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Identification of a pyroptosis-associated long non-coding RNA signature for predicting the immune status and prognosis in skin cutaneous melanoma

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Abstract. – OBJECTIVE: Pyroptosis is correlated with programmed tumor cell death and the tumor microenvironment. However, the prognostic value of pyroptosis-associated long non-coding RNAs (lncRNAs) in skin cutaneous melanoma (SKCM), a malignant tumor with a poor prognosis, has not been established.

PATIENTS AND METHODS: In this study, expression profiles and clinical data of patients with SKCM were downloaded from The Cancer Genome Atlas (TCGA) database to identify differentially expressed pyroptosis-related lncRNAs related to overall survival. A lncRNA risk signature was constructed by Cox regression analyses and its prognostic value was evaluated. Associations between the lncRNA signature and immune status, immune microenvironment, tumor stemness, immune checkpoints, and m6A-related genes were further evaluated.

RESULTS: Twenty-two pyroptosis-related lncRNAs were identified and incorporated into a prognostic risk signature. The signature was significantly correlated with overall survival, tumor growth, and metastasis in SKCM. The signature demonstrated better diagnostic accuracy than conventional clinicopathological characteristics. A gene set enrichment analysis indicated that the risk signature was enriched in several immune-related pathways. Furthermore, the risk signature was significantly correlated with the immune microenvironment, immune cell infiltration, and immune subtypes, as well as tumor stem cells and some m6A-related genes. The lncRNA expression levels were also significantly related to responses to several anti-tumor drugs. Finally, a nomogram based on the risk score was established.

CONCLUSIONS: Overall, a risk signature based on 22 pyroptosis-associated lncRNAs was generated, providing a novel perspective on the determinants of prognosis and survival in SKCM and a basis for the development of individualized treatments.

Key Words:
Skin cutaneous melanoma, Pyroptosis, Long non-coding RNA, Prognosis, Gene signature, Immune status.

Introduction

Skin cutaneous melanoma (SKCM) is a destructive malignant tumor and an important threat to human health[1]. In 2018, 287,723 new patients were diagnosed with melanoma worldwide, and 21.1% of these patients died from the disease[2]. Patients with SKCM have a 10-year overall survival rate of 75-98% when diagnosed at stages I and II[3] compared with 24-88% when diagnosed at stages IIIA to IIID, suggesting that early diagnosis is essential for favorable outcomes. It has been proposed that tumorigenesis and progression are related to skin pigmentation[4]. The pathogenesis is unclear but is associated with acquired melanocytic nevi, a family history, and genetic susceptibility[5,6]. Despite a recent work[7] focused on uncovering new diagnostic and prognostic biomarkers, only a few clinically relevant biomarkers and tools for SKCM are available, and the identification of additional biomarkers is needed.

Pyroptosis is a newly described type of lytic cell death characterized by bubble-like protrusions and cell swelling[8]. During the process of pyroptosis, the gasdermin family, the main executor of pyroptosis[9], can form pores of 10-20 nm in the cell membrane. The cell contents continuously flow out from the membrane pores, and cells produce apoptotic vesicle-like protrusions and gradually swell until rupture[10,11]. Pyroptosis plays a key role in the development and progression of

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cancer. Various pyroptotic components, such as gasdermin family genes, proinflammatory cytokines, and inflammatory vesicles, are closely related to tumorigenesis and metastasis[12]. Unlike apoptotic cells, pyroptotic cells can activate and release various danger-associated signaling cytokines to induce a strong inflammatory response and immune system activation[13]. In addition, the potent anti-tumor properties of pyroptosis are related to the modulation of the immune microenvironment (NK cells[14] and CD8+ T lymphocytes[15]) in tumors. Although the role of pyroptosis in tumor-related processes is widely known, its specific role in SKCM is unclear.

Long non-coding RNAs (lncRNAs) are a subset of non-coding RNAs longer than 200 base pairs. In addition to various cellular biological processes, lncRNAs contribute to tumor progression, including tumorigenesis, cell proliferation, and tumor metastasis[16,17]. A bioinformatics analysis has identified approximately 246 lncRNAs that are differentially expressed in melanoma, many of which are associated with overall survival[18]. LncRNAs in melanoma cells contribute to cell cycle progression, apoptosis, and cell invasion and migration. Furthermore, lncRNAs influence the chemotherapeutic sensitivity of melanoma cells[19]. Based on this, lncRNAs are key determinants of prognosis in SKCM. However, systematic analyses aimed at the identification of hub pyroptosis-associated lncRNAs associated with prognosis or progression are lacking.

Bioinformatics approaches have been used to identify disease-specific biomarkers for SKCM. However, effective prognostic signatures based on pyroptosis-related lncRNAs have not yet been developed. In this study, candidate pyroptosis-related lncRNAs related to SKCM prognosis were identified by a differential gene expression analysis and univariate Cox regression analysis. Subsequently, a Lasso penalized Cox regression analysis was used to characterize the hub lncRNAs and construct a risk signature. The functions and clinical significance of the newly developed lncRNA risk signature were explored. We also systematically investigated the association between the pyroptosis-related lncRNA signature and immune microenvironment, immune cell infiltration, cancer chemoresistance, tumor stemness, and m6A-related genes. To the best of our knowledge, our results provide the first pyroptosis-associated lncRNA signature for the prediction of prognosis, providing novel insights into the diagnosis and prognosis of SKCM.

Patients and Methods

Raw Data Acquisition

RNA and lncRNA sequencing data were collected for 471 SKCM tissues and one normal skin sample from The Cancer Genome Atlas (TCGA) database on June 30, 2020 (https://portal.gdc.cancer.gov). Clinical information is shown in Table I. The transcriptomic data for 812 normal skin samples were obtained from the Genotype-Tissue Expression database (GTEx; https://gtexportal.org/home/datasets). Log2-transformation and normalization were performed using the "sva" package in R to remove batch effects[20,21]. Furthermore, 146 protein domains for specific pyroptosis-related genes were collected from the GeneCards database (https://www.genecards.org) and are presented in **Supplementary Table I**.

Prognostic lncRNA Signature Construction

After assessing the association between pyroptosis-associated lncRNAs and SKCM by a Pearson correlation analysis ($|R^2| > 0.7$, $p < 0.001$), differentially expressed pyroptosis-associated lncRNAs were identified using the "limma" package. lncRNAs with |log2 fold change| > 1 and false discovery rate (FDR) < 0.05 between normal and tumor tissues were regarded as candidate pyroptosis-associated lncRNAs. Then, univariate Cox regression analyses were performed to identify prognostic lncRNAs among all pyroptosis-associated lncRNAs in SKCM using the "survival" package with a cutoff of $p < 0.05$. The overlap

Table I. The clinical characteristics of SKCM patients in the TCGA dataset.

