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ORIGINAL RESEARCH

# A CDKN2B-Associated Immune Prognostic Model for Predicting Immune Cell Infiltration and Prognosis in Esophageal Carcinoma

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**Objective:** Studies have indicated that cyclin dependent protein kinase inhibitor 2B (CDKN2B) deletion is one of the most common changes in esophageal cancer (EC) which affects its progression and prognosis. This study explored the association between CDKN2B deletion, immunophenotype, and the prognosis of EC.

**Methods:** We investigated CDKN2B status and RNA expression, identified differentially expressed immune-associated genes between wild-type CDKN2B ($CDKN2B^{WT}$) and deleted CDKN2B ($CDKN2B^{deletion}$) in Cancer Genome Atlas (TCGA) EC samples. We also a constructed an immune prognostic model (IPM) based on these genes. Thereafter, the effects of IPM on the immune microenvironment of EC were analyzed. Finally, we established a nomogram by integrating the IPM and other clinical factors.

**Results:** CDKN2B deletion leads to downregulation of the immune response in EC. A total of 136 immune-associated genes were identified based on the CDKN2B deletion status, and three genes with remarkable potential as individual targets were selected for model construction. An IPM was developed and validated, it showed good performance in differentiating patients with a low or high risk of poor prognosis, and its predictive ability was independent of traditional clinical features. High-risk patients with EC had increased T follicular helper cells (Tfh) and M0 macrophages, and lower infiltration levels of resting CD4 memory T cells resting, and naive B cells. The nomogram developed for clinical application showed good predictive performance.

**Conclusions:** Our results suggested that CDKN2B deletion was associated with the survival and immune microenvironment in EC. IPM is not only an effective indicator of the immune response and prognosis, but also suggest potential targets for immunotherapy in patients with EC.

**Keywords:** esophageal carcinoma, cyclin dependent protein kinase inhibitor 2B, immune prognostic model, tumor immunity, prognosis

## Introduction

Esophageal cancer (EC) ranks seventh in terms of incidence and sixth in overall mortality, accounting for 509,000 deaths annually.[1] Esophageal cancer is an aggressive malignancy owing to the variable area distribution between cases and deaths, and approximately half of all new cases worldwide occur in China each year.[2] Incidence rates are significantly higher in men than in women, with men accounting for 70% of all esophageal cancers worldwide.[2] Most patients have advanced disease at diagnosis because of the insidious nature of the early stages.[3,4] Despite remarkable advances in management and treatment, the prognosis for EC remains poor due to recurrence and metastasis, with 5-year survival rates below 30%.[4,5] In addition, patients with the same pathological type and clinical stage may have different outcomes due to genetic

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heterogeneity.[6] Studies have revealed that immune-inflammatory cell populations in the tumor microenvironment (TME) are associated with the development, therapeutic response, and prognosis of EC.[7,8] Over the past decades, immunotherapy has emerged as an effective treatment modality for various cancers including melanoma, lung, and bladder cancer.[9] Several immune-related studies have been conducted to predict the prognosis of patients with EC.[10–12] However, few studies have comprehensive explored the immune phenotype within the EC microenvironment and its relationship with prognosis, and developed IPM model according to gene mutation status in EC. Therefore, there is an urgent need to identify more predictive and efficient prognostic biomarkers for patients with EC receiving immunotherapy.

