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ORIGINAL RESEARCH

Nine Pyroptosis-Related lncRNAs are Identified as Biomarkers for Predicting the Prognosis and Immunotherapy of Endometrial Carcinoma

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Background: Endometrial carcinoma (EC) is one of the most common malignancies. Immunotherapy has shown promising effects in the treatment against specific subtypes of EC.

Methods: The RNA and clinical information of patients with EC were acquired from The Cancer Gene Atlas (TCGA) database. Firstly, the differentially expressed pyroptosis-related lncRNAs (PRLs) were screened between the tumor and normal control tissue. Secondly, the PRLs closely related to survival were identified by univariate and multivariate regression analysis, based on which, we evaluated the risk score for each EC patient to construct a risk signature. Moreover, we assessed the prognostic value, clinical relevance immunity, and immunotherapy based on this signature.

Results: We screened out 9 individual PRLs (AC087491.1, AL353622.1, AL035530.2, LINC02036, AL021578.1, AL390195.2, AC009097.2, AC004585.1, and AC244517.7) closely related to the prognosis of EC. Kaplan–Meier analyses showed a poorer prognosis for the patients in the high-risk FRLs signature ($P < 0.001$). The area under the curve (AUC) for 1 year, 2 years, 3 years was 0.693, 0.694, 0.750, respectively. Our risk model could be considered as an independent prognostic marker for EC ($P < 0.001$, HR:2.172, 95% CI:1.532–3.079). Moreover, immune functions and checkpoints were generally different in the 2 groups. Simulation analysis by termed immunophenoscores hinted that immunotherapy might bring optimal therapeutic effect in the low-risk group.

Conclusion: We successfully developed a novel signature with 9 lncRNAs related to pyroptosis, which may be used as biomarkers to evaluate the prognosis and immune treatment of EC.

Keywords: endometrial carcinoma, pyroptosis, immunotherapy, lncRNAs, immune infiltration

Introduction

Endometrial carcinoma (EC) is the sixth most prevalent cancer in women of 185 countries, with 417,000 patients diagnosed and 97,000 died in the past 2020.[1] Patients at stage I have the favorable clinical outcome, whereas the survival rate for stage III– stage IV is very poor.[2] Considering the low survival rate for the patients at a late stage, we need to find proper prognostic biomarkers to prolong the survival time of patients with EC. HE4, alone or associated with CA125, may be used to evaluate prognosis and survival of EC.[3,4] However, as far as we know, there is still a lack of accurate diagnostic and prediction models for EC.

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Pyroptosis is a type of programmed cell death driven by inflammatory caspases, accompanied with an intact nucleus and the formation of plasma membrane pore.[5] It is associated with various cancers. Wang et al found up-regulated caspase-1, IL-1β, and IL-18 in the esophageal cancer tissues, which indicated that pyroptosis could be involved in the development of esophageal cancer.[6] In gastric cancer cells, the caspase-3 dependent apoptosis induced by 5-FU was converted to pyroptosis through gasdermin E.[7] Docosahexaenoic acid (DHA) could lead to pyroptosis in breast cancer cells and might be a useful addition in breast cancer treatment.[8] Extracellular signal-regulated kinases (ERK) activation is pivotal in cancer cell survival through the upregulation of anti-apoptotic proteins and inhibition of caspase activity. In ovarian cancer, the inhibition of the c-Jun N-terminal kinase (JNK) pathway by targeting ERK or MEK leads to the suppression of tumor growth. Indeed, poly (ADP-ribose) polymerase 1 (PARP1) inhibition causes a loss of ERK2 stimulation by decreasing the activity of critical pro-angiogenic factors.[9]

The long non-coding RNAs (LncRNAs) are longer than 200 bp in the length of the transcription.[10] They do not encode proteins but play important roles in nearly every level of gene expression.[11] The expression levels of lncRNAs in tumor tissue possibly affect the tumor progression and metastasis.[12] At the same time, some special lncRNAs act in the pyroptosis of cancers. For example, the lncRNA RP1-85F18.6 put an impact on the proliferation, invasion, and pyroptosis of colorectal cancer cells by regulating the expression of ΔNp63.[13] The decreased long noncoding RNA growth arrest specific transcript 5 (lncRNA GAS5) was found accompanied with reduced pyroptosis in ovary cancer.[14]

We notice that both pyroptosis and certain types of lncRNAs influence the occurrence and development of cancers. Recent studies have reported that a group of lncRNAs, such as FRMD6-AS2, AL161431.1, LINC01133, LINC01243, PCAT1, MALAT1, and CARLo-5 were associated with EC.[15–20] We assumed that a risk model found based on pyroptosis-related lncRNAs might help us better identify EC and prolong the survival time of EC patients. Meanwhile, although a pyroptosis-related lncRNA signature showed potential predictive value in immune target therapy for head and neck squamous cell carcinoma,[21] the relationship between pyroptosis-related lncRNAs and immune target therapy in EC is still unknown. In our study, for the first time, we used The Cancer Gene Atlas (TCGA) data of patients with EC to identify a set of pyroptosis-related lncRNAs. Based on the lncRNAs, a risk signature was successfully constructed. We expect to use the risk model to judge prognosis and personalize the immune treatment of EC in the future.

Methods

Data Sources

The mRNA expression profiles and clinical information of patients with endometrial carcinoma (EC) were downloaded from The Cancer Gene Atlas (TCGA) database (https://tcga-data.nci.nih.gov/tcga/). We extracted pyroptosis-related genes from previous reviews.[22–25] We got immune infiltration data from the tumor immune estimation resource (TIMER) database (http://timer.comp-genomics.org). Scores for immune treatment and microsatellite instability status of EC were downloaded from The Cancer Immunome Atlas (TCIA) database (https://tcia.at/).