Variable	Number of samples
Age	
≤ 60/ > 60/Unknown	250/212/8
Gender	
Male/Female	290/180
T stage	
T0/ T1/ T2/ T3/ T4/Unknown	23/42/78/90/153/84
N stage	
N0/ N1/ N2/ N3/Unknown	235/74/49/55/57
M stage	
M0/ M1/Unknown	418/24/28
TNM stage	
0/ I/ II/ III/ IV/Unknown	7/77/140/171/23/52
Metastatic	
Metastatic/Primary	368/102
Metastatic/Primary	368/102

between the differentially expressed lncRNAs and prognosis-related lncRNAs was determined to obtain candidate pyroptosis-related lncRNAs, as visualized by a Venn diagram using the "VennDiagram" package in R. Thereafter, candidate pyroptosis-related lncRNAs were integrated into a Lasso penalized Cox regression analysis to identify hub lncRNAs and to generate a lncRNA risk signature. Next, patients with SKCM were categorized into low- and high-risk groups using the median risk score as a threshold. The risk score was calculated as follows:

$$\text{risk score} = \Sigma \text{explncRNAi} * \beta i$$

where exp lncRNAi is the relative expression of pyroptosis lncRNA i, and β is the regression coefficient[22].

Predictive Value of the LncRNA Signature

To explore the distribution of risk subgroups, t-SNE and PCA analyses of the constructed signature were performed using the "Rtsne" and "ggplot2" packages in R. The "survival" package was further applied to compare overall survival between the two risk subgroups according to risk scores. The "timeROC" package was also applied for both the lncRNA signature and traditional clinical features to verify the predictive accuracy of the signature,. Finally, univariate and multivariate Cox regression analyses were performed to evaluate the relationship between the risk score and clinical characteristics. The "ggpubr" package was applied to visualize these relationships, and a nomogram was constructed using the "rms" package to predict patient outcomes.

Gene Set Enrichment Analysis (GSEA)

For the hub pyroptosis-associated lncRNAs, GSEA 4.1 was used for a Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the two risk subgroups. Statistical significance was defined as FDR < 0.05.

Immune, Stem Cell-like Features, and M6A Correlation Analysis

A Spearman correlation analysis was performed to test the relationship between the risk score and stromal and immune scores. Two-way ANOVA was used to evaluate the connection between the immune infiltration subtype and risk score. A single-sample gene set enrichment analysis (ssGSEA) was used to compare immune cell infiltration in the two risk subgroups and to test immune functions. Potential immune checkpoints retrieved from a previous study were used to explore the connection between immune-related genes and risk signatures[23]. Next, correlations between the risk signature and several key immune regulators, including PD-L1, PD-L2, MRP1, and MRP3, were evaluated. Spearman correlation analyses were used to measure the relationship among the risk score, tumor stemness, and m6A-related genes.

Chemotherapy Sensitivity Analysis

The NCI-60 database and information on 218 FDA-approved chemotherapy drugs were obtained from the CellMiner interface (https://discover.nci.nih.gov/cellminer). A Pearson correlation analysis was then applied to investigate the association between drug sensitivity and the hub pyroptosis-related lncRNAs.

Results

Candidate Prognostic lncRNA Screening

The study workflow is illustrated in Figure 1. First, 77 lncRNAs associated with pyroptosis-related gene expression in patients with SKCM were identified. The correlations between levels of these lncRNAs and pyroptosis-related genes are summarized in **Supplementary Table II**. Based on a differential expression analysis and univariate Cox regression, 54 and 62 lncRNAs were identified as differentially expressed pyroptosis-associated lncRNAs and prognostic lncRNAs, respectively (**Supplementary Tables III and IV**). Based on the overlap, 46 lncRNAs were identified as candidate prognostic lncRNAs (Figure 2A).

Construction of a LncRNA Signature for SKCM

The 46 candidate prognostic lncRNAs were further analyzed by a Lasso penalized Cox regression analysis, and 22 hub pyroptosis-related lncRNAs (AC004847.1, USP30-AS1, AC082651.3, AL033384.1, AC138207.5, AC245041.1, U62317.1, AL512274.1, AC018755.4, MIR200CHG, LINC02362, LINC00861, AL683807.1, AC010503.4, AL512363.1, LINC02437, LINC01527, AL049555.1, AC245041.2, AL365361.1, AC015819.1, and MIR205HG) were ultimately used to construct the risk signature (**Supplementary Table V**). The ex-