The cyclin-dependent protein kinase inhibitor 2 B (CDKN2B) gene lies close to CDKN2A on human chromosome 9p21.3 and it is frequently mutated and deleted in a multitude of tumor types, including EC.[13–15] This gene encodes p15INK4B, which can inhibit the Rb1 regulators cyclin-dependent kinase 4(CDK4) and cyclin-dependent kinase 6 (CDK6), thereby inhibiting the cell cycle and causing G1 retardation in cells. It thereby inhibits tumor cell proliferation and facilitates tumor cell apoptosis.[16] CDKN2B has been regarded as functionally equivalent to CDKN2A and a bystander during 9p21.3 deletion because it is co-deleted with CDKN2A in many cancers.[17] Although studies have linked CDKN2B to cancer development, it was only recently that the definitive role of p15ink4b rather than p16Ink4a, in the development and proliferation of tumors was demonstrated.[18] Previous studies have suggested that CDKN2B (p15ink4b) deletion is necessary for pancreatic carcinogenesis and is associated with a poor prognosis.[19,20] Xia et al demonstrated that CDKN2B is a markedly stronger tumor suppressor than CDKN2A via dual inhibition of the cell cycle and aerobic glycolysis in bladder cancer. In addition, that CDKN2B deficiency plays a dominant role in driving the formation of low-grade non-invasive bladder cancer.[21] Therefore, exploring the effects of CDKN2B deletion on the pathogenesis of EC and other cancers is critical. Recent studies have demonstrated that CDKN2B deletions are associated with immune infiltrates and changes in the tumor-immune microenvironment of solid tumors.[22–24] Considerable data have shown that CDK4/6 inhibitors can overcome primary resistance to checkpoint immunotherapy.[25] Although studies have found that CDKN2B deletions are associated with poor prognosis in EC, its underlying function in the immune profile of EC remains unclear.[26] Growth hormone-releasing hormone (GHRH), Histone Cluster 1 h2B Family Member E (HIST1H2BE), Mucin 6 (MUC6), each of them plays a distinct role in cancer biology including EC. Therefore, we conducted a comprehensive analysis of the CDKN2B deletion status combined with RNA expression and constructed a there genes (GHRH, HIST1H2BE, MUC6) immune prognostic model (IPM), to explore the effects of CDKN2B deletion on immune responses in EC.

# Materials and Methods

## Cancer Genome Atlas (TCGA) Data Acquisition

The SNP6 Copy Number segment data for 173 EC samples were obtained from TCGA (http://firebrowse.org/). The mRNA sequencing data, somatic mutation status of 161 EC samples, and their matching clinical datasets were acquired from The Cancer Genome Atlas (TCGA) website (https://portal.gdc.cancer.gov/repository). Among these EC patients, 151 with both SNP6 Copy Number segment data and mRNA sequencing data, including 129 male, 22 female, and 122 male EC samples, were subjected to subsequent analyses.

## Somatic Copy Number Variation Analysis

To distinguish copy number changes in CDKN2B, we applied the Genomic Identification of Significant Targets in Cancer (GISTIC) to detect common copy number changes in all samples, including Copy Number Variation of the chromosome arm and minimum Common area between samples. The parameters of GISTIC were as follows: $Q \leq 0.05$ was appointed as being statistically significant. A confidence level of 0.95 was used to determine the peak interval, and a region longer than the 0.98*chromosome arm was used to analyze the variation of the chromosome arm. The corresponding MutSigCV module in the Broad Institute's online analysis tool GenePattern (https://cloud.genepattern.org/gp/pages/index.jsf) was used for the analysis.[27]

## Gene Set Enrichment Analysis (GSEA)

To determine the biological pathways associated with CDKN2B mutation and immunity between patients with CDKN2B mutated ($CDKN2B^{deletion}$) and CDKN2B wild-type ($CDKN2B^{WT}$) EC in the TCGA EC cohort, GSEA (http://software.broadinstitute.org/gsea/index.jsp) was performed.[28] The annotated gene set file (c5.bp.v7.0. symbols.gmt) was used as a reference file. Statistical significance was set at $P < 0.05$.