Identification of Differentially Expressed Long Non-Coding RNAs (lncRNAs) Related to Pyroptosis

Firstly, we screened out the pyroptosis-related lncRNAs by co-expressing relationships between pyroptosis-related genes and lncRNAs. Secondly, we identified differentially expressed pyroptosis-related lncRNAs between the EC and control tissues (with false discovery rate (FDR) < 0.05 and |log2 FC|≥ 1). The expression data of the pyroptosis-related lncRNAs were merged with the survival data of EC. (By employing the "limma" R package)

Development of a Pyroptosis-Related lncRNAs Signature

First, we applied univariate and multivariate Cox analysis to look out the pyroptosis-related lncRNAs associated with the survival of patients with EC. Then a risk signature was further developed with the least absolute shrinkage and selection operator (LASSO) cox regression model using the "glmnet", "survival", and "survminer" R packages. Risk scores= $\sum_{i}^{n} Xi \times Yi$ (X: coefficient of each lncRNA, Y: expression of each lncRNA). Based on the median score, the EC patients were separated into low- and high-risk groups. The results were visualized by employing "pheatmap" R package. We used the Kaplan–Meier survival curve analysis and the receiver operating characteristic (ROC) curve to evaluate the sensitivity and specificity of the pyroptosis-related lncRNAs

signature l (By employing the "timeROC", "survival" and "survminer" R packages). Both univariate Cox regression analysis and multivariate Cox regression analysis were applied to assess the prognostic relationship between risk score, age, and grade. We used Cytoscape 3.8.2 software to construct the pyroptosis-related genes-lncRNAs regulatory network.

Immunity Analysis of Pyroptosis-Related lncRNAs Signature

According to the risk scores, EC Patients were separated into 2 risk groups. The immune-related functions and immune checkpoints were assessed by applying enrichment analysis (By employing the "limma", "pheatmap" "GSVA", "GSEABase", "ggpubr", and "reshape2" R packages).

Immunotherapy Evaluation of Pyroptosis-Related lncRNAs Signature

Charoentong et al[26] created quantification by termed immunophenoscores (IPS) for 20 solid cancers through machine learning, which could be used to predict the response of those cancers to anti-programmed cell death protein 1 (PD-1) and anti-cytotoxic T lymphocyte antigen-4 (CTLA-4). In this model, higher IPS mean a better response to corresponding immunotherapy. We evaluated the immunotherapy of EC by employing "ggpubr" and "ggplot2" R package.

Statistical Analyses

Statistical analysis was applied by R version 4.0.2 (Institute for Statistics and Mathematics, Vienna, Austria). We used the Wilcoxon-test to compare the expression levels of pyroptosis-related lncRNAs between EC and adjacent normal tissues. The Pearson correlation was used to contrast the categorical variables. We compared the overall survivals (OS) of patients between the low- and high-risk groups by applying the Kaplan-Meier curve. Univariate and multivariate Cox regression analyses were used to find out the independent factors related to survival rate. A P-value less than 0.05 was considered statistically significant.

Results

Differential Expression of Pyroptosis-Related Long Non-Coding RNAs (lncRNAs)

The RNA-seq data of 35 paracancerous tissues and 552 endometrial carcinoma (EC) tissues and the clinical information of EC patients were downloaded from The Cancer Gene Atlas (TCGA) cohort. The 33 genes related to pyroptosis were extracted from the published reviews (Supplementary Table 1). We initially identified 170 significantly differently expressed pyroptosis-related lncRNAs between the EC and control tissues ($P < 0.05$).

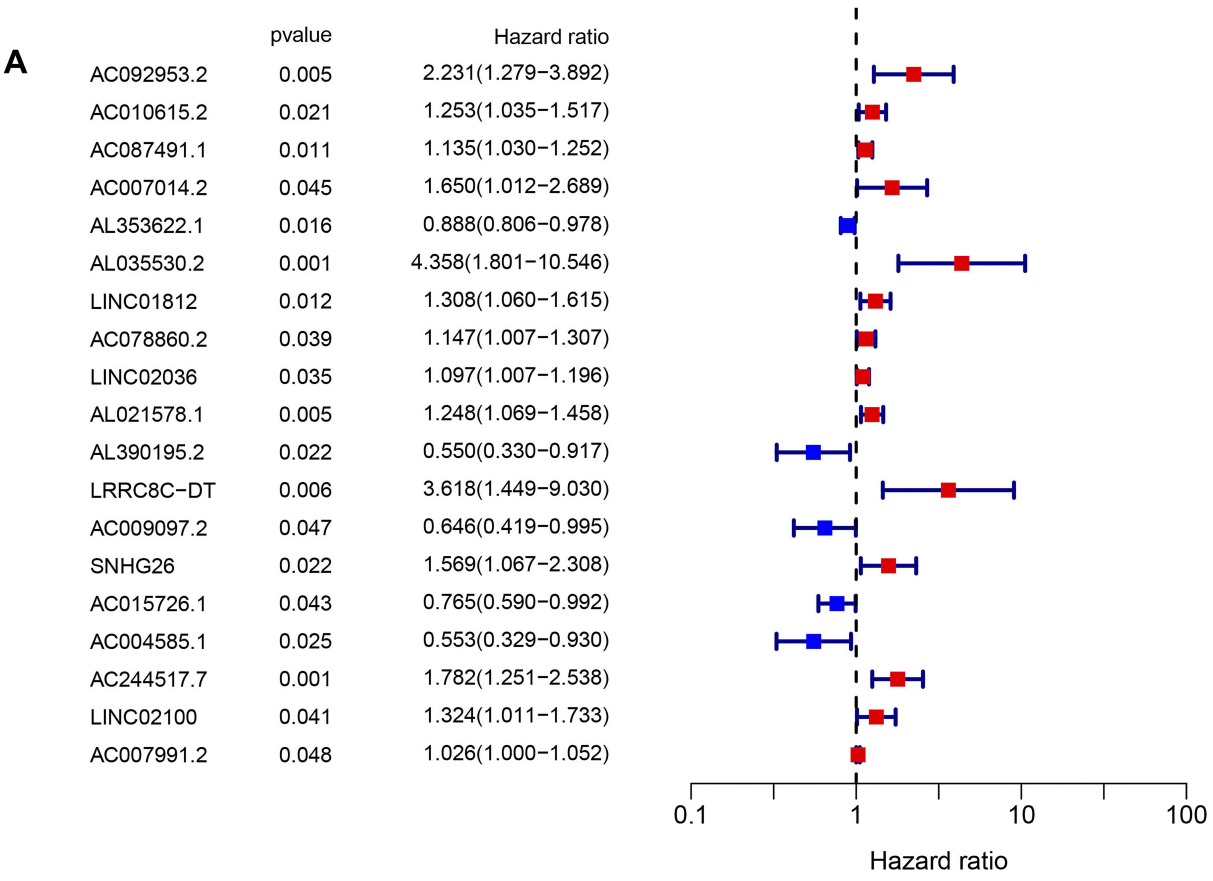
Identification of 9 Pyroptosis-Related lncRNAs Prognostic Signature for EC

Based on the survival information of EC patients, univariate Cox regression was applied to screen the expression profiles of the 170 lncRNAs related to pyroptosis. Nineteen differentially expressed and survival-related lncRNAs were determined based on $p < 0.05$ (Figure 1A). By multiple Cox regression analysis, 9 lncRNAs (AC087491.1, AL353622.1, AL035530.2, LINC02036, AL021578.1, AL390195.2, AC009097.2, AC004585.1, and AC244517.7) were further identified for the prognostic signature (Table 1). The regulatory network for these pyroptosis-related lncRNAs and pyroptosis-related genes (including GSDMB, PJVK, SCAF11, NLRP3, and AIM2) was constructed and visualized in Figure 1B.

