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Development and validation of an immune signature-based risk model for prognostic assessment in melanoma

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Melanoma is a highly invasive malignancy with poor prognoses in advanced stages. Developing a risk model that can accurately assess prognosis and guide personalized treatment is crucial for improving the clinical management of melanoma. This study aims to develop and validate an immune-based prognostic risk model for melanoma through comprehensive bioinformatics analysis. We collected transcriptomic data from multiple public databases and identified 9 immune features significantly associated with prognosis using single-sample Gene Set Enrichment Analysis (ssGSEA) and Cox regression. These features were utilized to construct the risk model, which was subsequently validated using relevant bulk transcriptomic datasets and single-cell transcriptomic datasets from the GEO database, encompassing diverse patient populations and sample types. The model effectively stratified patients into high-risk and low-risk groups with distinct survival outcomes. Further analysis revealed significant associations between the risk model and genomic heterogeneity indicators, such as tumor mutational burden (TMB), loss of heterozygosity (LOH), and immune checkpoint gene expression. The model robustness was confirmed using single-cell transcriptomic data, highlighting key genes with potential therapeutic relevance. Our findings provide a reliable prognostic tool and novel insights for personalized melanoma treatment, emphasizing the need for further clinical validation.

Keywords Melanoma, Prognosis, Immune, Tumor microenvironment, Risk model

Abbreviations

ARG	Anoikis-related genes
CXCL12	Chemokine 12
ECOG	Eastern Cooperative Oncology Group
HR	Hazard ratios
LDH	Lactate dehydrogenase
WBC	White blood cell
HRD	Homologous recombination deficiency
HER2	Human epidermal growth factor receptor 2
ICIs	Immune checkpoint inhibitors
IRRS	Immune-related risk score
IF	Immunofluorescence
LOH	Loss of heterozygosity
LYM	Lymphocytes
MIF	Macrophage migration inhibitory factor
MDA	Mean decrease accuracy
MMIMs	Melanoma macrophage immunomarkers
MSI	Microsatellite instability

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PDX	Patient-derived xenograft
PDCD1	Programmed cell death protein 1
SLAMF6	Signaling lymphocyte activation molecule family member 6
scRNA-seq	Single-cell RNA sequencing
ssGSEA	Single-sample Gene Set Enrichment Analysis
TME	Tumor microenvironment
TMB	Tumor mutational burden

Melanoma, a malignant tumor originating from melanocytes, is characterized by its high invasiveness and propensity for metastasis[1]. In 2024, it is estimated that approximately 100,640 new cases of melanoma will be diagnosed in the United States, with 8,290 deaths attributed to melanoma, accounting for 5% of all new cancer cases and 1.4% of all cancer-related deaths nationwide[2]. Moreover, the incidence of melanoma is increasing annually at a rate of 1.4% in men and 1.7% in women. By 2040, the global burden of melanoma is projected to reach 510,000 new cases annually, with 96,000 deaths attributed to the disease[3]. The current treatment options for melanoma include surgery, chemotherapy, and radiotherapy[4]. In recent years, advances in immunotherapy and targeted therapy have significantly improved patient survival and quality of life, but the prognosis for advanced melanoma remains poor[5]. This demonstrates the urgent need for more effective prognostic models and therapeutic strategies to improve patient survival and quality of life. In this context, understanding the molecular characteristics and immune microenvironment of melanoma is of paramount importance[6]. Recent studies have highlighted the significant heterogeneity within melanoma, suggesting that a deeper exploration of its molecular and immunological landscape could yield critical insights for clinical management[7].

The immune system plays a crucial role in the development and progression of melanoma[8]. Immune cells within the tumor microenvironment (TME) can influence tumor growth and metastasis, and changes in the expression of immune-related genes can serve as important diagnostic and prognostic markers[9]. However, existing prognostic tools, such as traditional clinical staging and biomarker testing, often lack sufficient specificity and sensitivity in predicting survival outcomes for melanoma patients and guiding treatment decisions[10]. These tools fail to fully account for the complex immune interactions within TME, overlooking the patient's immune status and the mechanisms of tumor immune evasion. Moreover, many existing therapeutic approaches primarily target tumor cells themselves while neglecting the critical role of the immune system in tumor progression[11]. Therefore, the development of immune-based models is particularly essential. By comprehensively analyzing immune cell infiltration levels, immune checkpoint expression, and the regulatory networks of immune-related genes, immune models can effectively predict the clinical outcomes of melanoma patients[12]. For instance, Li et al. integrated various bioinformatics approaches to develop and validate an immune-related prognostic model based on 10 melanoma macrophage immunomarkers (MMIMs), accurately reflecting the prognostic and immune infiltration characteristics of metastatic melanoma, thereby providing potential targets for immunotherapy[13]. Identifying and analyzing these immune characteristics are therefore essential for improving diagnostic accuracy and guiding therapeutic decisions[14]. The dynamic interplay between tumor cells and the immune system emphasizes the potential of immunological markers as both predictive and prognostic tools in melanoma[15].

With the advancement of bioinformatics and the progress of multi-omics approaches, comprehensive analyses of immune characteristics within TME have been increasingly deepened. For instance, single-cell RNA sequencing (scRNA-seq) enables detailed analyses of immune cell populations, revealing the heterogeneity and functional states within the TME[16]. Moreover, the integration of proteomics, epigenomics, and metabolomics with transcriptomic data offers a holistic view of the interactions between tumor cells and the immune system, thereby facilitating the identification of novel biomarkers and therapeutic targets[17,18]. These cutting-edge technologies have established a solid foundation for constructing robust prognostic models based on immune characteristics. High-throughput transcriptomic data and advanced computational methods enable researchers to systematically analyze the relationships between gene expression and immune features, leading to the development of models that can predict patient outcomes[19,20]. Particularly, immune-based models not only provide more accurate prognostic assessments but also reveal specific roles of the immune system in tumor progression, thereby offering novel insights for personalized therapy[21]. For example, Wang et al. constructed and validated an immune-related gene prognostic signature based on S100A13, MMP9, and SEMA3B, which effectively predicted overall survival, thus providing insights into TME and immunotherapy for uveal melanoma[22]. The integration of multi-omics data further enhances the ability to uncover complex biological interactions within the TME, highlighting the potential of these models in clinical applications[23].

Therefore, the primary objective of this study was to construct and validate an immune-based prognostic risk model for melanoma through comprehensive bioinformatics analysis. We collected transcriptomic data from multiple public databases and employed ssGSEA and Cox regression methods to identify immune features significantly associated with prognosis. Subsequently, we developed a risk model based on these immune features and validated it across several independent datasets. Additionally, we explored the relationships between the risk model and genomic heterogeneity, metabolic pathways, and immune infiltration, and further validated its robustness using single-cell transcriptomic data. Through this study, we aim to provide a reliable prognostic tool and offer new insights for the personalized treatment of melanoma.

Materials and methods

Data collection

The transcriptomic data for melanoma were collected from public databases. Firstly, RNA-seq data (log2-transformed FPKM) of tumor samples from the TCGA-SKCM project were downloaded and used as the conventional transcriptomic training set, along with the corresponding clinical information for these

samples. Secondly, datasets GSE65904 and GSE54467 were downloaded from the GEO database to serve as the conventional transcriptomic validation sets. Additionally, dataset GSE115978 was obtained from the GEO database to be used as the single-cell transcriptomic validation set. Furthermore, a list of 92 immune-related signature genes was sourced from Thorsson V et al. study results[24].

