Identification of an Eight-Cuproptosis-related IncRNA Signature as a Novel Prognostic Model and Prediction of Immunotherapy Response in Ovarian Cancer

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Research Article

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Abstract

Background

Cuproptosis-related long non-coding RNAs (lncRNAs) have been identified and constructed as new prognostic markers in several cancers. However, the role and prognostic value of Cuproptosis-related lncRNAs in ovarian cancer (OC) remain unknown.

Methods

RNA sequencing and clinical and tumor somatic mutation data from OC samples were downloaded from The Cancer Genome Atlas (TCGA) database. Patients with OC were randomly assigned to the training and testing groups. The least absolute shrinkage and selection operator regression analysis and Cox regression models were used to determine the prognostic model in the training cohort and confirmed in the testing cohort. In this study, a nomogram was constructed. Functional enrichment and immune function analyses were performed to investigate differences in biological functions. Tumor mutation burden (TMB) and tumor immune dysfunction and exclusion (TIDE) scores were used to predict response to immunotherapy.

Results

A total of eight Cuproptosis-related lncRNAs prognostic markers (AL732292.2, LINC00996, AC025287.2, AC022893.3, SUCLG2-AS1, AC245041.1, AL391832.3, and AC019080.5) were identified. The Kaplan–Meier survival curve revealed that the overall survival (OS) between the high- and low-risk groups was statistically significant. A mixed nomogram containing clinical characteristics and risk scores was constructed. The receiver operating characteristic curve and principal component analysis showed the accurate predictive ability of the model. Functional enrichment and immune function analyses confirmed that prognostic features were significantly correlated with the immune status of patients with OC. Patients in the high-risk group had a higher TIDE score and lower TMB, indicating a poor response to immunotherapy. The risk model can distinguish between the effects of antitumor therapy in patients with OC.

Conclusions

We identified an eight-Cuproptosis-related lncRNA signature of OC as a prognostic predictor and constructed a nomogram, which may be a reliable biomarker for predicting the benefit of OC immunotherapy.

1 Introduction
Ovarian cancer (OC) is the eighth most common cancer and the fifth leading cause of cancer-related deaths in females, with the highest mortality rate among gynecological cancers (1), in which 70% of patients initially present with advanced disease (2). However, high recurrence rates and drug resistance result in poor long-term survival among patients with advanced OC (only 17% at 5 years for those with advanced disease) (3). Approximately one-third of patients with OC do not respond to platinum-based primary chemotherapy, and up to 80% of patients develop resistance to platinum over time, making relapsed patients incurable (4). In recent years, poly (ADP-ribose) polymerase (PARP) inhibitors have become the maintenance treatment of choice for patients with OC with BRCA gene mutations or homologous recombination-deficient positivity (5). Combining immunotherapy with drugs that target different pathways, such as PARP inhibitors, may improve its efficacy and overcome cancer resistance; however, treatment is limited (6). Therefore, it is necessary to identify new and useful biomarkers to predict response to treatment and prognosis to inform clinical decision-making.

Copper (Cu) is an essential mineral nutrient and a biologically important cofactor for all organisms. It plays an important role in many biological processes, including mitochondrial respiration, iron uptake, antioxidant/detoxification, energy metabolism, autophagy, enzymatic reactions, and nerve signaling (7). A meta-analysis of 699 patients with OC showed that circulating Cu levels were higher in patients with OC than in normal tissues (8). Cu accumulation is associated with OC cell proliferation, metastasis, ascites formation, and angiogenesis (9, 10). Recently, Tsvetkov (11) showed, for the first time, the mechanism of Cuproptosis, which is involved in the development and progression of malignant tumors (7). Studies have shown that copper ions can reverse platinum drug resistance through Cuproptosis (12). Targeting Cuproptosis may be a new therapeutic strategy for cancer treatment.

Long noncoding RNAs (lncRNAs) have a length of more than 200 bps and no protein-coding function (13). It plays a role in important pathological and physiological processes such as autophagy, development, differentiation, apoptosis, and cell cycle (13). lncRNAs are involved in the mechanisms of OC progression, including carcinogenesis, proliferation, migration, invasion, metastasis, and angiogenesis (10, 14). Evidence has shown that lncRNAs can participate in the development, recurrence, and response to OC immunotherapy by regulating multiple pathways, such as ferroptosis (15), autophagy (16), pyroptosis (17), necroptosis (18).