Constructions and Validations of a Risk Signature Based on Pyroptosis-Related lncRNAs

We used least absolute shrinkage and selection operator (LASSO)-penalized cox regression model to construct a prognostic model based on the 9 pyroptosis-related lncRNAs. The risk score for each sample was calculated based on the expression levels of these 9 lncRNAs. Risk score = 0.27037 * AC087491.1 - 0.08116 * AL353622.1 + 1.17813 * AL035530.2 - 0.13151 * LINC02036 + 0.19490 * AL021578.1 - 0.74903 * AL390195.2 - 0.41496 * AC009097.2 - 0.43031 * AC004585.1 + 0.41271 * AL3 AC244517.7 (Table 1). 542 samples were defined as an entire cohort, which was randomly divided into a training cohort (379 samples) and a testing cohort (163 samples) according the ratio of 7:3. EC samples were divided into high- and low- groups according to the median risk score.

In the training cohort, compared with those in the high-risk group, patients in the low-risk group showed a lower rate of death and a higher rate of survival time (Figure 2A–C). The survival probability of patients in the two groups showed a significant difference (Figure 2D, $P < 0.001$). We employed the time-dependent receiver operating characteristic (ROC) curve to evaluate the predictive role of risk scores on overall survival (OS). The area under the curve (AUC) for 1 year, 2



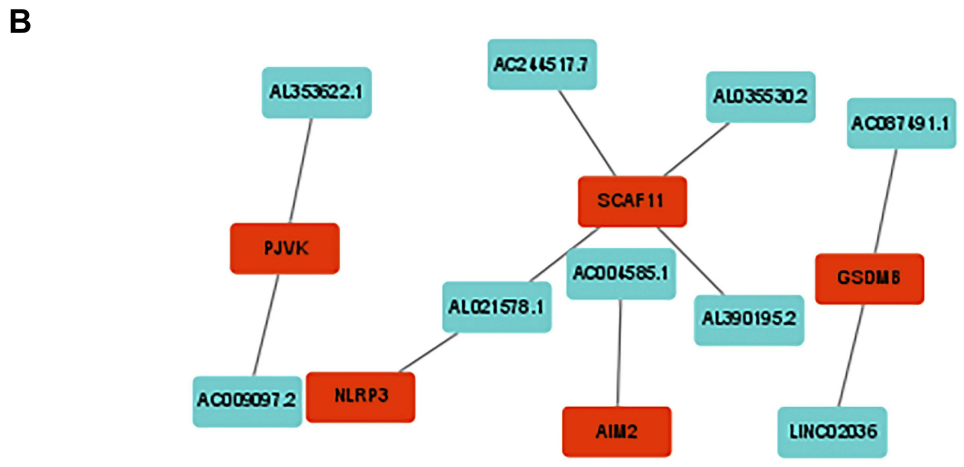


Figure 1 (**A**) Univariate cox regression analysis for each ferroptosis-related lncRNA. (**B**) The regulatory network for a multivariate cox regression analysis of pyroptosis-related lncRNAs and related genes.

Table 1 The Expression Levels of These 9 lncRNAs

LncRNA	Coefficient	HR	HR.95%L	HR.95%H	p-value
AC087491.1	0.27037	1.31045	1.08661	1.58042	0.00467
AL353622.1	−0.08116	0.92204	0.83616	1.01674	0.10372
AL035530.2	1.17813	3.24831	1.19453	8.83323	0.02099
LINC02036	−0.13151	0.87677	0.74584	1.03069	0.11101
AL021578.1	0.19490	1.215191	1.02056	1.44694	0.02864
AL390195.2	−0.74903	0.47283	0.26329	0.84912	0.01216
AC009097.2	−0.41496	0.66037	0.42205	1.03327	0.06926
AC004585.1	−0.43031	0.65031	0.41583	1.01699	0.05928
AC244517.7	0.41271	1.51091	0.99372	2.29729	0.05355

Abbreviations: HR, hazard ratio; L, low; H, high.

years, 3 years was 0.693, 0.694, 0.750, respectively (Figure 2E). We further used the multi-indicator ROC curve analysis to contrast the predictive efficacy between the risk score and the clinical features. We found that the predictive accuracy of our risk model was the best compared with age and tumor grade (AUC: 0.693, 0.576, and 0.657; respectively. Figure 2F). We validated the prognostic value of this risk signature in both the testing and entire cohorts. The distributions of expression of 9 lncRNAs, risk score, and survival status in the testing and entire groups were shown in Figure 3A–C and Figure 4A–C. The survival probability of patients in the two risk groups showed a significant difference in both testing (P = 0.002) and entire cohorts (P < 0.001) (Figures 3D and 4D). The ROC analysis showed satisfactory prognostic accuracy in the testing (Figure 3E) and entire sets (Figure 4E). Additionally, our risk model behaved better in predictive accuracy than age and tumor grade in the testing (Figure 3F) and entire sets (Figure 4F).

Independent Prognostic Value of the 9-lncRNAs Signature

The risk score was a reliable independent risk factor connected with OS (P < 0.001, HR:2.172, 95% CI:1.532–3.079) (Figures 5A and B). The result was validated in both the testing (Figures 5C and D) and entire (Figure 5E and F) cohorts.

Immunity Expression Based on the Risk Model

There was a significant difference between the 2 groups in the expression of immune checkpoints (Figure 6A). Correlation analysis between immune cell subpopulations and related functions based on ssGSEA showed that co-stimulation antigen-presenting cells (APC), chemokine receptors (CCR), checkpoint, cytolytic-activity, human leukocyte antigen (HLA), regulation of inflammation, co-stimulation and co-inhibition of T cells, type I and type II IFN response were significantly different between the low-risk and high-risk groups (Figure 6B).