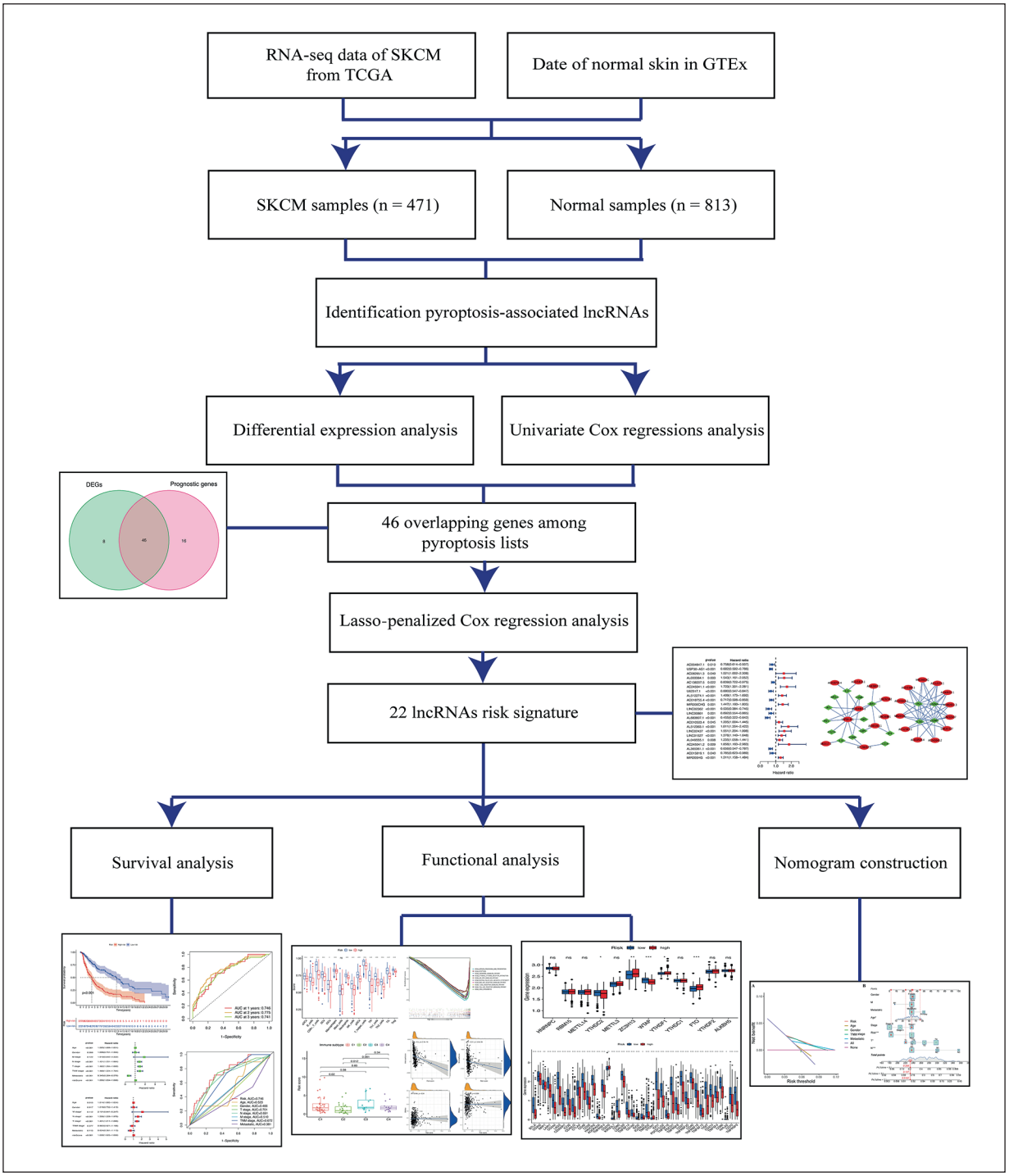

Figure 1. Schema of the study.

pression distribution of these lncRNAs is shown in Figure 2B. A univariate Cox analysis verified the associations between these lncRNAs and prognosis (Figure 2C). We further detected correlations between the levels of these lncRNAs (Figure 2D). A network of the prognostic lncRNAs and their associated mRNAs is shown in Figure 2E. We found significantly higher expression levels of the hub pyroptosis-associated lncRNAs AC004847.1, AC015819.1, AC018755.4, AC138207.5, AL365361.1, AL683807.1, LINC00861, LINC02362, U62317.1, and USP30-AS1 in SKCM samples than in control

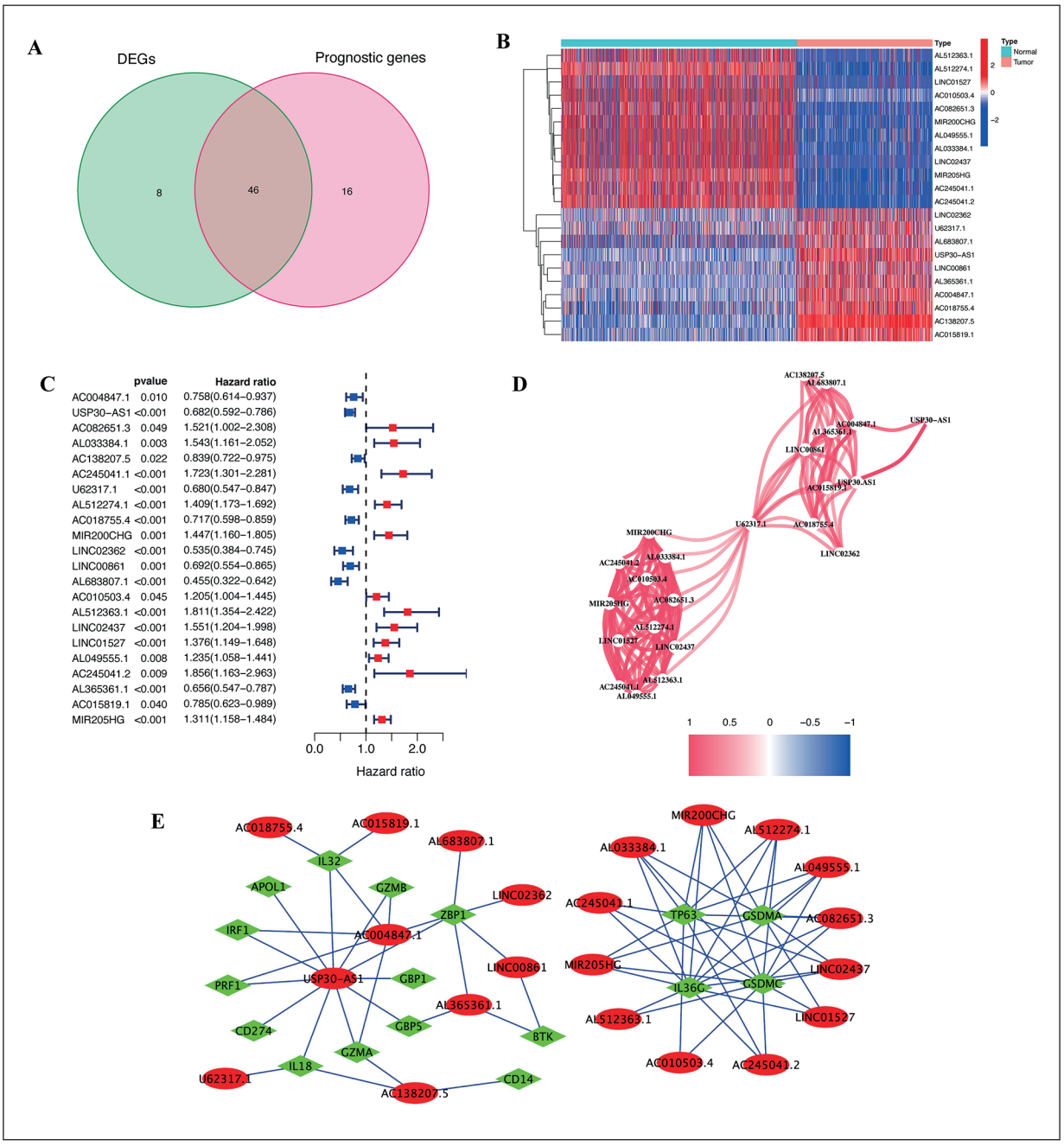

Figure 2. Identification of prognostic pyroptosis-related lncRNAs. **A**, Venn diagram of candidate pyroptosis-related lncRNAs determined by differential expression and univariate Cox analyses. **B**, Heatmap of hub pyroptosis-related lncRNAs. **C**, Forest plots of correlations between hub lncRNAs and overall survival of SKCM patients. **D**, Correlation network of hub lncRNAs. **E**, Correlation network of prognostic lncRNAs and their associated mRNAs.