Cuproptosis-related lncRNAs have been identified and constructed as new prognostic markers in colorectal cancer (19), head and neck squamous cell carcinoma (20), hepatocellular carcinoma (21), and gastric adenocarcinoma (22), providing a new perspective for its clinical treatment, especially immunotherapy. However, the role of Cuproptosis-related lncRNAs in OC remains unclear. This study aimed to identify Cuproptosis-related lncRNAs in patients with OC to construct new prognostic markers.

2 Methods

2.1 Public data collection
RNA-seq transcriptome dataset, clinical characterization data (survival time, survival status, age, grade, and stage), and tumor somatic mutation data comprising 381 OC samples were obtained from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) on August 12, 2022. The types of disease included cystic, mucinous, and serous neoplasms. There were no restrictions on vital status, race, age at diagnosis, number of days to death, or ethnicity. Nineteen Cuproptosis-related genes were selected according to the literature (11, 23).

2.2 Generation and assessment of a Cuproptosis-related lncRNAs signature

Finally, 258 patients with OC with complete data, who were randomly assigned to the training and testing groups, were included in the follow-up analysis. The training cohort was used to construct Cuproptosis-related lncRNA signatures, and the test set was used to validate the outcomes. In the training group, using univariate Cox regression analysis, least absolute shrinkage and selection operator (LASSO) regression analysis (1000-fold cross-validation), and multivariate Cox regression analysis, a Cuproptosis-related lncRNA signature was constructed. The risk scores for each patient were then calculated and the corresponding coefficients were obtained using the following formula: risk score = percent spliced in index (PSI) × βi, where β is the regression coefficient of Cuproptosis-related lncRNAs. The samples in the training and testing groups were then divided into high- and low-risk groups based on the cut-off criteria of $P < 0.05$ and $|\log_2 \text{FC}| \geq 1$. The threshold for co-expression analysis was set at |correlation coefficient| $>0.4$, $P < 0.001$ ($r > 0.4$, $P < 0.001$). Overall survival (OS) was compared using Kaplan–Meier (KM) analysis between the high-risk and low-risk groups in the training and testing groups. The correlation between the model and clinical characteristics was evaluated using the chi-square test.

2.3 Construction and evaluation of the risk model

A nomogram containing clinical parameters (grade, stage, and age) and risk scores of the lncRNA signature in TCGA-OV samples was constructed. All variates were calculated, and the 1-, 3- and 5-year survival probabilities of patients with OC were calculated, with higher scores indicating a worse prognosis. The prognostic capability of the risk model and clinical characteristics were evaluated and compared using receiver operating characteristic (ROC) analysis. We then constructed calibration curves and the concordance index (C-index) to assess the accuracy of the nomograms. In the calibration curves, a straight line at 45° indicates the best predictive power. The C-index was positively correlated with the predictive power of the nomogram.

2.4 Principal component analysis (PCA)

PCA is a method for reducing dimensionality and extracting features using a linear transformation (24). PCA was used to visualize the spatial distribution of Cuproptosis-related lncRNA with prognostic significance, all genes, Cuproptosis-related genes, or Cuproptosis-related lncRNAs in the high- and low-risk groups of TCGA-OV samples using the “scatterplot3d” R package.

2.5 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis
The GO and KEGG database pathways were used to explore the three molecular functions (biological process [BP], cellular component [CC], and molecular function [MF]) and key signaling pathways based on the limited conditions set to $P < 0.05$, false discovery rate (FDR) < 0.05.

2.6 Estimation of immune function and immunotherapy

To investigate differences in immune function between the high- and low-risk groups, the single-sample gene set enrichment analysis (ssGSEA) algorithm function package in R was used, and the results were visualized on a heatmap. The tumor immune dysfunction and exclusion (TIDE) score can be used to predict the effect of immunotherapy based on the simulation of the tumor immune escape mechanism (http://tide.dfci.harvard.edu)(25). Therefore, we investigated the effect of immunotherapy in the high- and low-risk groups using the TIDE score.

2.7 Sensitivity of antitumor drugs

The half-maximal inhibitory concentration (IC50) value refers to the semi-inhibitory concentration of the antagonist. The IC50 value of the antitumor drugs was calculated using R, and the differences in the IC50 values of each drug in the high- and low-risk groups were compared using the Wilcoxon signed-rank test based on $P < 0.001$. The outcomes were visualized using boxplots using the R package “ggplot2.”