Immunotherapy Assessment of the Pyroptosis-Related lncRNAs Signature

In our risk model, the relative probabilities of response to anti-PD-1 and anti-CTLA-4 treatments in the low-risk group were higher compared with those in the high-risk group (p < 0.05) (Figure 7A–C). The risk scores in the high-frequency microsatellite instability (MSI-H) group were lower than that in the microsatellite stability (MSS) group (p < 0.05) (Figure 7D). No significant difference was found between neither the low-frequency microsatellite instability (MSI-L) group and MSS group nor the MSI-H group and MSI-L group (Figure 7D).

Discussion

In the present study, a prognostic signature was developed based on long non-coding RNAs (lncRNAs) related to pyroptosis using The Cancer Gene Atlas (TCGA) dataset. This signature consisted of 9 pyroptosis-related lncRNAs. Among the 9 lncRNAs, AC004585.1 has been reported to predict the outcomes of patients with breast cancer.[27] However, there is a lack of detailed information for other lncRNAs.

The development of risk models might be beneficial to predict the prognosis of various cancers. More and more studies try to establish signatures based on lncRNAs and improve the clinical outcome of related diseases. For example, lncRNAs can provide valuable information in diagnosis and prognosis and offers predictive value in

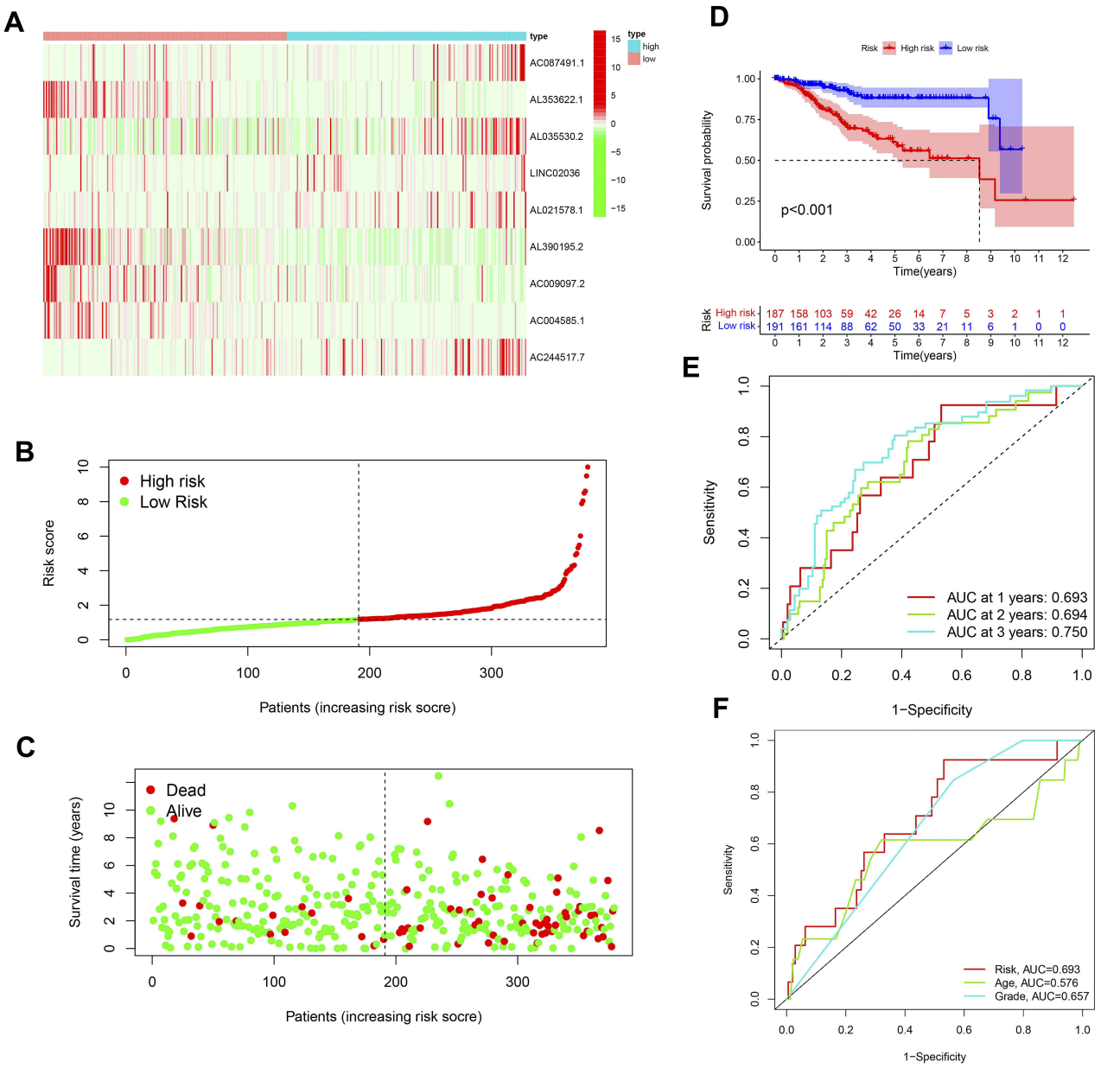


Figure 2 Identification of the pyroptosis-related lncRNA signature in the training cohort. (**A**) Distributions of related lncRNAs in the signature. (**B**) Distribution of patients based on the median risk score. (**C**) The survival status for each patient. (**D**) Kaplan–Meier curves for the EC of patients in the high- and low-risk groups. (**E**) The AUC for the prediction of 1, 3, 5-year survival rate of EC. (**F**) Multi-indicator ROC curves for risk score, age, and tumor grade.
Abbreviation: AUC, area under the curve.