samples ($p < 0.05$; Supplementary Figure 1A-J). In contrast, the lncRNAs AC082651.3, AL033384.1, AC245041.1, AL512274.1, MIR200CHG, AC010503.4, AL512363.1, LINC02437, LINC01527, AL049555.1, AC245041.2, and MIR205HG were expressed at lower levels in SKCM than in normal tissues ($p < 0.05$; Supplementary Figure 1K-V). Patients with SKCM in TCGA cohort were divided into low- and high-risk subgroups based on the median risk scores (Figure 3A and B). t-SNE and PCA analyses indicated that patients in the two risk subgroups were clearly separated (Figure 3C and D).

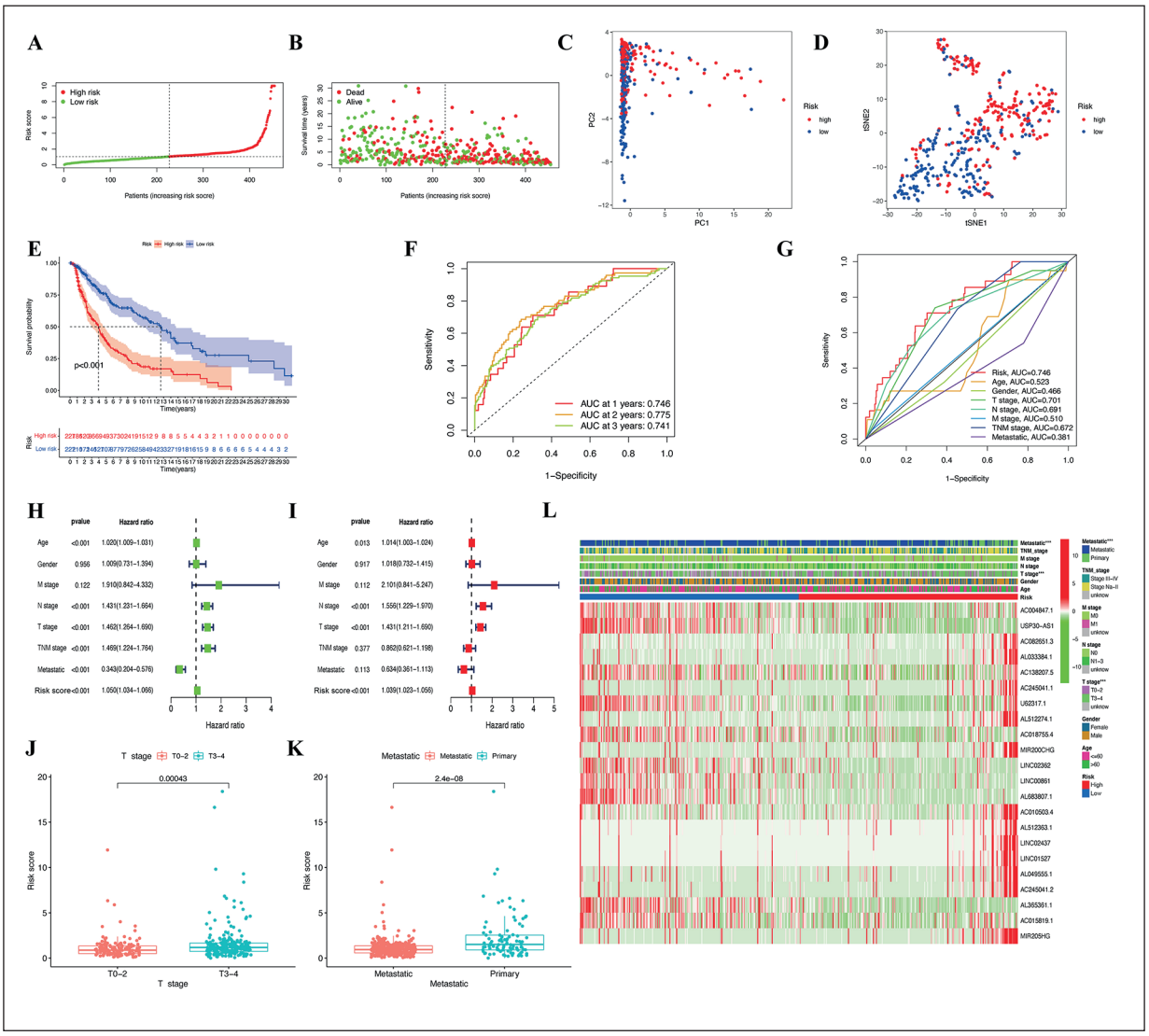

Figure 3. Associations between risk signature and clinicopathological factors. Risk score distribution (**A**), survival status (**B**), PCA plot (**C**), and t-SNE (**D**) analysis of TCGA-SKCM cohort. **E**, Survival curve of SKCM patients. TimeROC (**F**) and ClinicalROC (**G**) curves to forecast overall survival of patients. Univariate (**H**) and multivariate Cox (**I**) regression of clinicopathological features in TCGA-SKCM cohort. Correlations between risk scores and T stage (**J**) and metastatic capacity (**K**). **L**, The heatmap of clinicopathological features and hub lncRNAs expression in two risk subgroups.

Associations Between Clinical Characteristics and Risk Scores in SKCM

The overall survival was lower in the high-risk SKCM group than in the low-risk group in TCGA cohort (Figure 3E). A receiver operating characteristic (ROC) curve analysis indicated that the risk signature had moderate predictive accuracy at 1 (ROC = 0.746), 2 (ROC = 0.775), and 3 (ROC = 0.741) years (Figure 3F). A decision curve analysis and ROC analysis proved that the signature has greater accuracy than other traditional clinicopathological features (Figure 3G and 4A), revealing that our risk signature is a sensitive and specific predictor of overall survival in SKCM.