2.8 Calculation of tumor mutation burden (TMB) scores

Mutation data from TCGA-OV samples were obtained from TCGA and extracted using Pearl (version 5.30.0, https://www.perl.org/). The waterfall diagrams and accordion diagrams were then drawn using R to show the TMB in the high- and low-risk groups. KM analysis was used to analyze OS in patients with low and high TMB.

2.9 Statistical analysis

All statistical analyses were performed using R version 4.2.1 (R packages: tidyverse, ggplot2, ggExtra, survival, survminer, pheatmap, timeROC, dplyr, rms, pec, regplot, org.Hs.eg.db, DOSE, clusterProfiler, enrichplot, ComplexHeatmap, colorspace, stringi, RColorBrewer, ggpubr, limma, GSVA, GSEABase, reshape2, maftools, and BiocManager). A two-tailed $P < 0.05$ was considered statistically significant.

3 Results

3.1 Construction of a Cuproptosis-related lncRNA signature

A total of 258 patients with OC were randomly assigned to the training (n = 132) and testing groups (n = 126) in a 1:1 ratio. In the two cohorts, 68.62% of the patients aged < 65 years old and 93.09% of the population had advanced OC (stage III−IV) and 85.64% of the patients were grade 3, indicating that the tumor was highly malignant; however, there were no significant differences in clinical characteristics (age, grade, and stage) between the two cohorts (Table 1, $P > 0.05$).
Table 1
Comparison of clinical characteristics between two cohorts of ovarian cancer

<table>
<thead>
<tr>
<th>Variates</th>
<th>Type</th>
<th>Total N (%)</th>
<th>Testing group N (%)</th>
<th>Training group N (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;=65</td>
<td>258 (68.62)</td>
<td>126 (67.02)</td>
<td>132 (70.21)</td>
<td>0.5784</td>
</tr>
<tr>
<td></td>
<td>&gt; 65</td>
<td>118 (31.38)</td>
<td>62 (32.98)</td>
<td>56 (29.79)</td>
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</tr>
<tr>
<td>Grade</td>
<td>1</td>
<td>1 (0.27)</td>
<td>0 (0.00)</td>
<td>1 (0.53)</td>
<td>0.0875</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42 (11.17)</td>
<td>27 (14.36)</td>
<td>15 (7.98)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>322 (85.64)</td>
<td>155 (82.45)</td>
<td>167 (88.83)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>unknow</td>
<td>11 (2.93)</td>
<td>6 (3.19)</td>
<td>5 (2.66)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>I</td>
<td>1 (0.27)</td>
<td>0 (0.00)</td>
<td>1 (0.53)</td>
<td>0.5079</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>22 (5.85)</td>
<td>13 (6.91)</td>
<td>9 (4.79)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>292 (77.66)</td>
<td>146 (77.66)</td>
<td>146 (77.66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>58 (15.43)</td>
<td>26 (13.83)</td>
<td>32 (17.02)</td>
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<td>unknow</td>
<td>3 (0.80)</td>
<td>3 (1.60)</td>
<td>0 (0.00)</td>
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</tr>
</tbody>
</table>

Information about Cuproptosis-related genes and 16,876 lncRNAs of OC was obtained from the TCGA database. Furthermore, lncRNAs associated with Cuproptosis-related genes were screened using co-expression analysis (Fig. 1A). In total, 430 Cuproptosis-related lncRNAs were identified (Table S1).

To investigate the relationship between Cuproptosis-related lncRNAs and survival outcomes in patients with OC, lncRNAs with prognostic significance were further screened. First, a univariate Cox regression analysis was performed, and 16 Cuproptosis-related lncRNAs with prognostic significance were selected (Table S2, Figure S1). Fourteen Cuproptosis-related lncRNAs were screened using LASSO regression and cross-validation (Fig. 1B, 1C). Next, eight Cuproptosis-related lncRNAs that were closely related to the prognosis of OC were confirmed using multivariate Cox regression analysis (Table S3). Among them, five lncRNAs (AL732292.2, LINC00996, AC025287.2, AC022893.3, and SUCLG2-AS1) were protective indicators in patients with OC, while the other three lncRNAs (AC245041.1, AL391832.3, and AC019080.5) were risk factors (Table S2, Fig. 1D).