Screening of prognosis-related immune features

Firstly, the R package GSVA was utilized to perform ssGSEA analysis on the 92 immune-related signature genes, calculating 92 immune scores for each cancer sample. Secondly, univariate Cox regression was applied to predict the prognostic capabilities of the 92 immune scores across the 3 conventional transcriptomic datasets, and meta-analysis was used to compute the Hazard Ratios (HR). From this, a total of 9 prognostic immune features were identified based on the criteria of $p < 0.05$ and $HR > 1$. Finally, a prognostic risk model associated with immune features was constructed using the Lasso-Cox regression method. The fitted output values were used as risk scores, which were then divided into high and low-risk groups based on the median value. Principal component analysis, Kaplan-Meier survival analysis, and survival time ROC analysis were subsequently performed.

Association analysis related to the risk model

To further investigate the clinical value of the risk model, we first performed a differential analysis between the high-risk and low-risk groups using genomic heterogeneity data from TCGA-SKCM. Additionally, we analyzed the correlation between the risk score and PD-L1-related genes (PDCD1 and CD274). Using the previous findings[25], we obtained the expression levels of 594 energy metabolism-related genes. Differential expression and correlation analyses were employed to identify significantly different energy metabolism genes in melanoma samples between the high and low-risk groups. Then, the GeneMANIA database was used to predict the interaction network of these energy metabolism genes and identify significantly enriched energy metabolism pathways. Additionally, GSEA analysis was conducted to identify significantly altered pathways. Finally, the CIBERSORT algorithm was used to perform immune infiltration analysis on 26 types of immune cells in melanoma samples.

Single-cell transcriptome validation and communication analysis

Based on the single-cell transcriptomic data from GSE115978, we utilized the Seurat package for preprocessing to facilitate subsequent analyses. Initially, the NormalizeData method was employed to normalize the raw values. Following this, dimensionality reduction was performed using RunPCA and RunTSNE on the data matrix. Next, genes related to 9 immune features were extracted for ssGSEA analysis on single-cell samples, and a clustering heatmap was generated. The CellChat R package was utilized for single-cell transcriptomic cell communication analysis. The single-cell transcriptomic data from the early-onset group was first converted into a CellChat object. Subsequently, we predicted cell communication pathways using the built-in signaling pathway information in the CellChat package, calculated the communication probability of each signaling pathway between pairs of cell types, and visualized the results using network diagrams and heatmaps.

Identification of key genes in the risk model

Using the gene sets associated with the 9 immune features from the risk model, we constructed a random forest model to distinguish between high- and low-risk groups. The contribution of each gene to the model classification ability was calculated using Mean Decrease Accuracy (MDA). The top 50 genes, ranked by MDA, were considered key genes. Cross-validation was performed using three datasets, including TCGA-SKCM, GSE65904, and GSE54467. This process ultimately identified 9 key genes, including CD2, GZMK, HLA-DPB1, GZMA, TNFRSF9, CD3G, ITK, CD247, and HLA-B. Finally, the protein-protein interaction network for these 9 genes was constructed using the STRING database, and their mutation frequencies in melanoma were analyzed using the GSCA database.

Validation of key genes in the risk model

To investigate the expression differences of key genes and the infiltration levels of CD8 + T cells between high-risk and low-risk groups, we performed immunofluorescence (IF) staining on melanoma tissue samples confirmed by pathological diagnosis. All materials used in this study were approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, with consent obtained from the patients and their families. None of the patients had undergone adjuvant treatments such as radiotherapy or chemotherapy before surgery.

Statistical methods

Gene expression differences were assessed using the Wilcoxon test, and the significance of survival analysis was evaluated with the log-rank test. Gene expression correlations were calculated using the Pearson method, and hierarchical clustering was employed for clustering analyses. All statistical methods in this study were implemented using R software.

Results

Construction of an immune feature-based prognostic model for melanoma

Initially, we calculated risk scores using a set of 92 immune-related genes previously reported in the literature across 3 independent melanoma cohorts, as shown in Fig. 1A. Subsequently, by filtering for p-values < 0.05 and HR > 1, we identified nine immune features that were significantly associated with prognosis across all three cohorts. These features are as follows: MHC-II, lymphocytes (LYM), IL-12, human epidermal growth factor receptor 2 (HER2), signaling lymphocyte activation molecule family member 6 (SLAMF6), chemokine 12 (CXCL12),

