Survival time analyses in the high-TMB score and low-TMB score groups. (E) Survival time analyses of patients based on TMB score and signature. TMB, tumor mutational burden; H, high; L, low.

As an essential mineral nutrient for all organisms and the basis of a large number of BPs, Cu serves as a factor in signaling to regulate or trigger several biological pathways under external stimuli (18,19). A growing number of studies have shown that greater Cu accumulation exists in cancers compared to normal tissues and promotes proliferation, growth, angiogenesis, and metastasis (20). In addition, Cu also plays a major role in promoting angiogenesis, which is essential for tumor progression via the activation of many angiogenic factors, including angiogenin and VEGF (21). Cuproptosis is a novel type of programmed cell death and is different from other known death forms, including apoptosis, ferroptosis, and necroptosis. A previous study showed that cuproptosis is involved in the development and progression of malignancies (22). As cuproptosis is a newly discovered type of programmed cell death, there are limited data and studies related to its clinical value in BLCA. Thus, we conducted this comprehensive analysis to explore the clinical value of cuproptosis-related lncRNAs in BLCA.

We identified 95 prognostic-associated cuproptosis-related lncRNAs and constructed a prognostic risk signature with 15 lncRNAs (AL031429.2, AL390236.1, AC007365.1, LINC01184, LINC02159, RAP2C-AS1, AL731537.1, MIR181A2HG, AC010186.3, LINC-PINT, BDNF-AS, LINC02443, AC006160.1, AC005954.1, and AC090948.1) that could accurately forecast the prognostic outcome and

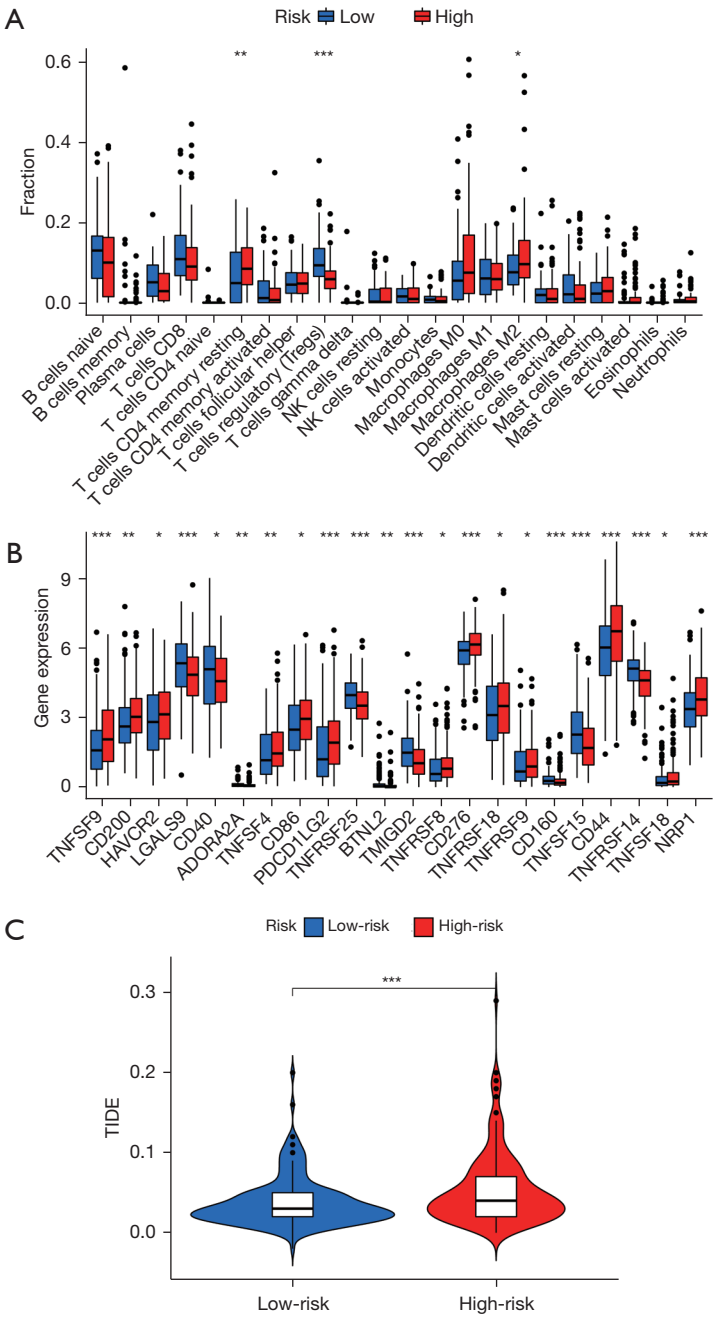

Figure 8 Evaluation of immune microenvironment based on the signature. (A) The difference of immune cells infiltration in risk groups. (B) The difference of checkpoints expression in risk groups. (C) The difference of TIDE scores in risk groups. *, P<0.05; **, P<0.01; ***, P<0.001. NK, natural killer; TIDE, tumor immune dysfunction and exclusion.

immunotherapy response of patients with BLCA. Principal component analysis (PCA) based on the 15 signature-related lncRNAs discriminated high- and low-risk patients better than did those based on all genes, cuproptosis-related lncRNAs, and cuproptosis-related mRNAs (Figure S2A-S2D).