3.2 The risk score can serve as an independent prognostic factor and guide the prediction of clinical outcome for TCGA-OV samples

The patients were divided into high- and low-risk groups in the training and testing groups according to the cut-off criteria. Figure 2 shows the expression, risk score, and survival status (dead/alive) of Cuproptosis-related lncRNAs from top to bottom in each sample (Fig. 2A-C, 2E-G). The results showed
that the expression of AC245041.1, AL391832.3, and AC019080.5 was higher than in the high-risk group in both the training and testing groups. The higher the risk score for Cuproptosis-related lncRNAs, the worse the prognosis for patients with OC. KM analysis showed that OS of patients with OC in the high-risk group was worse in both cohorts ($P < 0.05$, Fig. 2D, 2H).

To further construct the prognosis model for patients with OC, univariate and multivariate analyses were used to study the predictive ability of Cuproptosis-related lncRNAs. The results showed that Cuproptosis-related lncRNAs were independent risk factors for the prognosis of patients with OC (Fig. 3A, 3B). The area under the curve (AUC) of the risk score was 0.692 at 1 year, 0.648 at 3 years, and 0.651 at 5 years, indicating that Cuproptosis-related lncRNA has a good ability to predict the prognosis of patients with OC (Fig. 3C).

We compared the predictive ability of clinical parameters (grade, stage, and age) and risk scores for OS in patients with OC. The AUC of the risk score and age were similar (0.692 vs. 0.710), which was better than that of the grade and stage in predicting OS in patients with OC (Fig. 3D). The C-index showed that the risk score had the highest predictive accuracy (Fig. 3E). These results suggested that the risk score of Cuproptosis-related lncRNAs can be used as a reliable predictor of OS in patients with OC.

The clinical parameters (grade, stage, and age) and risk score were used to draw a nomogram to predict the survival outcome of patients with OC. The total score on the nomogram was the sum of the corresponding scores assigned to each risk factor. The higher the score, the worse the prognosis of the patients with OC (Fig. 3G). Calibration curves confirmed that the prognostic model had a good ability to predict the 1-, 3-, and 5-year OS (Fig. 3F).

3.3 PCA verified the prediction ability and the grouping power of the prognostic signature

PCA was used to verify whether Cuproptosis-related lncRNAs of prognostic significance could distinguish between the high- and low-risk groups of patients with OC. The results showed that PCA of Cuproptosis-related lncRNAs (Fig. 4B), Cuproptosis-related genes (Fig. 4C), and all genes (Fig. 4D) could not distinguish between the high- and low-risk groups. LncRNAs, which were used to construct prognostic models, were able to better distinguish between the high- and low-risk groups (Fig. 4A).

3.4 Functional enrichment analysis and immune function analysis

By comparing gene expression between the high- and low-risk groups, we further screened 268 DEGs for GO analysis and KEGG analysis (Table S4). GO analysis showed that the functions of these DEGs were mainly related to skeletal system development, collagen-containing extracellular matrix, receptor-ligand activity, and signaling receptor activator activity (Fig. 5A). KEGG analysis showed that DEGs were mainly enriched in the PI3K-Akt signaling pathway and neuroactive ligand-receptor interactions (Fig. 5B).

To explore whether Cuproptosis was related to the immune function of patients with OC, we studied differences in immune function between the high- and low-risk groups. The results suggested significant differences in Type_I_IFN_Reponse, Type_IL_IFN_Reponse, T_cell_co-inhibition, T_cell_co-stimulation, HLA,
APC_co_stimulation, cytokines and cytokine receptors (CCR), and inflammation between the two groups, and all were more enriched in the high-risk group than in the low-risk group. Furthermore, the difference in CCR and Type_I_IFN_Response was the most significant between the two groups \( (P < 0.001, \text{Fig. 5C}) \). These results suggest a significant correlation between Cuproptosis and immune function in OC.

### 3.5 Significance of the risk model in drug therapy

A high TIDE score indicates that the curative effect of immune checkpoint blockade (ICB) treatment was poor, and the survival time was short after ICB treatment \( [4] \). The TIDE score was higher in the high-risk group, suggesting that this group of patients may have had a worse response to ICB treatment \( (P > 0.05, \text{Fig. 6A}) \).

Therefore, we further investigated the sensitivity of patients with OC in the high- and low-risk groups to 19 antitumor drugs (Figs. 6 and 7). Patients in the high-risk group were more sensitive to THZ-2-49, (5Z)-7-Oxozeaenol, WH-4-023, A-770041, saracatinib, pazopanib, dasatinib, cytarabine, CGP-60474, BEZ235, and AP-24534 \( (P < 0.05, \text{Fig. 6B-L}) \). Patients in the low-risk group were more sensitive to TAK-715, rTRAIL, CP724714, NSC-207895, PD-0325901, MP470, gefitinib, and FH535 \( (P < 0.05, \text{Fig. 7A-7H}) \). These results indicated that the risk model could distinguish the effects of antitumor therapy in patients with OC.