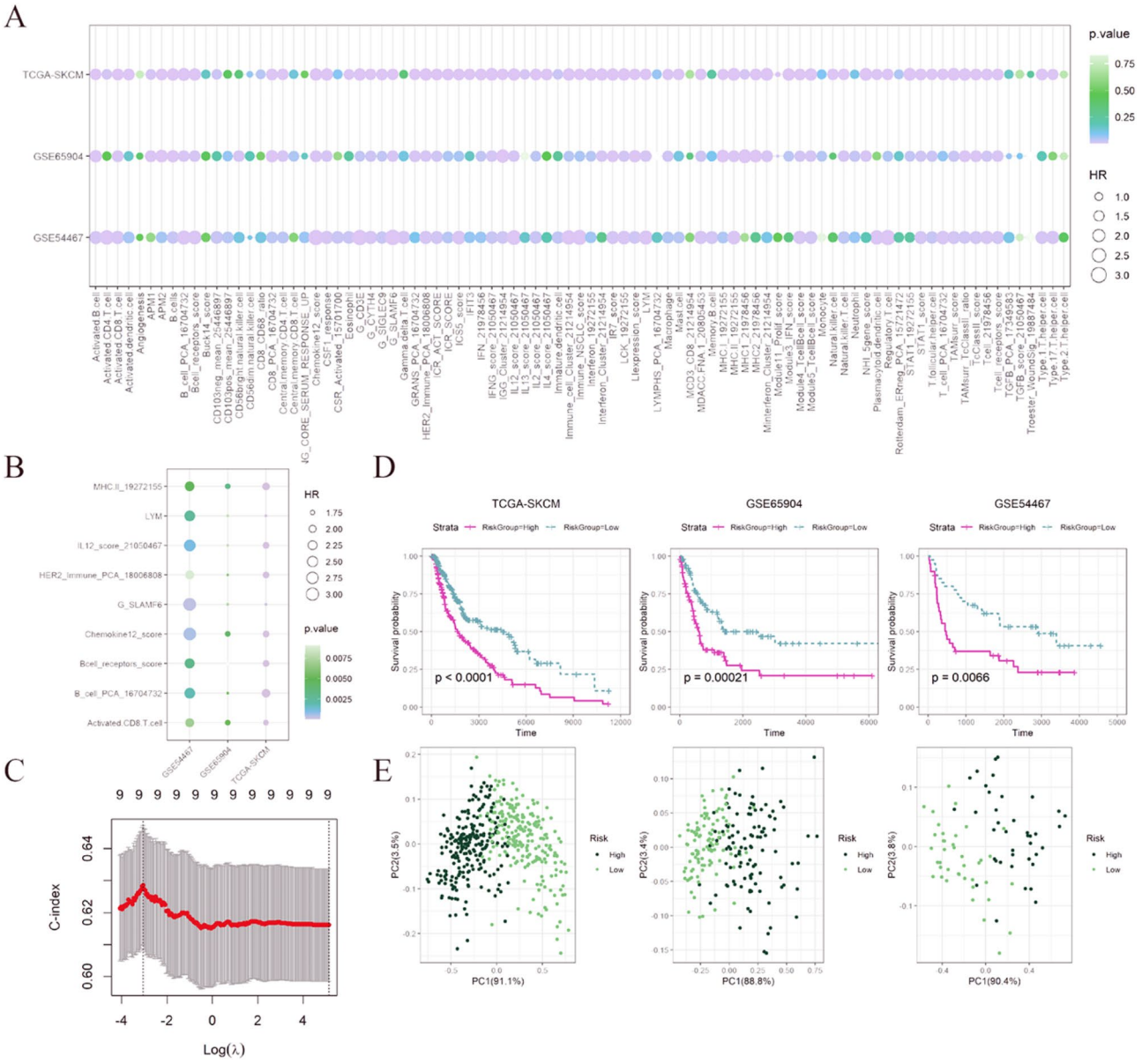

Fig. 1. Analysis and construction of immune feature-based prognostic risk model. **(A)** Univariate Cox regression analysis of immune features. Each column represents an immune feature, and each row represents an independent melanoma transcriptome dataset. The size of the dots indicates the hazard ratio from the meta-analysis, while the color represents the significance level (p-value). **(B-E)** Construction of the immune feature-associated prognostic risk model. **(B)** Univariate Cox regression analysis identified 9 immune features significantly associated with higher risk in melanoma across all datasets. **(C)** Selection of the optimal λ value in the Lasso-Cox regression model for risk calculation. **(D)** Kaplan-Meier survival curves stratifying patients into high- and low-risk groups based on the median risk score, demonstrating significantly poorer prognosis in the high-risk group. **(E)** Two-dimensional principal component analysis (2D-PCA) scatter plot illustrating clear separation between high- and low-risk groups.

B cells, B cell receptors, and activated CD8 + T cells (Fig. 1B). A growing body of research has revealed that lymphocytes, as central cells of the immune system, play a crucial role in melanoma. The degree of lymphocyte infiltration serves as an important indicator of the strength of the antitumor immune response[26]. High levels of lymphocyte infiltration are generally associated with better prognosis. Among these, activated CD8 + T cells are the key effector cells in antitumor immunity, capable of directly killing melanoma cells by recognizing tumor antigens presented by MHC class I molecules[27]. B cells may exhibit dual roles in melanoma. On the one hand, B cells can directly eliminate tumor cells in most cases through antibody-mediated immune responses. On the other hand, B cells may differentiate into immunosuppressive B cells under certain circumstances, thereby facilitating immune evasion in melanoma[28]. MHC-II plays a pivotal bridging role in the immune system, linking antigen recognition with immune activation[29]. Aberrant expression of MHC-II in melanoma attracts inflammatory

tumor-specific CD4 + T cells, which subsequently impair the antitumor reactivity of CD8 + T cells[30]. IL-12 is a key pro-inflammatory cytokine that promotes Th1-type immune responses and enhances CD8 + T cell activity[31]. In melanoma, high expression of IL-12 contributes to the suppression of tumor growth and metastasis[32]. CXCL12 is a chemokine whose aberrant expression may facilitate immune evasion in melanoma[33]. HER2 is a tyrosine kinase receptor, and SLAMF6 is a member of the signaling lymphocytic activation molecule family[34,35]. Although both are closely associated with TME and immune regulation, their roles in melanoma require further investigation. Lasso-Cox regression was then applied to determine the coefficients for these 9 immune features (Fig. 1C), which were used to construct a risk score model. Based on these risk scores, cancer samples from the 3 cohorts were stratified into high- and low-risk groups. Survival analysis confirmed that the high-risk group had a significantly poorer prognosis (Fig. 1D). Additionally, a clear separation between the high- and low-risk groups was observed in the PCA model (Fig. 1E).

Prognostic performance of the risk score model and immune feature activity analysis

Subsequently, we evaluated the predictive performance of the risk score model for 1-year, 2-year, and 3-year survival using time-dependent ROC analysis. An AUC value closer to 1 indicates stronger predictive performance of the model. In our analysis, the highest AUC value reached 0.77, suggesting that the model possesses good predictive capability (Fig. 2A-C). The ssGSEA results of the 9 immune feature gene sets revealed that immune feature activity was inversely correlated with risk scores, suggesting that corresponding immune pathways might be persistently suppressed in high-risk patients (Fig. 2D-F).

Analysis of the immune feature risk model concerning genomic heterogeneity indicators

Next, we explored the relationship between the immune feature risk model and genomic heterogeneity indicators, including TMB, microsatellite instability (MSI), homologous recombination deficiency (HRD), and LOH. As shown in Fig. 3A, the box plot revealed that the level of LOH was significantly higher in the high-risk group compared to the low-risk group ($p < 0.05$). Higher levels of LOH may lead to abnormalities in the antigen presentation system, including reduced expression of MHC-I, which impairs the immune activity to recognize and attack tumor cells, thereby enabling tumors to evade immune surveillance[36]. Subsequently, the correlation analysis demonstrated a significant negative correlation between the immune feature risk score and the expression of two immune checkpoint inhibitor-related genes, programmed cell death protein 1 (PDCD1) and CD274 (Fig. 3B-C).

Association between immune risk model and altered energy metabolism in tumor progression

Recently, the role of altered energy metabolism in tumorigenesis and tumor progression has garnered widespread attention. Compared to normal cells, tumor cells often exhibit distinct metabolic characteristics, such as enhanced glycolysis, fatty acid synthesis, and amino acid metabolism[37]. The metabolic reprogramming fulfills the substantial demands for energy and various biosynthetic materials during rapid proliferation, thereby providing the necessary foundation for tumor growth and dissemination. Consequently, we investigated the relationship between the immune risk model and changes in energy metabolism. Differentially expressed genes in the high-risk group were significantly enriched in several metabolic pathways, including sulfur compound biosynthetic process, glycosaminoglycan metabolic process, aminoglycan metabolic process, aminoglycan biosynthetic process, and glycosaminoglycan biosynthetic process (Fig. 4A). Subsequent GSEA analysis revealed that TCA cycle was notably upregulated in the high-risk group, indicating a higher degree of altered energy metabolism compared to the low-risk group (Fig. 4B-C). The TCA cycle is one of the central processes in cellular energy metabolism. Moreover, cancer cell growth and proliferation depend on de novo nucleotide synthesis from intermediates in the TCA cycle, glucose-derived ribose sugars from the pentose phosphate pathway, and amino acids that generate purines and pyrimidine nucleotides[38]. Therefore, patients in the high-risk group might have a worse prognosis.