melanoma. In particular, levels of UCA1 and MALAT-1 are significantly higher in patients with melanoma and are correlated to the stage of the disease.[28] In a pan-cancer study, a signature based on 5 lncRNAs was found to behave well in predicting survival outcomes of cancers based on the TCGA, TARGET, and National Cancer Institute cohorts.[29] After exploring the lncRNAs in colorectal cancer, HOXA11-AS, MEG3, SLCO4A1-AS1, SPINT1-AS1, and DANCR lncRNAs were discovered strong power for the diagnosis.[30] Four-methylated LncRNAs were considered as biomarkers for predicting the survival of osteosarcoma.[31] The prognosis of diffuse large B-cell lymphoma might be predicted by a signature based on 6 lncRNAs.[32] In bladder cancer of TCGA cohort, 7 lncRNAs related to immune could be used to predict the prognosis of patients with bladder cancer, and the immune statuses were different in 2 risk groups.[33] Besides, up-regulated lncRNA ZFAS1 was connected with increased cell proliferation and epithelial-mesenchymal transition, and it could be a poor prognostic indicator for endometrial

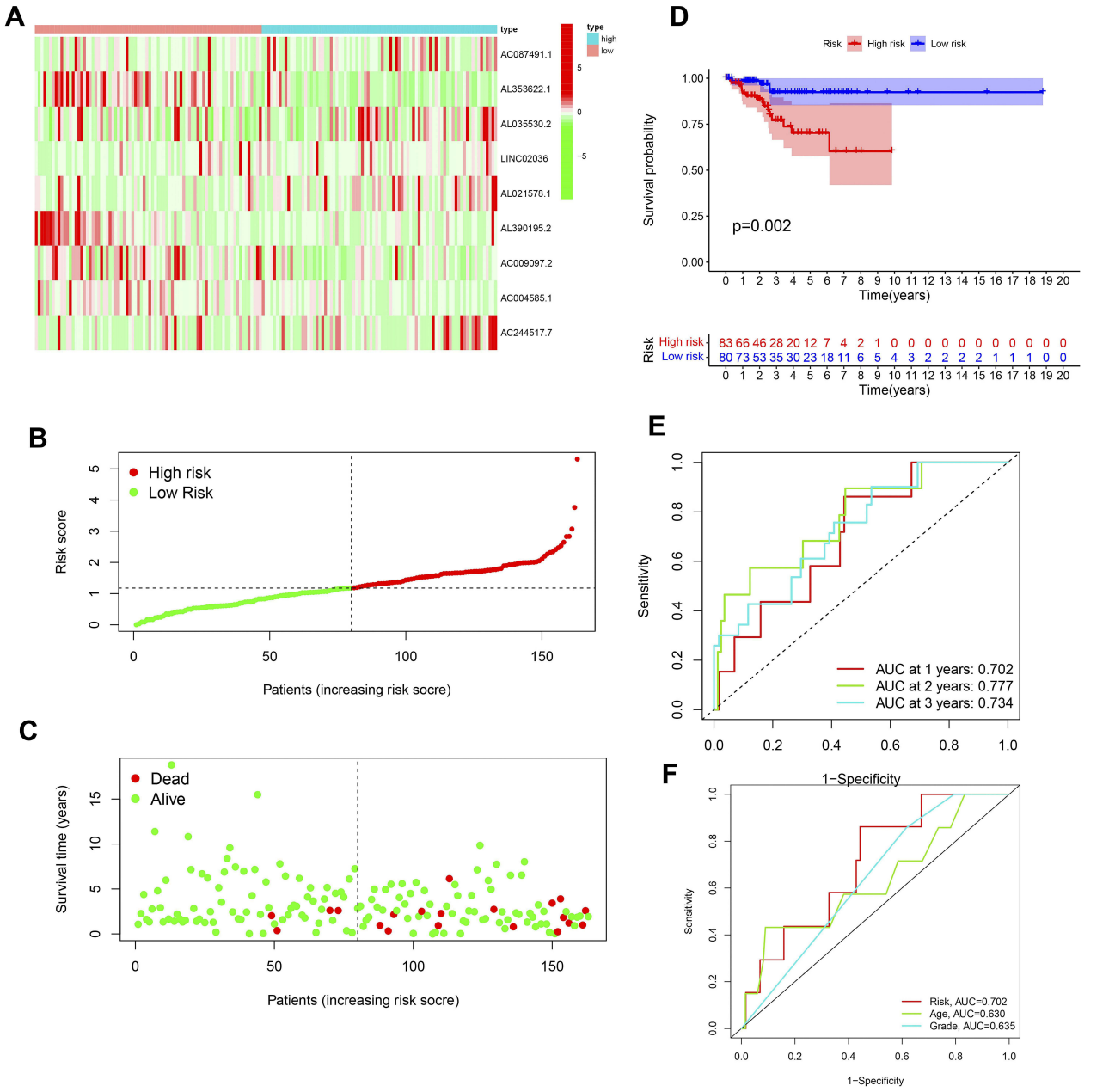


Figure 3 Identification of the pyroptosis-related lncRNA signature in the testing cohort. (**A**) Distributions of related lncRNAs in the signature. (**B**) Distribution of patients based on the median risk score. (**C**) The survival status for each patient. (**D**) Kaplan–Meier curves for the EC of patients in the high- and low-risk groups. (**E**) The AUC for the prediction of 1, 3, 5-year survival rate of EC. (**F**) Multi-indicator ROC curves for risk score, age, and tumor grade.
Abbreviation: AUC, area under the curve.

carcinoma (EC).[34] Similarly, the risk model based on 9 pyroptosis-related lncRNAs could be considered as an independent prognostic marker for the prognosis of EC, and the predictive accuracy of the risk model was superior to age and tumor grade.

The tumor microenvironment (TME) is comprised of extracellular matrix, fibroblasts, endothelial cells, neurons, and multiple immune cells.[35] Among them, the Interaction between lymphoma cells and the TME is essential for the survival and proliferation of a large number of tumors.[36] In recent years, people have been trying to find novel treatment methods for EC. To our delight, immunotherapy brings new insight into it. Growing evidence shows that the status of tumor-infiltrating lymphocytes (TILs) plays important roles in the prognostic and has potentially predictive significance for various tumor types. A chronic inflammatory reaction represented by TILS and plasma cells is associated with an improved prognosis of the