Multivariate and univariate Cox regression analyses revealed that the newly identified risk signature is an independent prognostic factor for patients with SKCM (Figure 3H and I). Interestingly, a heatmap of clinical features and risk subgroups showed that our gene signature was significantly associated with tumor T stage and the metastatic state (Figure 3L). Patients with SKCM of stages T3–4 had significantly greater risk scores than those with stages T1–2 (Figure 3J). Patients with SKCM who were diagnosed with primary melanoma also showed significant-

ly higher risk scores than patients without primary melanoma ($p < 0.05$; Figure 3K). Finally, the risk signature was used to construct a nomogram to predict patient outcomes (Figure 4B). Overall, the risk signature was clearly associated with the development of SKCM and might be a valuable tool for the clinical management of patients.

Associations with Immunity, Tumor Stemness, and M6A-related Genes

The proportion of nearly all immune cell subpopulations, levels of components of related pathways, and functions were significantly reduced in the high-risk subgroup compared with the low-risk subgroup (Figure 5A and B). Only the scores for mast cells and type II IFN responses did not differ significantly between the two subgroups ($p > 0.05$). Similar results were obtained using EPIC, XCELL, MCP counter, QUANTISEQ, CIBERSORT, and TIMER (Figure 5C). Furthermore, immune infiltrates corresponding to tumor suppression and promotion[24], namely C1 (wound healing), C2 (INF-g dominant), C3 (inflammatory), and C4 (lymphocyte-depleted), were evaluated to understand the connection between immune components and the risk signature. The calculated risk score was significantly lower for the C2 subtype than for other subtypes (Figure 5H).

The immune microenvironment (including immune and stromal scores), tumor stemness (including the RNA stemness score and DNA methylation pattern), and m6A-related genes are key regulators of tumor progression. The constructed risk signature was significantly negatively correlated with the immune and stromal scores (Figure 5D and E) but positively correlated with RNA methylation patterns (RNAss; Figure 5F and G). Expression levels of the m6A-related genes *ZC3H13, YTHDF1*, and *FTO* were significantly higher and *YTHDC2* and *WTAP* were lower in the high-risk subgroup than in the low-risk subgroup (Figure 5I).

With respect to immune checkpoints, the expression levels of all identified immune-related genes were low in the high-risk subgroup, except for *CD276* (Figure 6A). Moreover, considering the roles of the immune checkpoint protein PD-L1 (also known as CD274) and PD-L2 (also known as PDCD1LG2) in immune evasion, the relationship between these loci and the lncRNA signature was analyzed comprehensively. The gene expression levels of *PD-L1* and *PD-L2* were both significantly lower in the high-risk subgroup than in the low-risk group (Figure 6B and C). Expression levels were also significantly negatively correlated with the risk score based on the lncRNA signature (Figure 6D and E).

GSEA

A KEGG pathway enrichment analysis of the two risk subgroups was performed. The lncRNA signature was significantly enriched in 44 pathways (FDR < 0.05) (**Supplementary Table VI**), including antigen processing and presentation, apoptosis, and the chemokine signaling pathway (Figure 7). Several immune-related pathways,

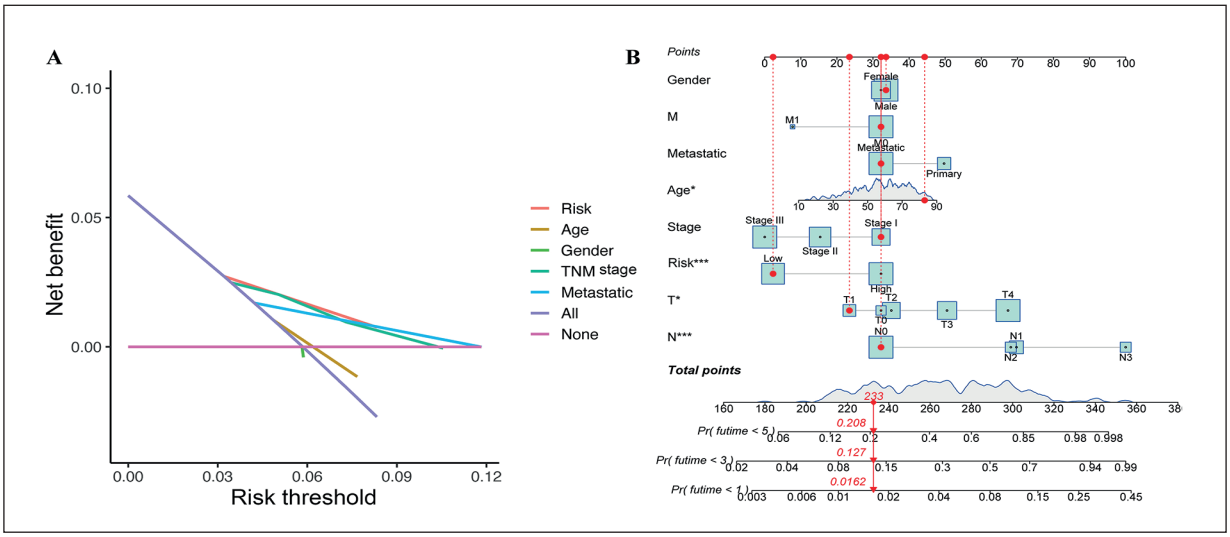

Figure 4. Construction of nomogram. **A**, Decision curve analysis of risk signature and other clinicopathological features. **B**, Nomogram for predicting SKCM 1-, 3-, and 5-year overall survival in TCGA cohort.