Previous studies suggest that some of these lncRNAs are involved in tumor immunity and are relevant to prognosis. For example, LINC01184 was found to be associated with tumor-infiltrating B lymphocytes in BLCA (23) and to promote the proliferation and invasion of colorectal cancer

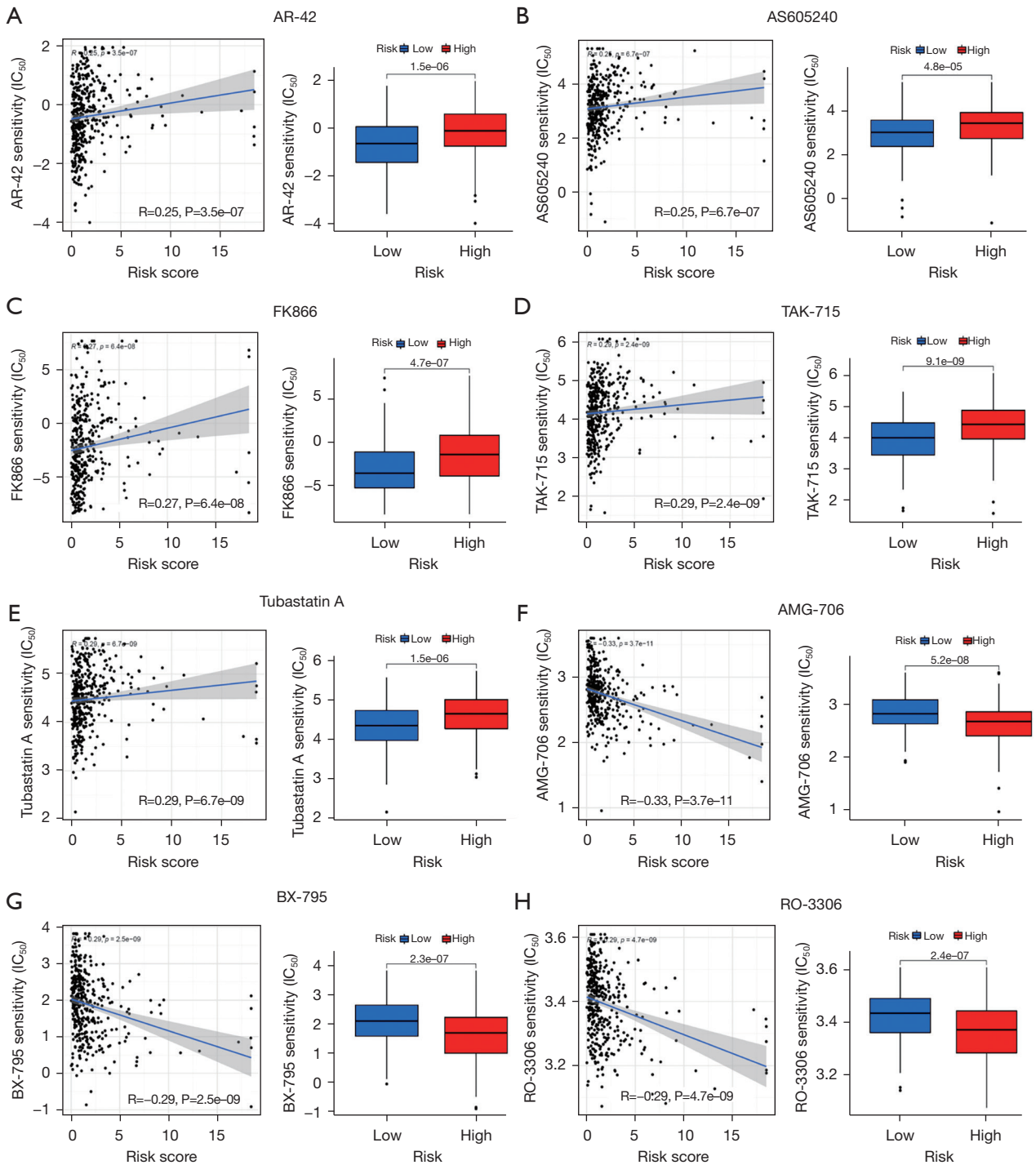

Figure 9 Evaluation of antitumor drug sensitivity based on the signature. The IC_{50} and sensitivity of (A) AR-42, (B) AS605240, (C) FK866, (D) TAK-715, (E) tubastatin A, (F) AMG-706, (G) BX-795, and (H) RO-3306. IC_{50}, half maximal inhibitory concentration.