### 3.6 TMB of the Cuproptosis-related IncRNAs prognostic marker in patients with OC

TMB is related to the sensitivity of ICB treatment; therefore, we further explored TMB in the high- and low-risk groups. The results showed that the TMB in the low-risk group was higher than in the high-risk group \( (P < 0.05, \text{Fig. 8E}) \), indicating that patients in the high-risk group were less sensitive to ICB treatment. The waterfall map showed that 89.16% of the samples in the low-risk group contained mutations, and the three genes with the highest mutation frequencies were \( TTN \) (57%), \( TP53 \) (39%), and \( MUC16 \) (36%) (Fig. 8A); 90.26% of the samples in the high-risk group had mutations, and the three genes with the highest mutation frequencies were \( TTN \) (45%), \( TP53 \) (45%), and \( MUC16 \) (25%) (Fig. 8B). In particular, the frequency of the common cancer mutation gene, \( MUC16 \), was higher in the low-risk group, while the mutation frequency of the tumor suppressor gene, \( TP53 \), was higher in the high-risk group. KM analysis showed that the OS of patients with low TMB was shorter, and the prognosis was worse than that of patients with low TMB \( (P < 0.05, \text{Fig. 8C}) \), which was consistent with the conclusions of previous studies. In addition, the prognosis of the high-risk group was worse in the high TMB and low TMB groups \( (P < 0.05, \text{Fig. 8D}) \).

### 4 Discussion

Cu is an indispensable trace element in all organisms and is normally maintained at very low levels in mammalian cells \( [7] \). Cuproptosis may occur because copper exceeds the steady-state threshold \( [9] \). Compared to normal cells, cancer cells are usually preferentially induced to die from Cuproptosis \( [26] \). A comprehensive analysis of Cuproptosis-related gene expression in 33 cancers showed that Cuproptosis-related genes \( FDX1, LIAS, DLD, DLAT, PDHA1, PDHB, GLS, LIPT1, MTF1, \) and \( CDKN2A \) were highly
expressed in 33 tumors (27). Cuproptosis-related genes were differentially expressed in 18 cancer types and adjacent normal tissues (27). In OC, high expression of ATP7A and ATP7B can regulate the intracellular concentration of platinum by mediating the uptake and efflux of platinum in cells, thus improving platinum resistance, which is expected to be a predictive marker of platinum resistance (4). Disulfiram mediates cell death in OC cells by promoting a pro-oxidative intracellular environment via a copper-dependent mechanism (28). In summary, Cuproptosis-related gene target therapy may be an effective way to treat many types of cancers in the future.

We identified eight lncRNAs that were associated with OC prognosis. Among them, five lncRNAs (AL732292.2, LINC00996, AC025287.2, AC022893.3, and SUCLG2-AS1) were protective indicators in patients with OC, while the other three lncRNAs (AC245041.1, AL391832.3, and AC019080.5) were risk factors. Studies have shown that high expression of LINC00996 is a good prognostic factor for patients with lung adenocarcinoma, colorectal cancer, head and neck squamous cell carcinoma, and OC (18, 28–30). High expression of SUCLG2-AS1 is associated with better OS in clear cell renal cell carcinoma and triple-negative breast cancer (31, 32). High expression of AC245041.1 is a poor prognostic factor in patients with gastric adenocarcinoma and cutaneous melanoma (15, 33). High expression of AC019080.5 is a risk factor for poor OS in patients with endometrial cancer (34, 35). Our results were consistent with these findings. However, there are no reports on AL732292.2, AC025287.2, AC022893.3, and AL391832.3; therefore, it is necessary to further determine their mechanism of action in Cuproptosis in our future studies.