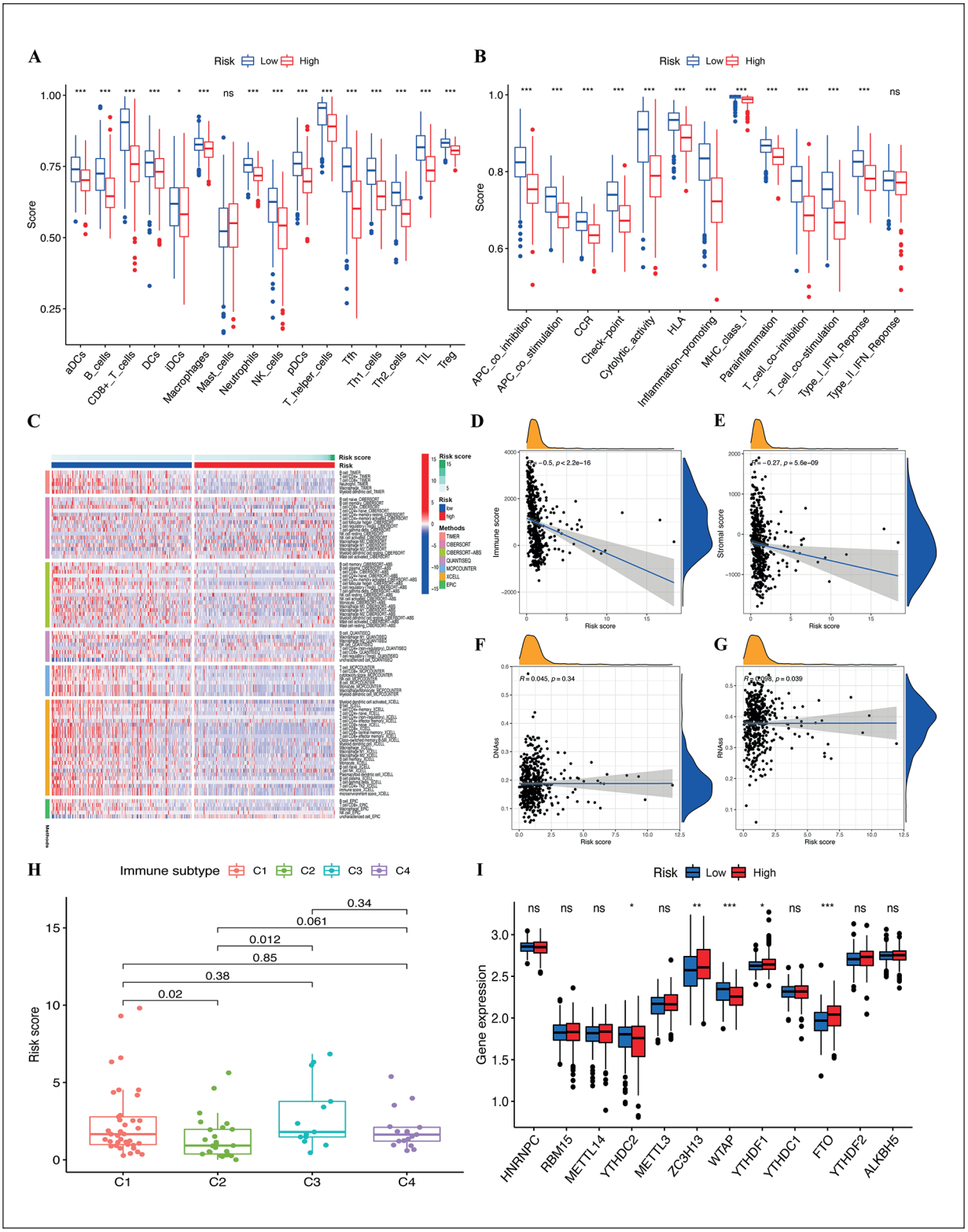

Figure 5. Potential role of risk signature in SKCM immune status, tumor stemness, and m6A-related genes. Boxplots of scores of immune cells (**A**) and immune-associated functions (**B**) in risk subgroups. **C**, Heatmap for immune responses based on EPIC, XCELL, MCP counter, QUANTISEQ, CIBERSORT, and TIMER among two risk subgroups. Associations between risk signature and immune scores (**D**), stromal scores (**E**), DNAss (**F**), RNAss (**G**), immune infiltration subtypes (**H**), and m6A-related genes (**I**).

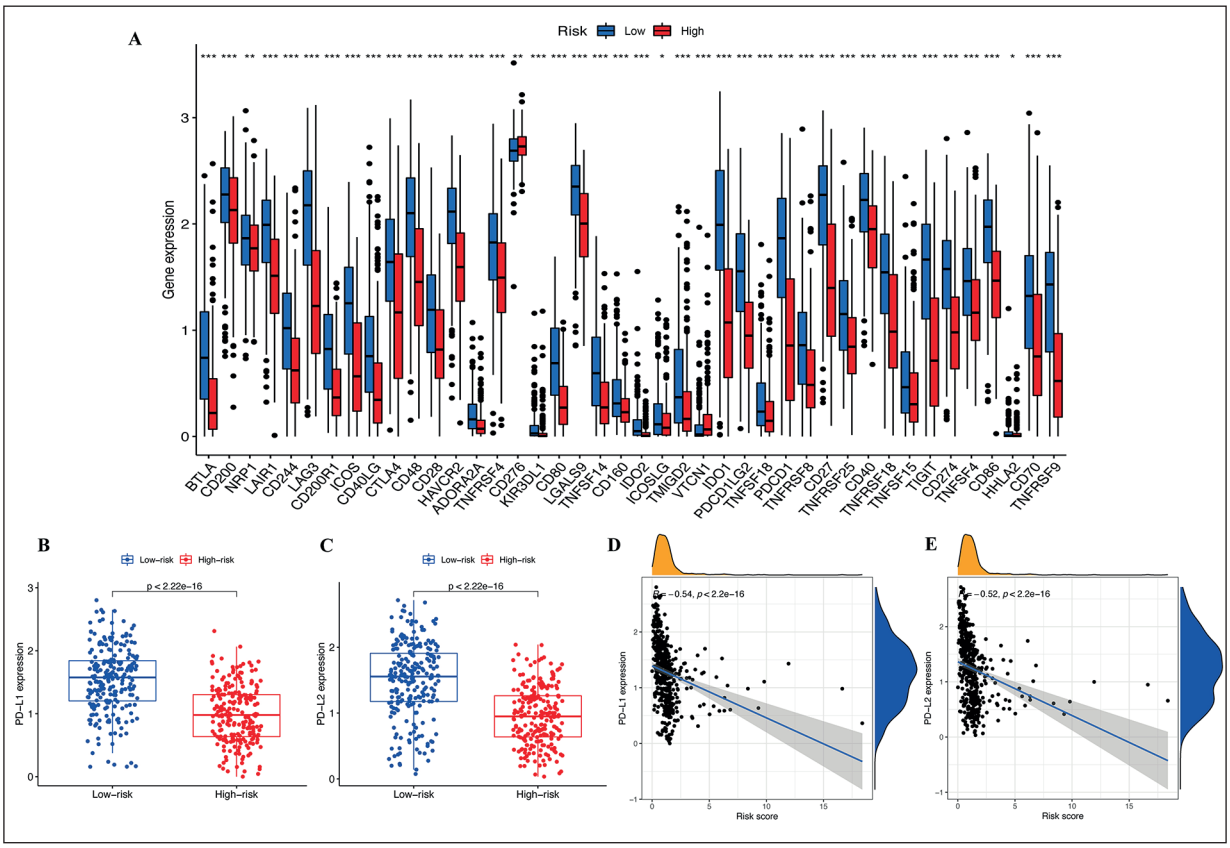

Figure 6. Associations between risk signature and immune checkpoints. **A**, Expression of immune checkpoints among two risk subgroups in SKCM patients. Expression levels of genes PD-L1 (**B**) and PD-L2 (**C**) in risk subgroups. Correlation analysis between risk score, PD-L1 (**D**), and PD-L2 (**E**).