via phosphorylated Ser/Thr kinase (p-Akt) pathway (24). Moreover, MIR181A2HG was shown to impair vascular endothelial cell proliferation and migration via the dysregulation of the microRNA (miRNA)/AKT2 axis (25) and to be involved in prognosis and immunotherapeutic response in BLCA (26). AC010186.3 is a autophagy-related lncRNA , which predicts the prognosis of ovarian cancer (27). LINC-PINT acts as a tumor suppressor in colon cancer (28), and inhibits DNA repair and increases radiotherapeutic response in nasopharyngeal cancer (29). Furthermore, the overexpression of LINC-PINT inhibits the proliferation, invasion, and migration of BLCA cells via miR-155-5p (30). BDNF-AS regulates brain-derived neurotrophic factor (BDNF) expression, which is involved in neurodevelopmental diseases. BDNF-AS was reported to be downregulated in multiple cancers, including colorectal cancer, esophageal cancer, and prostate cancer (31). The biological function of the other lncRNAs have not been studied systematically and is expected to become the targets of future research and treatment. In this study, we explored the respective expression and role of 15 cuproptosis-related lncRNAs on prognosis in BLCA. The results were consistent with *Figure 2D*, suggesting AL031429.2, AL390236.1, AC007365.1, LINC01184, LINC02159, RAP2C-AS1, and AL731537.1 appear to be detrimental factors; Meanwhile, MIR181A2HG, AC010186.3, LINC-PINT, BDNF-AS, LINC02443, AC006160.1, AC005954.1, and AC090948.1 appear to be protective factors (Figures S3,S4).

Immunotherapy has become an essential treatment for advanced BLCA (32). However, immunotherapy is only effective in about 25% of patients with advanced BLCA (33). Thus, it is worth searching for more valuable biomarkers of immunotherapy response. TMB is a biomarker of tumorigenesis and immune response. However, research suggests that they have a limited prediction power in some subpopulations (34). In our study, patients in the low-risk group had higher levels of TMB, and the risk score had synergistic effects with TMB level in predicting the prognosis of patients with BLCA. The somatic mutation accumulation promotes the formation of neoantigens which activate the immunogenicity of T cells to kill cancer cells. The cancers with high TMB often generate new antigens to recruit the immune cells (35). Consistent with the previous studies, our results showed that higher TMB tends to predict worse survival. Meanwhile, patients with high-TMB had higher levels of CD4 memory activated T cells and CD8 T cells, which can inhibit cancer progression and form T cell immunogenicity (Figure S5). Overall, the patients in low-risk group with higher TMB tended to benefit from immunotherapy.

Drug resistance remains a challenge for the clinical management of those with tumors. Many patients may experience a lack of drug availability after multiline antitumor therapy. The development of new drugs is a tortuous, expensive, and highly uncertain process. Computational drug repositioning or repurposing is a promising and efficient tool for discovering new uses for existing drugs (36). We demonstrated that the sensitivities of multiple antitumor drugs were correlated with risk score, with positive correlations being found for AR-42 (a histone deacetylase inhibitor), AS605240 (a PI3Kγ inhibitor), FK866 (a Nampt inhibitor), TAK-715 (a p38 MAPK inhibitor) and tubastatin A (a HDAC6 inhibitor), and negative ones being found for AMG-706 (a VEGFR1/2/3 inhibitor), BX-795 (a PDK1 inhibitor), and RO-3306 (a CDK1 inhibitor). These results point to candidate drugs for the preclinical and clinical treatment of different BLCA patient subgroups.

This is the first study to explore the relationship between cuproptosis-related lncRNAs and the prognosis of patients with BLCA. However, there are some limitations involved in our research. The cuproptosis-related lncRNA signature needs to be verified with prospective, multicenter studies and real-world data. Furthermore, the biological mechanisms associated with cuproptosis-related lncRNAs also require verification. In future study, the experiments *in vivo* and/or *in vitro* based on the cell lines, mouse model and patient samples are planned to elucidate the underlying mechanisms of the signature and validate the predictive performance.

Conclusions

Our study established and verified a novel prognostic risk signature with cuproptosis-related lncRNAs that may be a potential indicator for immunotherapy response and chemotherapy sensitivity, and thus capable of estimating the prognosis of BLCA patients.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5294/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5294/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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