Immune infiltration analysis in the risk model

The TME plays a crucial role in the immune evasion of cancer cells. Therefore, we utilized a deconvolution method to reconstruct the immune infiltration landscape in melanoma tissues to explore its relationship with the immune risk model. The results revealed significant differences in 16 types of immune cells between the high-risk and low-risk groups (Fig. 5A). Subsequent correlation analysis identified CD8 + T cells and NK cells as the most correlated cell types, both of which showed decreased infiltration levels in high-risk patients (Fig. 5B-C). NK cells belong to the innate lymphoid cell family and represent a group of cells involved in innate immune responses[39]. NK cells can directly recognize and kill tumor cells without the need for prior sensitization. CD8 + T cells, as cytotoxic T lymphocytes, can directly kill tumor cells by recognizing tumor-associated antigens or tumor-specific antigens on the surface of tumor cells[40]. Additionally, CD8 + T cells can secrete cytokines, such as IFN-γ, to enhance immune responses, thereby inhibiting tumor growth. Higher levels of CD8 + T cell and NK cell infiltration in tumors are potentially more beneficial for controlling tumor growth and progression. Therefore, the significant negative correlation between the infiltration levels of CD8 + T cells and NK cells and the risk score further suggested poor prognosis in high-risk patients.

Single-cell transcriptomics analysis and immune risk model application

Subsequently, applying the immune risk model to single-cell transcriptomics, CD8 + T cells were consistently identified as low-risk cells (Fig. 6A-C). The cell communication network analysis revealed that CD8 + T cells were the most actively communicating cell type (Fig. 6D). Furthermore, MIF signaling pathway was identified as the primary communication route between CD8 + T cells and other cell types (Fig. 6E-G). Macrophage migration

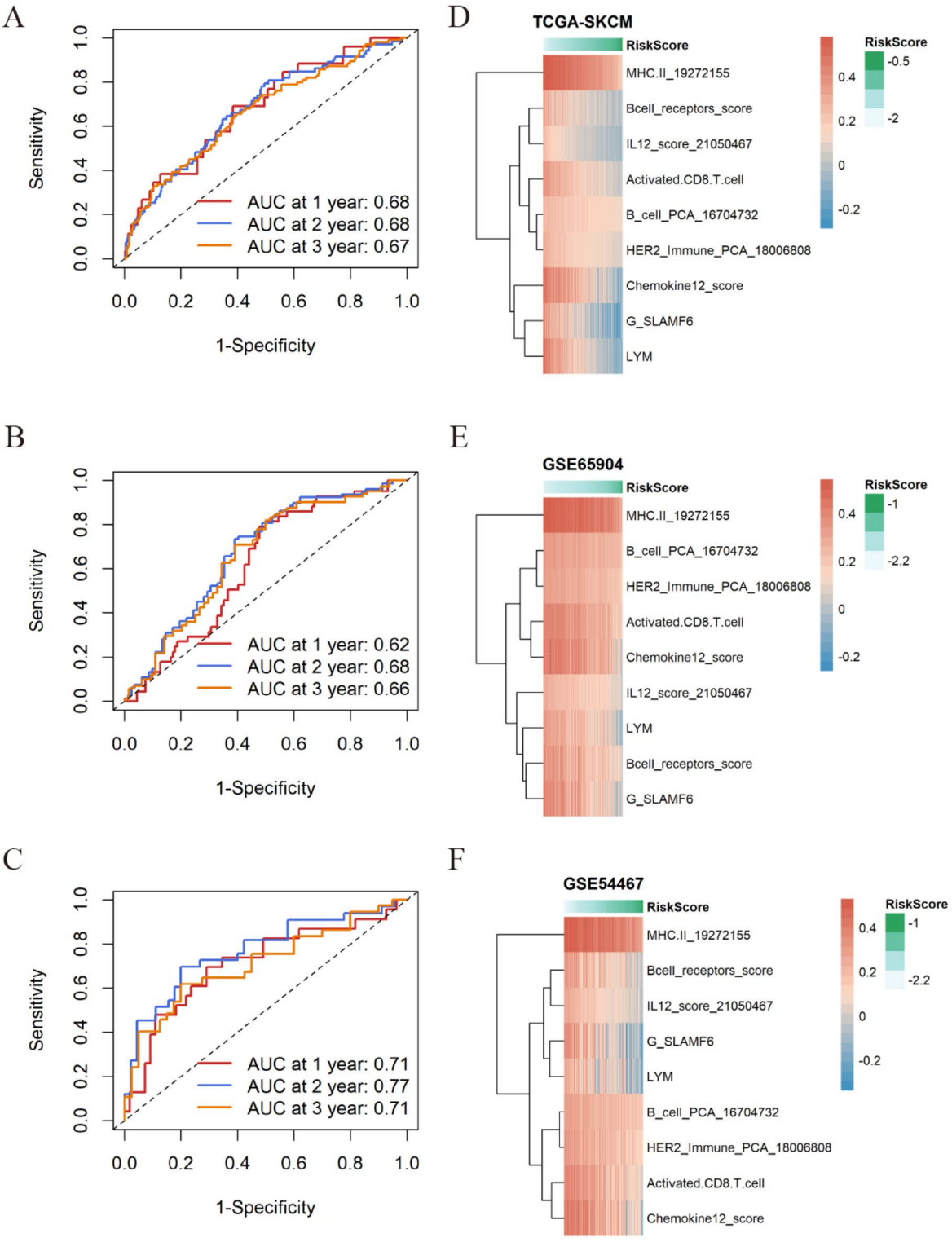

Fig. 2. Prognostic performance of the risk score model and hierarchical clustering of immune features. **(A-C)** ROC curves for predicting 1-, 2-, and 3-year survival times using the TCGA-SKCM, GSE65904, and GSE54467 datasets, respectively. **(D-F)** Hierarchical clustering heatmaps of ssGSEA scores for 9 immune features using the TCGA-SKCM, GSE65904, and GSE54467 datasets, respectively. The heatmaps illustrate the inverse relationship between immune feature activity and risk scores, indicating potential long-term suppression of immune pathways in high-risk patients.