Significant differences in Type_I_IFN_Reponse, Type_II_IFN_Reponse, T_cell_co-inhibition, T_cell_co-stimulation, HLA, APC_co_stimulation, CCR, and inflammation were found between the two groups, and all of them were more enriched in the high-risk group than in the low-risk group. In addition, the difference in CCR and Type_I_IFN_Reponse was the most significant between the two groups. The results suggested a significant correlation between Cuproptosis and immune function in patients with OC. Immune functions in the tumor microenvironment can promote or inhibit the progression of malignant tumors. Type I interferons have become a double-edged sword for cancer treatment. The prevailing wisdom is that type I interferon (IFN-I) proteins enhance antitumor immune responses by stimulating the maturation of dendritic cells, enhancing T cell cytotoxicity, and even inducing tumor cell senescence and apoptosis by providing the necessary inflammatory signals (36). Simultaneously, feedback inhibition is initiated in immune cells and cancer cells, and prolonged IFN-I signaling can lead to immune dysfunction, thus preventing cancer control (37). For example, IFN-I can also activate NF-κB, which leads to immunosuppression and tumor progression through the induction of pro-tumoral cytokines, such as IL-10, IL-6, and TNF (38). The response of IFN-1 is generally considered the primary signaling activity of the stimulator of the interferon gene (STING), and tumor-induced T cell death is partially dependent on the IFN-independent activity of STING in T cells (39). Low doses of STING agonists induce favorable antitumor immunity, whereas high doses of STING agonists cause massive T cell death and impaired antitumor immunity (40, 41). Over the past 40 years, CCRs have been extensively investigated as cancer targets or cancer therapeutics (41). Cytokines are key mediators of cell communication in the tumor microenvironment (TME). Some cytokines contribute to hosting antitumor responses, but the production
and function of many cytokines are dysregulated in cancer, participating in all stages of carcinogenesis and response to therapy (42). The coexistence of T cell co-stimulation and co-inhibition in the immune microenvironment seems contradictory; however, it is common. These results indicate that the patients with OC in the high-risk group were more immunocompromised.

TMB, an important biomarker that represents the degree of tumor mutation, is becoming a key indicator to predict the efficacy of tumor immunotherapy (43). Cancers with higher TMB are more likely to respond to immune checkpoint blockade inhibitors (ICIs) (43). Cumulative mutations with an increased neoantigen potential resulting from high TMB lead to elevated immunogenicity that can induce an immune response in humans, suppressing tumor growth and subsequently resulting in relatively high patient survival (44). The results of this study showed that TMB was lower in the high-risk group, suggesting that OC patients in this group may have a poor response to immunotherapy. KM analysis showed that patients with high TMB had longer OS than those with low TMB, suggesting that patients with high TMB had a better prognosis, which is consistent with the above studies.

MUC16, also known as CA125, is frequently mutated in various tumors and is one of the top three genes with the highest mutation frequency (45). Balachandran et al. (46) found that neoantigens generated by MUC16 mutations were responsible for long-term survival in patients with pancreatic cancer, mainly by the enrichment and activation of CD8-positive T cells, which play a role in killing tumors. Zhang et al. (47) analyzed the mRNA expression profile of 9850 samples of 30 solid tumor types in the TCGA database and found that the tumor microenvironment with MUC16 mutations had more abundant immune cell infiltration. GSEA also showed that MUC16 mutations were mainly enriched in immune-related pathways. A high level of the MUC16 mutation predicts a good ICIs treatment response and is expected to be a biomarker to predict the efficacy of immunotherapy. TP53 encodes a tumor suppressor protein. Donehower et al. (48) analyzed TP53 mutations in whole-exome sequences from 10 of 225 TCGA patients with 32 different cancer types and identified 3786 patients with TP53 mutations. The frequency of TP53 mutations varies according to cancer type, with mutation rates greater than 90% for ovarian and uterine carcinomas. It can be seen that TP53 is a frequently mutated oncogene in human tumors and affects the occurrence and development of various cancers. However, the relationship between TP53 mutations and tumor prognosis remains unclear. Mutant TP53 RNA expression signature has been shown to be significantly associated with reduced survival in 11 types of cancer (48), while mutations of TP53 may be associated with poor prognosis in breast, head and neck, liver, hematopoietic, and lymphatic cancers. There were no statistically significant differences in the number of studies that were associated and not associated with poor prognosis in bladder cancer, brain cancer, lung cancer, colon cancer, esophagus cancer, and OC, but this remains controversial (49). TP53 can increase the level of T cells to enhance the immune response in the pancreatic cancer microenvironment, thus strengthening the role of dendritic cells (DC) to inhibit tumor effects. Loss of the normal activity of TP53 (TP53 mutation) changes the pancreatic tumor immune microenvironment and promotes inflammation, which promotes cancer and accelerates tumor progression and metastasis (50, 51). MUC16 and TP53 mutations are common among other Cuproptosis-related IncRNA signatures. For example, in the TMB analysis of Cuproptosis-related IncRNA signatures in colorectal cancer, the mutation frequency of MUC16 in the high-
risk group was lower than that of the low-risk group (24% vs. 35%), and the mutation frequency of TP53 in the high-risk group was higher than that of the low-risk group (64% vs. 49%) (19). The frequency of MUC16 mutations was higher in the low-risk group, suggesting that these patients responded well to immunotherapy. However, the frequency of TP53 mutation was higher in the high-risk group, which may be related to a poor prognosis.