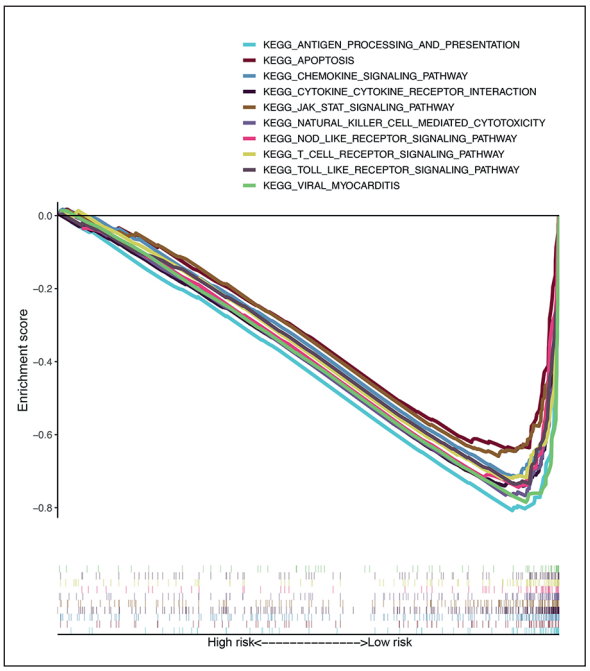

Figure 7. GSEA of top 10 enriched pathways in risk signature.

such as natural killer cell-mediated cytotoxicity, the T cell receptor signaling pathway, and the Toll-like receptor signaling pathway, were also enriched.

Relationship Between Pyroptosis-associated LncRNAs and Drug Sensitivity

As shown in **Supplementary Table VII**, the prognosis-associated lncRNAs were correlated with sensitivity to several drugs ($p < 0.05$). For example, the expression levels of LINC00861 and USP30-AS1 were positively associated with increased tumor cell sensitivity to imatinib, isotretinoin, bendamustine, nilotinib, fluphenazine, nelfinavir, oxaliplatin, megestrol acetate, ifosfamide, palbociclib, etoposide, alectinib, and dromostanolone propionate (Figure 8). In contrast, sensitivity to the chemotherapy drug irofulven was negatively associated with the expression levels of LINC00861 and USP30-AS1 in tumor cells.

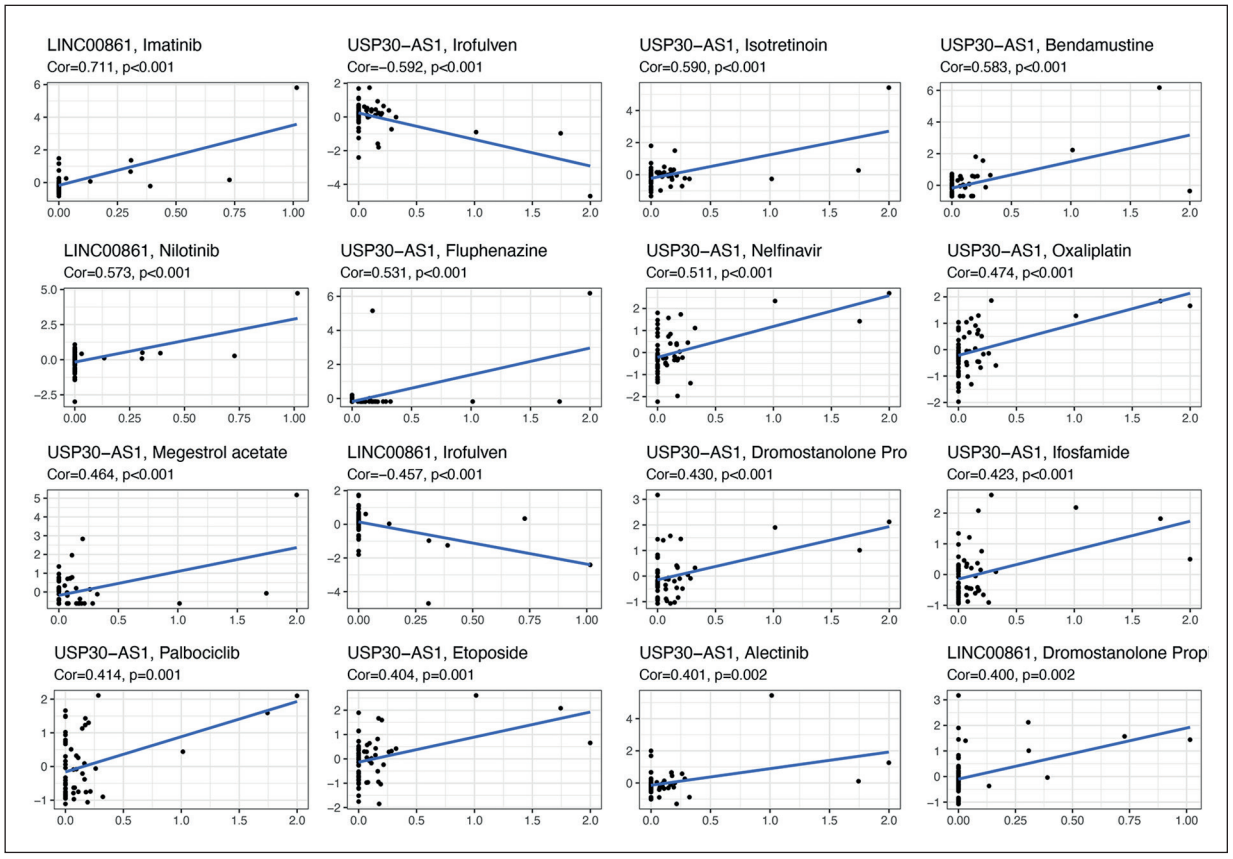

Figure 8. Scatter plots of top 16 classes of associations between hub pyroptosis lncRNAs and drug sensitivity.