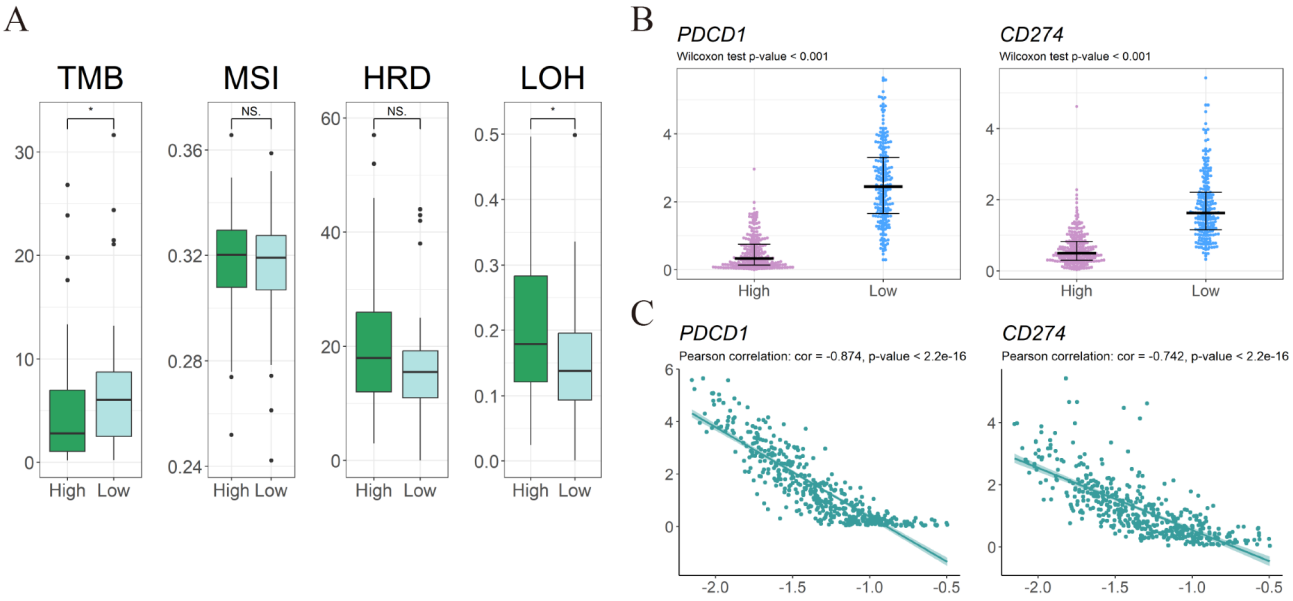

Fig. 3. Risk model in relation to genomic heterogeneity and immune checkpoint therapy. **(A)** Differential distribution of tumor genomic heterogeneity markers between high-risk and low-risk groups, highlighting significant differences in Tumor Mutation Burden (TMB) and Loss of Heterozygosity (LOX). **(B)** Box plot of immune checkpoint-related gene expression, highlighting significantly lower PDCD1 and CD274 expression in the high-risk group. **(C)** Scatter plot depicting the correlation between PD-L1 gene expression and risk scores.

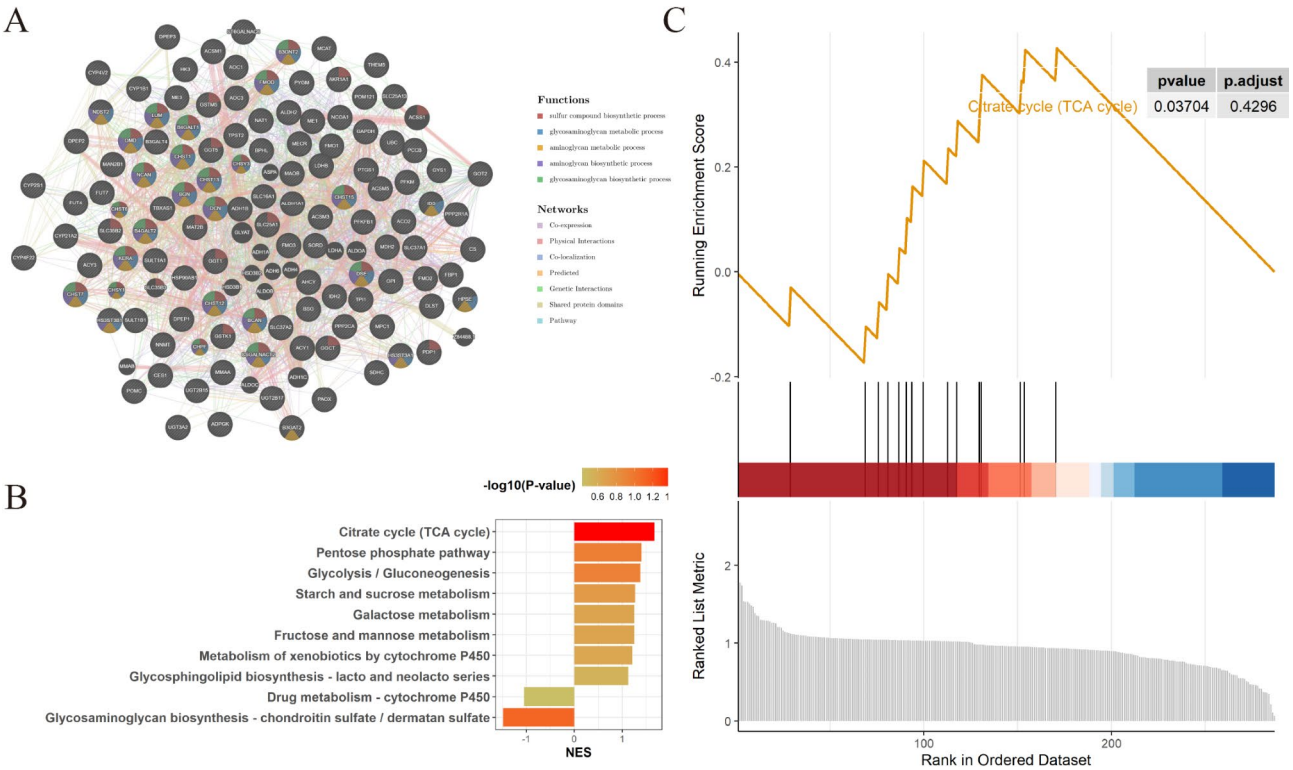

Fig. 4. Analysis of energy metabolism associations. (**A**) Network of differentially expressed energy metabolism genes between high-risk and low-risk groups, with significant enrichment in metabolic pathways. (**B**) KEGG pathway enrichment results for differentially expressed energy metabolism genes. (**C**) GSEA enrichment analysis results for differentially expressed energy metabolism genes.