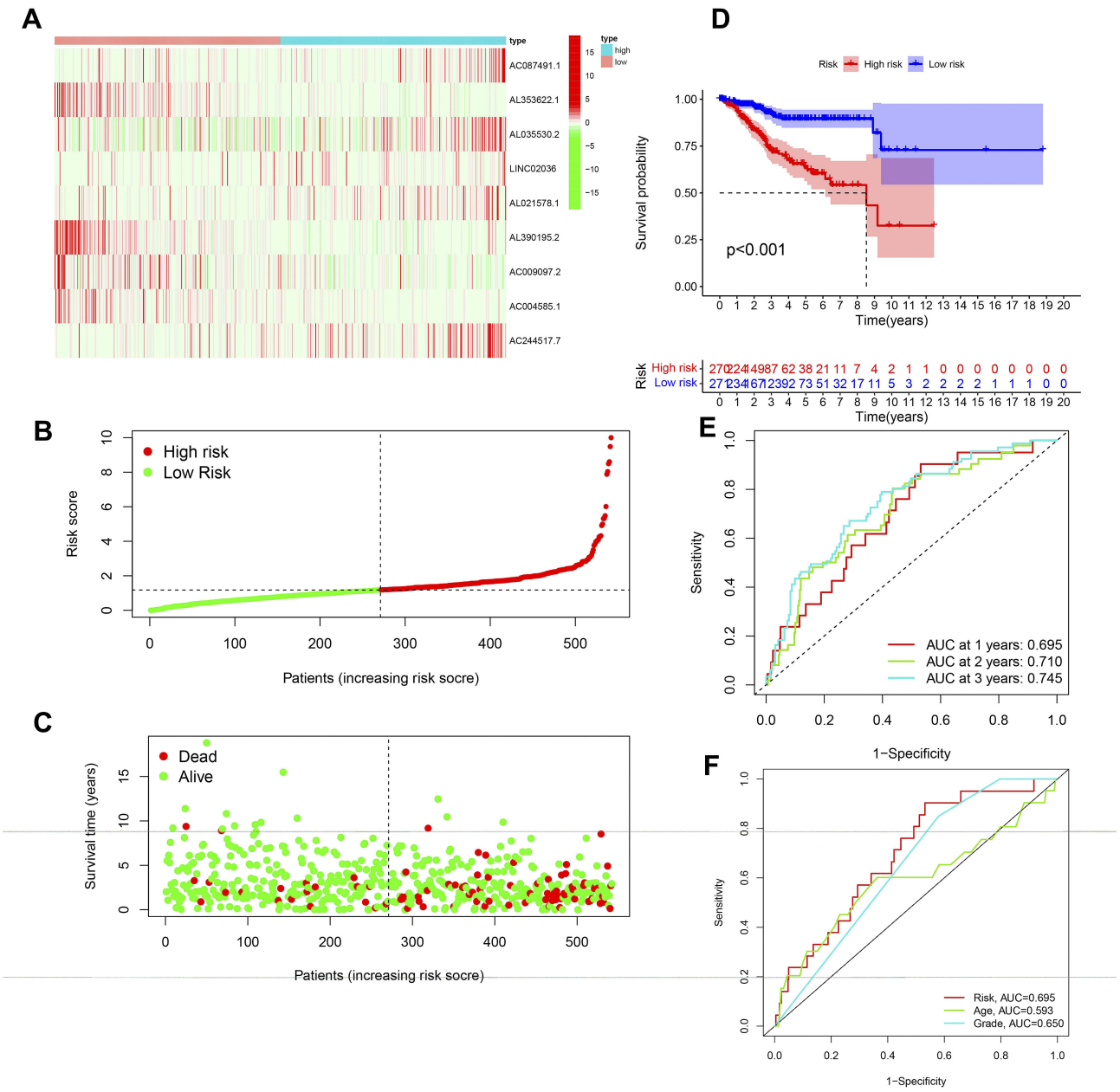


Figure 4 Identification of the pyroptosis-related lncRNA signature in the entire cohort. (**A**) Distributions of related lncRNAs in the signature. (**B**) Distribution of patients based on the median risk score. (**C**) The survival status for each patient. (**D**) Kaplan–Meier curves for the EC of patients in the high- and low-risk groups. (**E**) The AUC for the prediction of 1, 3, 5-year survival rate of EC. (**F**) Multi-indicator ROC curves for risk score, age, and tumor grade.
Abbreviation: AUC, area under the curve.

malignant mesothelioma that could be responsive to immunotherapy.[37] Clinical outcomes of EC were found improved with the increased tumor-infiltrating of CD8 (+), FoxP3(+), and CD45R0(+) T-lymphocytes.[38] The infiltrated number of intraepithelial CD8(+) T, or CD3(+) lymphocytes at the invasive border was considered an independent prognostic factor of survival for EC patients.[39,40] Identification of immune cells in the EC is beneficial for prognosis and clinical improvement. In the present risk model, different prognoses were found and various cellular components were shown in the high-and low-risk groups, which might be applied to guide clinical diagnosis and treatment.

ECs are genetically divided into DNA polymerase epsilon (POLE), high-frequency microsatellite instability (MSI-H), copy number high, and copy number low groups.[41] In 27 types of cancers, EC was found with the highest microsatellite instability (MSI) by using an MSI-