Discussion

Although next-generation sequencing technology has resulted in the discovery of various biomarkers for melanoma, there is still a need for novel markers that are more closely associated with early detection and prognosis in SKCM. Pyroptosis, which is significantly correlated with programmed cell death, has good prognostic performance in ovarian cancer[25]. However, its role in SKCM has not been systemically studied, and a pyroptosis-associated lncRNA signature has not been reported. Similar to previously established risk signatures, such as immune checkpoint-related signatures[26], ferroptosis-related signatures[27], and hypoxia-related signatures[28], our newly identified risk signature showed high predictive accuracy for overall survival in SKCM. The lncRNA signature was also associated with the immune status, tumor microenvironment, immune components, m6A-related genes, tumor stemness, and chemotherapeutic drug sensitivity, presenting an advantage over other risk signatures.

In the present study, 146 pyroptosis-related genes were systematically analyzed to identify those associated with overall survival. Next, 22 hub lncRNAs, including AC004847.1, USP30-AS1, AC082651.3, AL033384.1, AC138207.5, AC245041.1, U62317.1, AL512274.1, AC018755.4, MIR200CHG, LINC02362, LINC00861, AL683807.1, AC010503.4, AL512363.1, LINC02437, LINC01527, AL049555.1, AC245041.2, AL365361.1, AC015819.1, and MIR205HG, were used to construct a novel prognostic signature for SKCM. The prognostic value for SKCM was verified by various approaches. Furthermore, the signature was correlated with metastasis and T stage.

The American Joint Committee on Cancer (AJCC) staging system is a widely used clinicopathological parameter for tumor evaluations. However, there is increasing evidence that the AJCC staging model is not suitable for comprehensive analyses of tumor behavior and is not accurate in SKCM diagnostics[29]. Compared with the TNM stage, irrespective of whether T, N, and

M stages are considered separately or together, the constructed risk signature not only showed higher accuracy for the prediction of prognosis but also could be used to predict SKCM growth and metastatic potential. A nomogram analysis revealed the effectiveness of this lncRNA signature for predicting the outcomes of patients with SKCM.

Based on a GSEA, the risk signature was enriched in several immune-related pathways, such as natural killer cell-mediated cytotoxicity, the T cell receptor signaling pathway, and the Toll-like receptor signaling pathway. Thus, the prognostic value of the lncRNA signature might be attributed to its association with immune processes. Interestingly, nearly all immune cells showing reduced infiltration and immune functions were inhibited in the high-risk subgroup. Given the critical roles of these immune cells in stimulating anti-tumor immunity[30], it is reasonable to conclude that the degree of anti-tumor immunity in patients with SKCM in the high-risk subgroup is substantially reduced. In addition, the ESTIMATE algorithm demonstrated that the stromal cell and immune cell scores were both negatively correlated with the risk score, confirming that immune cell infiltration was poor in the high-risk subgroup.

Cancer immunotherapies targeting immune checkpoints have improved outcomes in various cancers[31]. However, they have different effects depending on the tumor type. PD-L1 and PD-L2 are key regulators of immune responses[32]. Some tumors express immune-inhibitory checkpoint cytokines, which contribute to the inhibition of T cell-mediated immune responses. The immune escape of tumor cells and exhaustion of T cells can be promoted by the binding of PD-L1 to its receptor PD-1[33]. However, positive PD-L1 expression is correlated with better clinical outcomes in melanoma. Clinical trials have demonstrated that PD-1/PD-L1 pathway-targeted monoclonal antibodies result in impressive outcomes in SKCM by preventing the inhibition of the PD-L1 pathway and enhancing the function of T cells[34,35]. The significantly differential expression of PD-L1 and PD-L2 in the two risk subgroups as well as the fact that they are both negatively correlated with the risk score were also verified in this study. Levels of nearly all immune checkpoints were significantly lower in the high-risk subgroup than in the low-risk subgroup, suggesting that immune responses were dramatically altered in this group. The prognostic lncRNA signature could predict the expression of immune checkpoints in SKCM and potentially guide the implementation of immunotherapy. However, the specific connections between pyroptosis lncRNAs and immune-related genes warrant further study.

The role of the lncRNA signature in immune infiltration was tested to evaluate the association between immune components and SKCM. C2 was significantly associated with low risk scores. Considering the predictive value of the risk signature in overall survival, C2 might be a protective factor in SKCM.

Cancer stem cell-like cells (CSCs) promote tumor growth owing to their self-renewal and invasion abilities. CSCs are also the main determinant of chemotherapy drug resistance[36,37]. In the present study, the risk signature was positively correlated with the stem cell score, confirming that our newly constructed gene signature was a risk factor for SKCM. M6A-related genes have also been a focus of recent tumor research[38]. Our pyroptosis-related lncRNA signature could effectively predict the expression levels of the m6A-related genes *ZC3H13, YTHDF1, FTO, YTHDC2*, and *WTAP* in SKCM, although the specific mechanisms underlying these relationships need further exploration.

Despite the prognostic value of the risk signature, this study had several limitations. First, this was a retrospective analysis; thus, prospective studies are needed to confirm the results. Second, there was a lack of experimental assays to validate the results of bioinformatics analyses. In the future, functional studies are needed to gain mechanistic insights into the pyroptosis-associated lncRNAs and their role in the development of SKCM.

Conclusions

A novel pyroptosis-associated prognostic risk signature consisting of 22 hub pyroptosis-associated lncRNAs was identified to have high predictive accuracy. The constructed gene signature was valuable in predicting parameters related to immune cell infiltration, immune functions, the tumor microenvironment, immune components, tumor stemness, m6A-related gene expression, and drug sensitivity in SKCM. To the best of our knowledge, this is the first pyroptosis-related lncRNA signature for SKCM. These results

provide a novel basis for understanding the specific effects of pyroptosis-related lncRNAs in SKCM. Therefore, we believe that this study makes a significant contribution to the literature and can contribute to improvements in outcomes and individualized treatments for patients with SKCM.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Conceived and designed the experiments: Liang Wu and Zhengyuan Wu. Performed the experiments: Gang Liu. Analyzed the data: Youwu He. Contributed reagents/materials/analysis tools: Rong Chen. Wrote the paper: Zhengyuan Wu.

Data Availability Statement

The datasets analysed during the current study are available sourced from the publicly available TCGA and GTEx database (https://portal.gdc.cancer.gov, https://gtexportal.org/home/datasets).

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