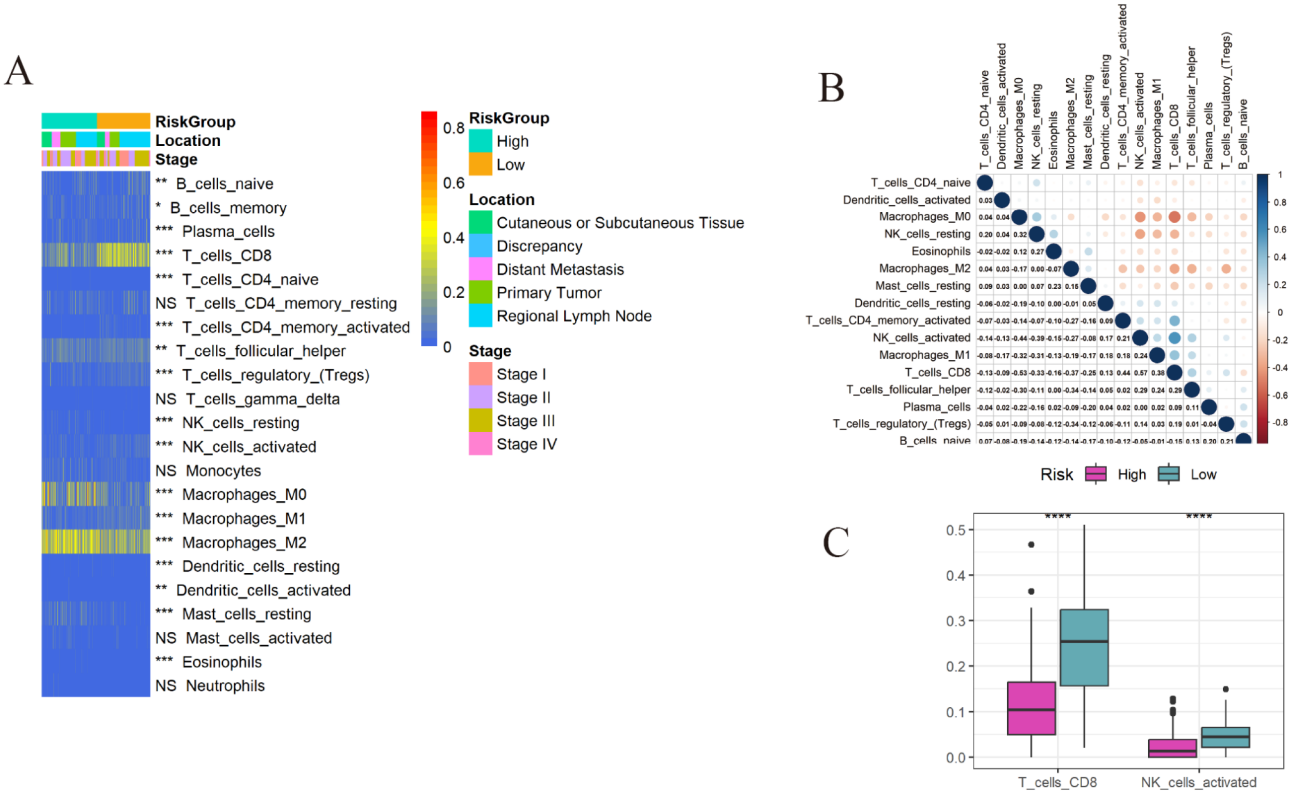

Fig. 5. Immune infiltration analysis in the risk model. (**A**) Heatmap of immune infiltration clustering, with significant markers on the right indicating differences in immune cell infiltration levels between high-risk and low-risk groups, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and NS, non-significant differences. (**B**) Correlation heatmap of significantly differentially infiltrated immune cells, showing that CD8 + T cells and NK cells have the highest correlation. (**C**) Box plots illustrating the differences in infiltration levels of CD8 + T cells and NK cells between high-risk and low-risk groups.

inhibitory factor (MIF) is a multifunctional cytokine involved in both innate and adaptive immune responses[41]. MIF initiates multiple signaling pathways, including MAPK, PI3K/Akt, and NF-κB, through binding to membrane receptors, such as CD74, CXCR2, CXCR4, and CXCR7. MIF is overexpressed in the majority of solid and hematological malignancies[42]. Although traditionally regarded as a mediator of pro-inflammatory innate immune responses, MIF promotes the development of anti-inflammatory, immune-evading, and immune-tolerant phenotypes in both innate and adaptive immune cells within TME. Studies have shown that inhibiting the MIF/CD74/AKT survival pathway significantly suppresses melanoma cell growth and induces apoptosis[43]. Therefore, the MIF signaling pathway holds potential as a therapeutic target for melanoma patients.

Identification and validation of key genes in the immune risk model

Finally, we identified key genes within the immune risk model, which consistently showed significantly lower expression in the high-risk group across three independent datasets (Fig. 7A). Protein-protein interaction predictions revealed strong co-expression relationships among these genes (Fig. 7B). Additionally, mutation analysis using the TCGA database indicated that these genes predominantly exhibited missense mutations in melanoma, with CD2, ITK, and GZMA possessing the highest mutation frequencies at 34%, 34%, and 21%, respectively (Fig. 7C). The immunofluorescence staining results indicated that CD248 expression was relatively lower and CD8 + T cell infiltration was diminished in the high-risk group (Fig. 7D). These observations were consistent with the findings from previous bioinformatics explorations.

Discussion

Multi-omics-based immune feature mining provides a valuable application scenario for tumor diagnosis and treatment[44]. In this study, we constructed and validated an immune-based prognostic risk model for melanoma through comprehensive bioinformatics analysis. By employing transcriptomic data from multiple public databases, ssGSEA, and Cox regression methods, we identified immune features significantly associated with prognosis. Our risk model, based on these immune features, demonstrated robust predictive performance across several independent datasets. Additionally, our analysis revealed significant associations between the risk model and genomic heterogeneity, metabolic pathways, and immune infiltration, further validated by single-cell transcriptomic data.