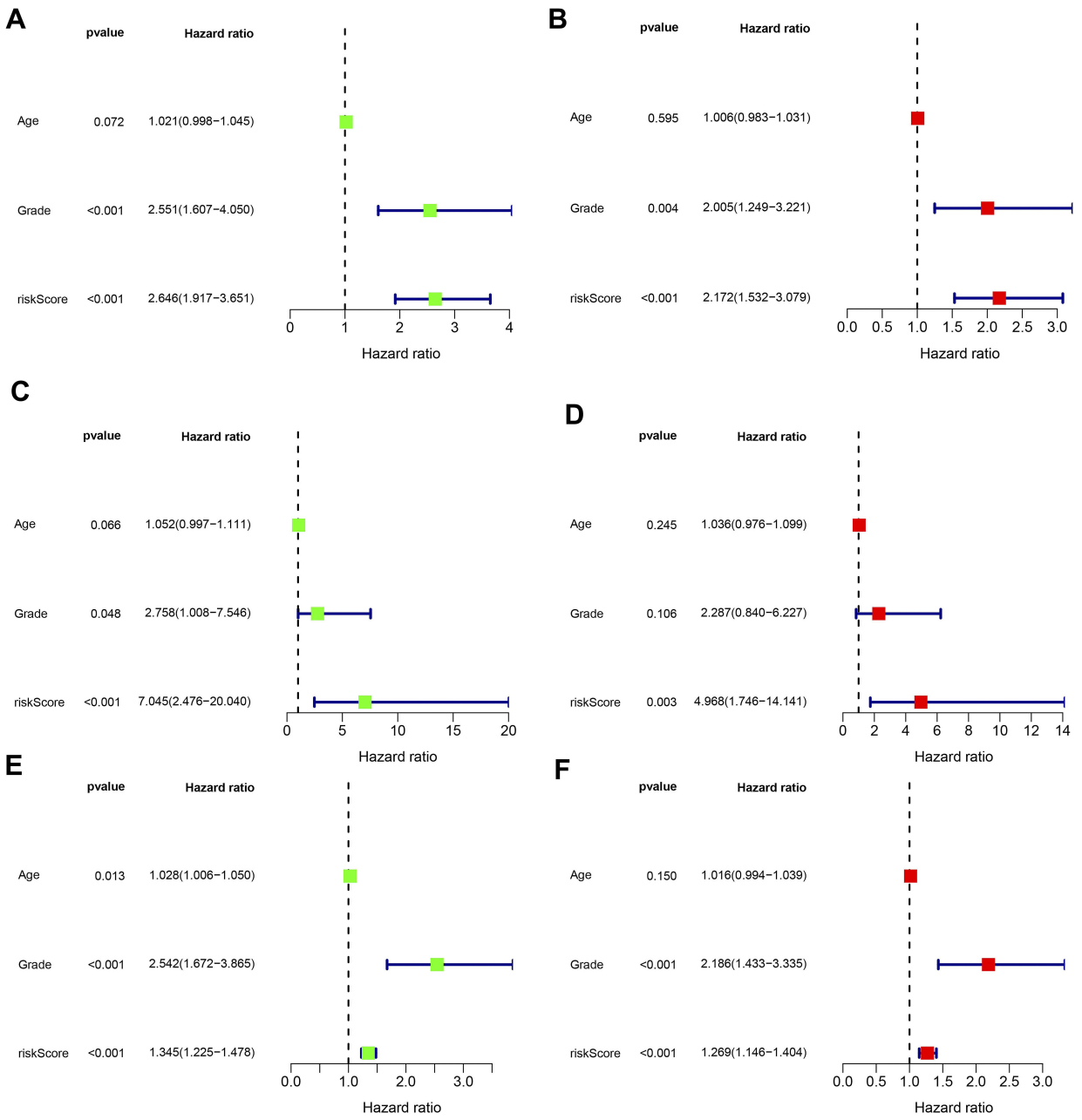


Figure 5 Cox regression analysis was used to assess the independent prognostic value of the risk score. Training cohort: (**A**) univariate cox regression analysis, (**B**) multivariate Cox regression analysis. Testing cohort: (**C**) univariate cox regression analysis, (**D**) multivariate cox regression analysis. Entire cohort: (**E**) univariate cox regression analysis, (**F**) multivariate cox regression analysis.

calling software.[42] Overexpressed PD-1 and PD-L1 were found in ultramutated polymerase e and microsatellite instability (MSI) ECs, which were accompanied by high neoantigen loads and a large number of tumor-infiltrating lymphocytes.[43] That makes ECs with POLE-mutation and MSI suitable for immunotherapies. Immune checkpoint therapy (ICT) has been developed as a novel treatment method, including monotherapy, combination with cytotoxic chemotherapy, other immunotherapy, or targeted agents.[44] The immune tolerance in ECs with a high mutation rate or a high mutation burden is less likely to occur, which makes they are more easily recognized and targeted by ICT-induced T cells. In our risk signature, the relative probabilities of response to ICT treatment in the low-risk group were higher than those in the high-risk group. At the same time, the risk scores in the high-frequency microsatellite instability (MSI-H) group were lower than those in the microsatellite stability (MSS) group. The