This study had some limitations. First, the Cuprotosis-related lncRNA signature and immune function must be further studied through biological experiments in vivo and in vitro. Second, the relationship between the eight Cuprotosis-related lncRNAs and survival outcomes in OC patients should be validated in clinical samples.

In conclusion, our study identified an eight-Cuprotosis-related lncRNA signature of OC as a prognostic predictor and constructed a mixed nomogram containing clinical characteristics and risk score, which may be a reliable biomarker to predict the benefit of OC immunotherapy.

**Declarations**

**Ethics approval and consent to participate**

Not necessary, because all data were downloaded from online data repository.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The original contributions of this study are included in this article. Further inquiries can be directed to the corresponding authors. RNA-seq transcriptome dataset, clinical characterization data, and tumor somatic mutation data of OC samples can be obtained from the TCGA database (https://portal.gdc.cancer.gov/).

**Competing interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors’ contributions

JT Fan and ZF Zhi conceived and designed the study. D Sun and HY Qin drafted the manuscript. D Sun, SS Lin, and JR Tong produced the figures and tables. Y Yang and ZF Zhi analyzed the data and formatted the article. All authors have contributed to the manuscript and approved the submitted version.

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References


Figures
Figure 1

Construction of prognostic Cuproptosis-related IncRNAs in TCGA-OV samples. (A) Co-expression outcomes (Sankey diagram) of 19 Cuproptosis-related genes and 430 Cuproptosis-related IncRNAs. (B-C) Construction of prognostic prediction models using LASSO regression analysis. (D) Correlations between 19 Cuproptosis-related genes and eight prognostic Cuproptosis-associated IncRNAs. *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure 2

Prognostic performance of the eight Cuproptosis-associated IncRNAs of the signature. Risk score distribution of the prognostic Cuproptosis-associated IncRNAs signature in the training and testing cohort, including the expression heatmap (A, E), risk scores (B, F), and survival status (C, G). (D, H) Kaplan-Meier curves for overall survival in the training and testing groups.
Figure 3

Construction and validation of the prognostic model. Univariate Cox analysis (A) and multivariate Cox analysis (B) of clinical variates and prognostic models. (C) ROC curves for 1-, 3-, and 5-year OS of patients with OC. The ROC curves (D) and C-index (E) showed the predictive accuracy of the risk model, age, grade, and stage. (F) Calibration curves to validate the 1-, 3-, and 5-year OS of TCGA-OV. *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure 4

PCA between the high- and low-risk groups. (A) Risk model. (B) Cuproptosis-related lncRNAs. (C) Cuproptosis-related genes. (D) All genes.
Figure 5

Analysis of function enrichment. (A) GO enrichment analysis. (B) KEGG pathways analysis. (C) Analysis of 13 immune-related functions in the high- and low-risk groups. BP, biological process; CC, cellular component; MF, molecular function. *p < 0.05, **p < 0.01, and ***p < 0.001.
Figure 6

The TIDE score and the comparison of IC50 of 11 antitumor drugs between the two groups. (A) TIDE score in high- and low-risk patients. (B-L) Eleven antitumor drugs are more sensitive to patients in the high-risk group. IC50, half maximal inhibitory concentration. *p< 0.05.
Figure 7

Comparison of IC50 of eight antitumor drugs between the two groups. (A-H) Eight antitumor drugs are more sensitive to patients in the low-risk group. IC50, half maximal inhibitory concentration.
Figure 8

TMB differences between the high- and low-risk groups in the TCGA-OV cohort. (A-B) Waterfall diagram of the top 15 mutant genes in the two groups. (C) Overall survival (OS) of the high and low TMB groups. (D) TMB risk combined with OS in patients with OC. TMB, tumor mutational burden.

Supplementary Files
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- SupplementaryMaterials.docx