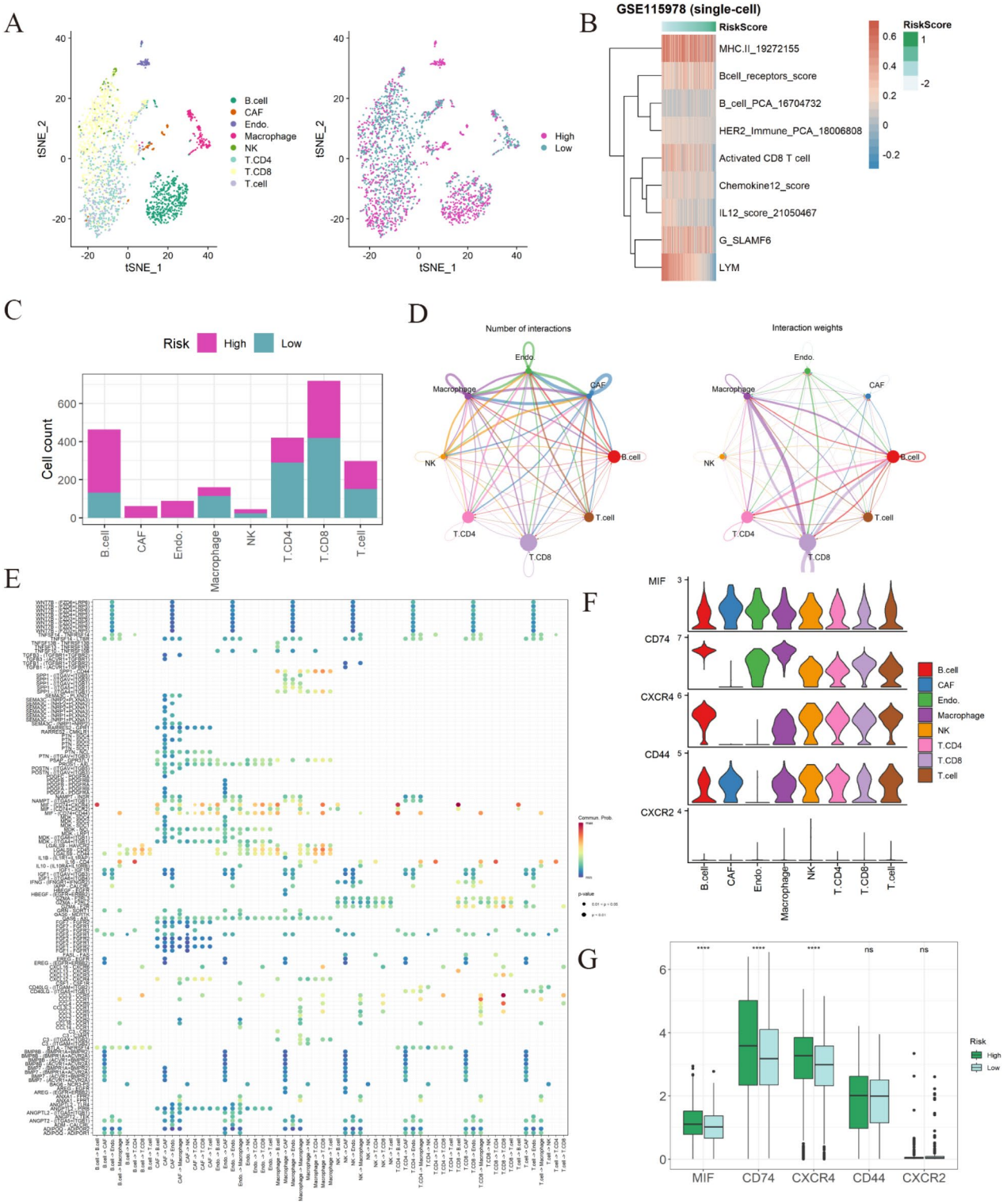

Fig. 6. Validation with single-cell transcriptomics. (**A**) Single-cell tSNE plots colored by cell type (left) and by high/low-risk groups (right). (**B**) Heatmap of 9 immune features associated with the risk model. (**C**) Stacked bar chart showing the relative proportions of different cell types in high-risk and low-risk groups. (**D**) Network diagrams illustrating the number of signaling pathways (left) and the weight of these pathways (right) between cells; the width of the connections represents the number and weight of signaling pathways, respectively, indicating that CD8 + T cells are the most actively communicating cell type. (**E**) Heatmap showing the probabilities of communication-related signaling pathways between cells; the x-axis represents receptor-ligand cell pairs, the y-axis represents receptor-ligand gene pairs, and the color indicates the probability of communication, with the MIF signaling pathway being the most prominent in CD8 + T cells. (**F**) Violin plots depicting the expression levels of MIF pathway-related genes in different cell types. (**G**) Box plots showing the differential expression of MIF pathway-related genes in cells from high-risk and low-risk groups.

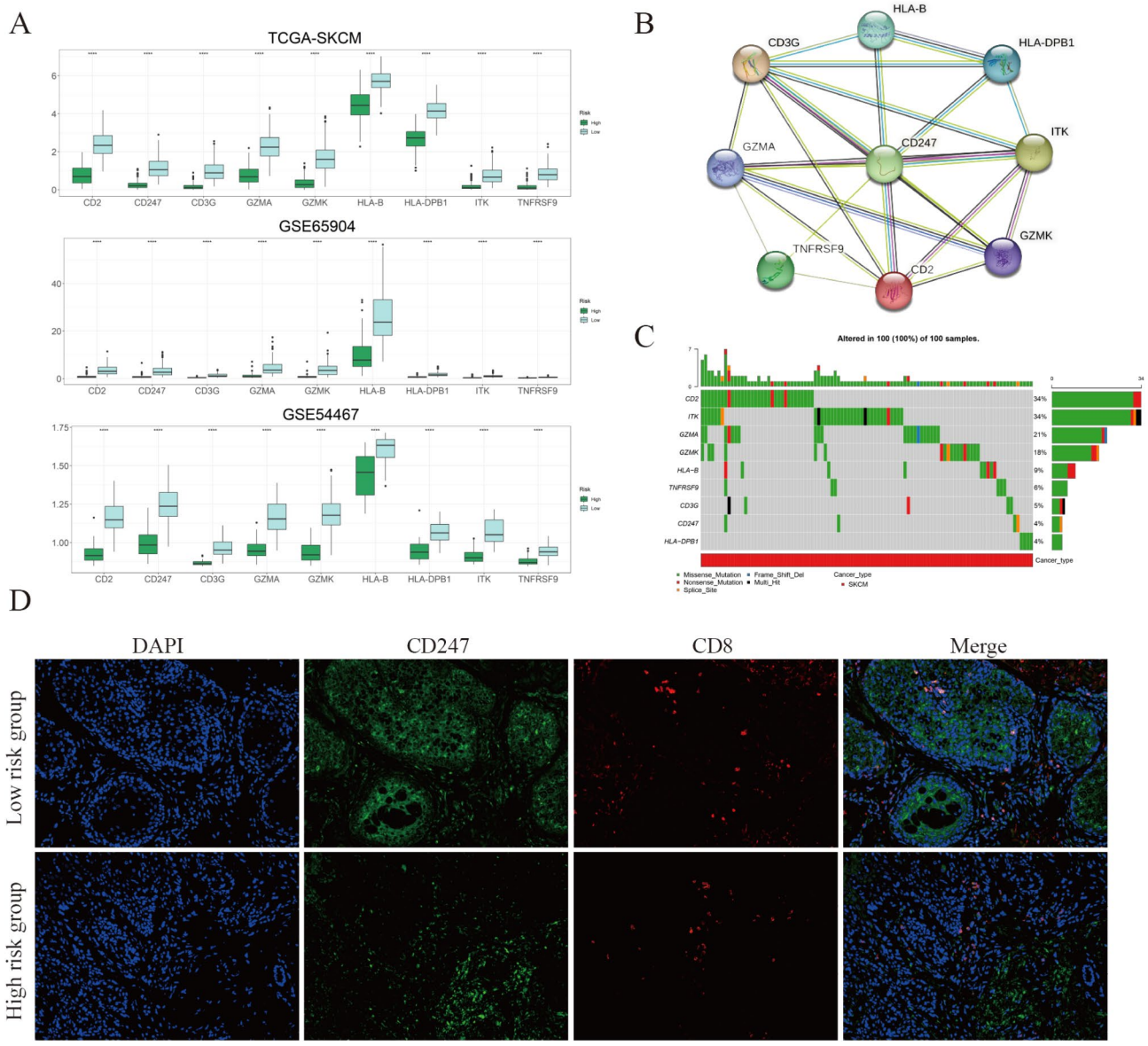

Fig. 7. Identification and Validation of Key Genes in the Immune Risk Model. (**A**) Box plots illustrating the differential expression of key genes between high-risk and low-risk groups. (**B**) Protein-protein interaction network of the key genes, showing strong co-expression relationships. (**C**) Mutation waterfall plot of key genes in melanoma, predominantly featuring missense mutations, with the highest mutation frequencies observed in CD2 (34%), ITK (34%), and GZMA (21%). (**D**) Immunofluorescence staining of CD247 and CD8 in high-risk and low-risk groups.