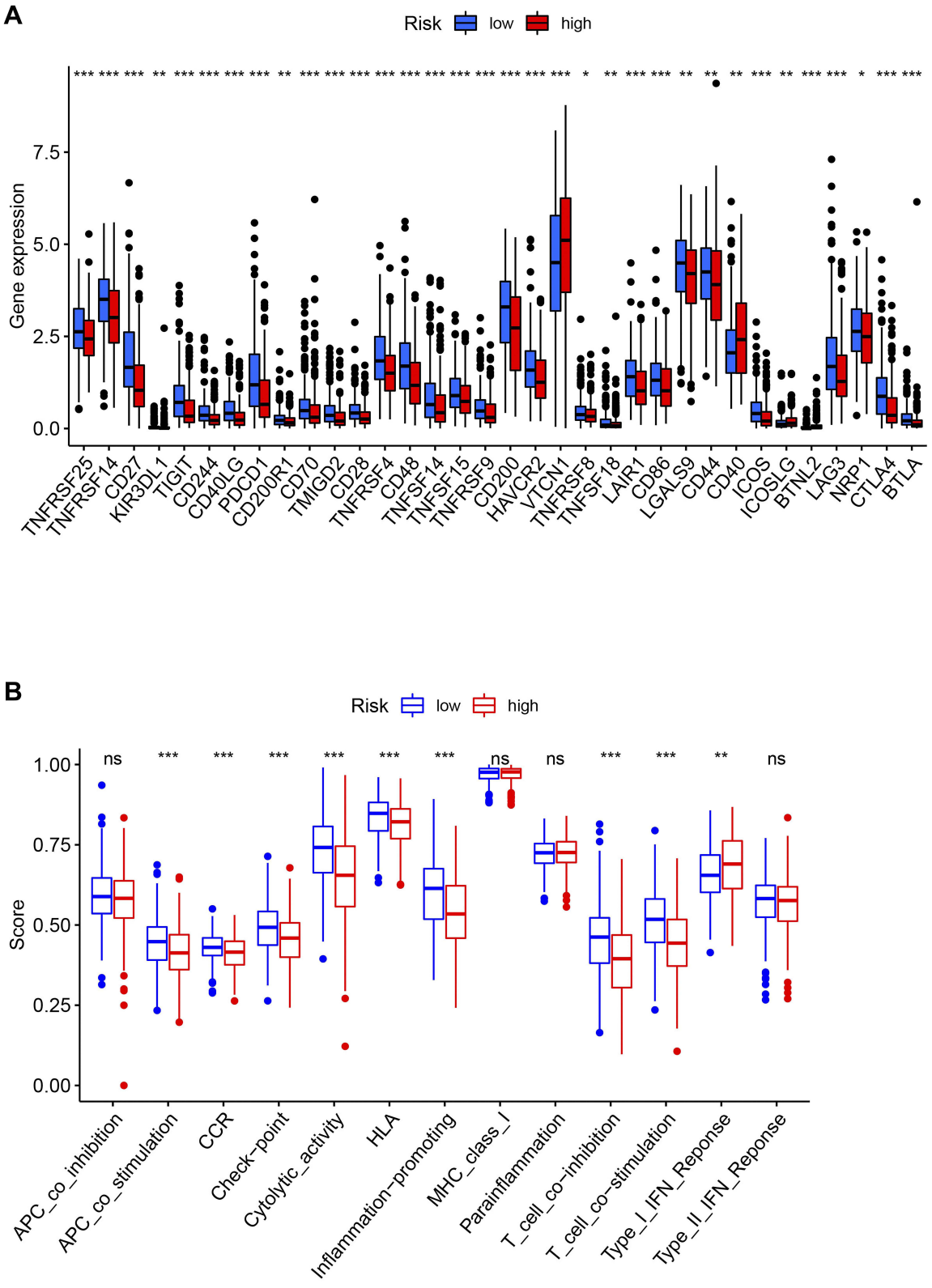


Figure 6 (**A**) Immune checkpoints between high- and low-risk groups. (**B**) ssGSEA for the association between immune cell subpopulations and related functions. *p<0.05, **p<0.01, ***p<0.001.

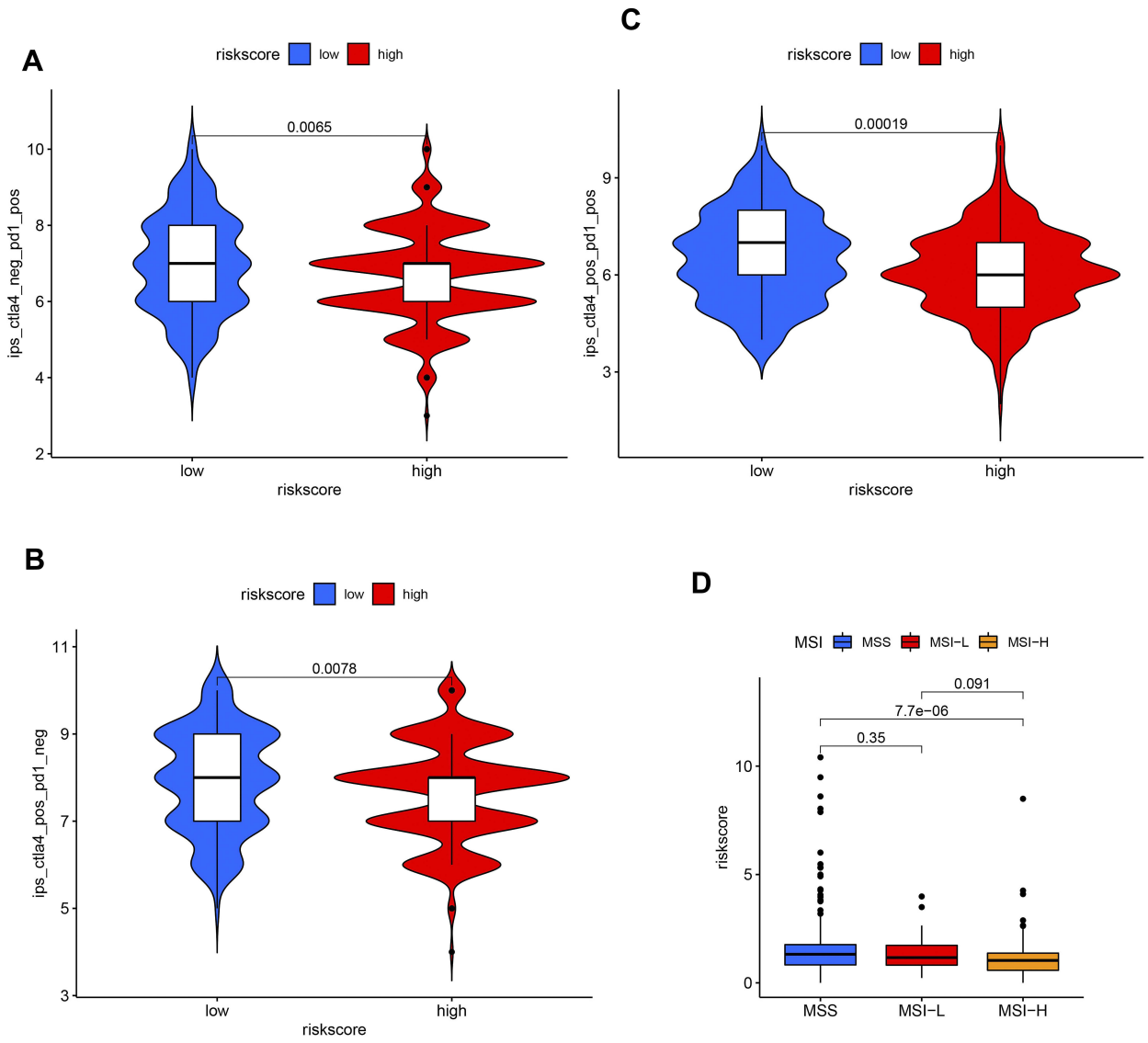


Figure 7 The immunotherapy evaluation based on the risk score. (**A**) With negative CTLA4 and positive PD-1, (**B**) with positive CTLA4 and negative PD-1, (**C**) with positive CTLA4 and positive PD-1, (**D**) risk scores in different microsatellite statuses.
Abbreviations: Ips, immunophenoscore; neg, negative; pos, positive.

results implied that patients with EC in the low-risk group might be with ideal targets for Immunotherapy.

There are some limits to the present study. First, our signature needs further validation by prospective large-scale randomized controlled studies with more clinical samples in the future. Second, work to explore the potential functions of pyroptosis-related lncRNAs is needed to explain the mechanisms of those lncRNAs in EC.

Conclusion

In summary, 9 pyroptosis-related lncRNAs were found useful in predicting the prognosis and immunotherapy of EC. The risk signature based on the 9 pyroptosis-related lncRNAs could well class EC patients and may be applied in guiding immunotherapy for patients with EC.

Data Sharing Statement

All data can be acquired from the corresponding author.

Ethics Approval and Consent to Participate

Our study had been reviewed by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. Ethical approval and informed consent were waived.

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Disclosure

The authors declare that they have no competing interests.

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