Firstly, our study identified 9 immune features that were significantly associated with prognosis in melanoma patients. These features were used to construct a risk model that effectively stratified patients into high- and low-risk groups. Survival analysis confirmed that the high-risk group had a significantly poorer prognosis, demonstrating the clinical relevance of these immune features. The inverse correlation between immune feature activity and risk scores suggests that specific immune pathways may be persistently suppressed in high-risk patients, potentially contributing to their poorer outcomes. This highlights the importance of targeting these suppressed pathways in the development of new therapeutic strategies. Similarly, Zhou et al. developed and validated a prognostic risk model based on anoikis-related genes (ARGs) to predict the prognosis and immune infiltration of melanoma[45]. Their study revealed the impact of immune-related signaling pathways on melanoma and provided potential insights for individualized immunotherapy. Although the model developed by Zhuo et al. emphasized anoikis and included analyses of immunotherapy response and drug sensitivity, it lacks, compared with our study, detailed investigations at the single-cell level and comprehensive analyses of specific immune cell types and intercellular communication mechanisms. Moreover, our study not only focuses on the prognostic predictive ability of the risk model but also conducts an in-depth analysis of the associations between the risk model and various clinical features. These efforts help uncover the biological mechanisms underlying the risk

model and provide valuable guidance for clinical treatment decision-making. Although the focus of the two studies differs, both highlight the critical role of the immune microenvironment in melanoma prognosis. Li et al. developed an immune-related risk score (IRRS) model based on immune cell pairs to predict the prognosis and immunotherapy response of patients with cutaneous melanoma by analyzing differences in the relative abundances of various immune cells within TME[46]. Stukalin et al. developed and validated a prognostic risk model to assess overall survival in patients with advanced melanoma treated with immune checkpoint inhibitors (ICIs), based on five baseline characteristics: high white blood cell (WBC) count, high lactate dehydrogenase (LDH) levels, low albumin levels, Eastern Cooperative Oncology Group (ECOG) performance status ≥ 1, and the presence of liver metastases[47]. The diverse modeling approaches and feature selections demonstrate various research pathways, all contributing valuable insights for personalized treatment strategies. Future research should continue to explore the clinical application potential of these models and further validate their effectiveness across different patient cohorts.

Secondly, our analysis revealed a significant relationship between the immune feature risk model and genomic heterogeneity indicators, such as TMB, microsatellite instability (MSI), HRD, and LOH. Notably, the high-risk group exhibited higher levels of LOH, suggesting a potential mechanism for immune evasion and tumor progression. Furthermore, the significant negative correlation between the risk scores and the expression of immune checkpoint inhibitor-related genes (PDCD1 and CD274) suggests that high-risk patients may be in an immunosuppressive state, with the functions of immune cells in the TME being markedly impaired. The immunosuppressive state may lead to poor responses to existing immune checkpoint inhibitors, such as PD-1/PD-L1 inhibitors, making it difficult to effectively restore anti-tumor immune function using a single immune checkpoint inhibitor. Therefore, for high-risk patients, it may be necessary to explore more comprehensive therapeutic strategies, including combinations of targeted therapy, immunotherapy, and chemotherapy. We also noted that Lin et al. developed and validated a seven-immune-gene risk model with significant prognostic value, effectively predicting survival outcomes and guiding suitable treatments for cutaneous melanoma patients, while also emphasizing its association with the tumor immune microenvironment[48]. Liu et al. employed single-cell analysis technology to reveal the heterogeneity of CD8 + T cells in TME and identified marker genes of exhausted CD8 + T cells[49]. Using these genes, the study constructed a subtype classification system and prognostic model for breast cancer, demonstrating that the key hub genes CRTAM, CLEC2D, and KLRB1 were closely associated with patient survival and responses to immunotherapy. These studies emphasize the importance of integrating genomic and immune features in prognostic models to better understand the complex interactions within TME and to tailor more effective, individualized treatment strategies.

The innovative aspects of our study include the integration of multi-omics data and the application of advanced bioinformatics techniques to construct and validate the prognostic model. The use of single-cell transcriptomic data to validate our findings is particularly noteworthy, as it provides a more granular understanding of TME and the interactions between different cell types. The identification of key genes within the immune risk model and their mutation frequencies in melanoma further enhance the potential clinical utility of our model, providing novel targets for therapeutic intervention.

In recent years, pan-cancer research has emerged as a rapidly growing field. The core objective of pan-cancer research is to deeply explore the shared characteristics across different cancer types, aiming to identify more universal biomarkers and therapeutic targets[50]. From a pan-cancer perspective, the immune characteristics we identified encompass multiple aspects, including immune cell infiltration and immune-related signaling pathways, which may also be closely associated with the occurrence and development of other tumors[51]. Therefore, these immune characteristics hold promise as potential biomarkers and therapeutic targets in pan-cancer research, providing new insights and directions for the development of more broadly applicable cancer diagnosis, prognosis assessment, and therapeutic strategies[52].

Despite the strengths of our study, several limitations should be noticed. First, our study is indeed based on retrospective data obtained from multiple public databases. Although retrospective data provide a large sample size for the preliminary construction and validation of models, the use of such data may introduce selection bias due to variations in sample collection protocols, heterogeneity in patient population characteristics, and the inherent limitations of historical data[53]. We utilized transcriptomic data for relevant analyses in study. While transcriptomic data provide valuable insights into gene expression patterns and disease mechanisms, data analysis is hindered by various technical and biological biases[54]. Technical biases mainly stem from the limitations of different sequencing methods, such as microarrays, RNA-seq, and nanopore sequencing. Biological biases are associated with factors such as tumor heterogeneity and sample complexity, which may obscure cell-specific expression patterns. Therefore, prospective studies are required to validate our findings in clinical settings. Secondly, although our model demonstrated robust predictive performance across multiple independent datasets, the samples in these datasets were primarily derived from specific public databases. These samples may have certain limitations in terms of characteristics such as race, age, and disease stage, which makes it challenging to fully represent all patient populations[55]. Therefore, further validation is required to assess the model's generalizability across diverse patient groups. Lastly, the functional mechanisms underlying the identified immune features and the interactions of these features with other cellular processes remain to be fully elucidated, which limits a comprehensive understanding of the complex dynamic network within the tumor immune microenvironment. However, patient-derived xenograft (PDX) models, which more accurately replicate the biological characteristics of human tumors, are emerging as powerful tools for research in this field[56]. In future studies, further exploration and validation will be conducted using PDX models or larger cohorts. In conclusion, our study provides a comprehensive immune-based prognostic risk model for melanoma, offering valuable insights into the molecular and immunological landscape of the disease. While our findings hold promise for improving prognostic accuracy and guiding personalized treatment, further research is needed to address the limitations and fully realize the clinical potential of our model.

Conclusion

Our study developed an immune-based prognostic risk model for melanoma, identifying 9 key immune features that significantly predict patient outcomes. The model demonstrated robust performance across multiple independent datasets, effectively distinguishing between high- and low-risk groups with distinct survival differences. Additionally, it revealed important associations between risk scores, genomic heterogeneity, immune checkpoint gene expression, and altered energy metabolism. These findings provide a promising tool for personalized prognosis and highlight potential therapeutic targets, warranting further clinical validation.

Data availability

The data used in this study are available from public databases (TCGA and GEO). We utilized RNA-seq data from tumor samples and the associated clinical information from the TCGA-SKCM project. The datasets GSE65904, GSE54467, and GSE115978 were obtained from the GEO database.

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Author contributions

R.T., J.R., and X.C. participated in the research design, data collection, analysis, and initial drafting of the manuscript. S.L. and B.P. conducted in-depth data mining and prepared relevant figures. S.A. and S.Z. reviewed and revised the manuscript. Z.S. and Z.Z. were responsible for the overall guidance, supervision, and final review of the entire research project.

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Declarations

Competing interests

The authors declare no competing interests.

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