## Differentially Expressed Gene (DEG) Analysis

The edgeR package was used to analyze the differentially expressed genes (DEGs) between patients with $CDKN2B^{deletion}$ (n = 135) and $CDKN2B^{WT}$ (n = 197) EC; absolute |log2-fold change (FC)|>1.0 and FDR<0.05, were considered thresholds.[29]

## Development and Validation Immune Prognostic Model (IPM)

We constructed an immune prognostic model (IPM) to predict the prognosis of patients with EC. The expression matrix of the DEGs from $CDKN2B^{WT}$ and $CDKN2B^{deletion}$ patients were analyzed using univariate Cox regression analysis. The prognostic value of the DEGs was defined via univariate Cox regression analysis, and $p<0.05$ was regarded as statistically significant. The highly correlated genes were subjected to least absolute shrinkage and selection operator (LASSO) analysis with L1 penalty to determine the critical genes for survival prediction in patients with EC. The key immune-associated genes that were significant in the univariate Cox regression analysis were screened using the LASSO method.[30] Finally, a relatively small proportion of genes with non-zero weights remained. Most of the potential indicators decreased to zero. Thus, the number of immune genes was reduced using LASSO-penalized Cox regression. We constructed a prognostic model using the regression coefficients of multivariate Cox Regression analysis of these key prognostic genes. The maxstat R package was used to determine the best cutoff for classifying patients with low-risk or high-risk EC. The log-rank test and Kaplan-Meier survival analysis were conducted to evaluate the predictive ability of the prognostic model.

## Estimation of Immune Cell Type Fractions

CIBERSORT is a method for characterizing cell composition based on gene expression profiling of complex tissues.[31] The LM22 signature matrix file consisted of 547 genes and was used to distinguish 22 types of human immune cells, including natural killer (NK) cells, seven T cell subtypes, plasma cells, naïve and memory B cells, and myeloid subtypes. A combination of CIBERSORT and the LM22 signature matrix was used to estimate the distribution of 22 human hematopoietic cell phenotypes in $CDKN2B^{WT}$ and $CDKN2B^{deletion}$ EC samples. For each sample, the sum of all estimates of the immune cell subtype fraction was equal to 1.

## Functional Enrichment Analysis

The clusterprofiler R package (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html) was used to perform Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.[32] Significant biological immune processes were visualized using the GOplot R package (https://cran.r-project.org/web/packages/GOplot/index.html).[33]

## Validation Independence of the IPM

In total, 103 of 129 EC samples with complete clinical information (sex, age, and pathological staging) were included in the subsequent analyses. We performed univariate and multivariate Cox regression analyses to verify whether the predictions of IPM were independent of traditional clinical features.

## Construction and Assessment of the Nomogram

A nomogram was used to predict the survival probability at 0.5, 1, and 3 years, based on the results of the multivariate analysis. To generate a nomogram containing significant clinical features and calibration plots, we used the rms R package (https://CRAN.R-project.org/package=rms).[34] Concordance index (C-index) and receiver operating characteristic (ROC)

analyses were performed to compare the predictive accuracy of the nomogram and the independent prognostic factors. All statistical tests used in this study were two-tailed, $p < 0.05$ was used as statistical significance.

## Immunohistochemistry

Human tissue arrays (ZL-EsoS961) containing 37 EC and matched adjacent normal esophageal tissues were obtained from Shanghai Outdo Biotech Co., Ltd. These arrays included sex, age, pathology, and tumor-node-metastasis (TNM) stage. This study was approved by the Institutional Review Board (IRB) of The Second Affiliated Hospital of Jianghan University. Immunohistochemistry was performed as previously described.[35] The anti-HIST1H2BE protein antibody (1:1000 dilution, ab52599, abcom) was obtained from Abcam. Immunohistochemistry (IHC) staining was assessed separately by two experienced researchers using a semi-quantitative scoring system based on the staining intensity, as previously described.[35] SPSS version 20.0 (IBM Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software, CA, USA) were used for data analyses. Statistical significance was set at $P < 0.05$.

# Results

## Association Between Immunotype and CDKN2B Deletions in EC

CDKN2B deletion is one of the most common mutations in ECs (Figure 1A). Previous studies have demonstrated that CDKN2B mutations are associated with lymph node metastasis and poor prognosis.[15,36] However, their influence on the immune profile of EC has not been thoroughly explored. In the present study, we investigated the association between immune-related biological processes and CDKN2B status in EC using gene expression data and matching clinical data of patients with EC from TCGA (Supplementary Tables 1 and 2). GSEA was performed in patients with EC without (n =

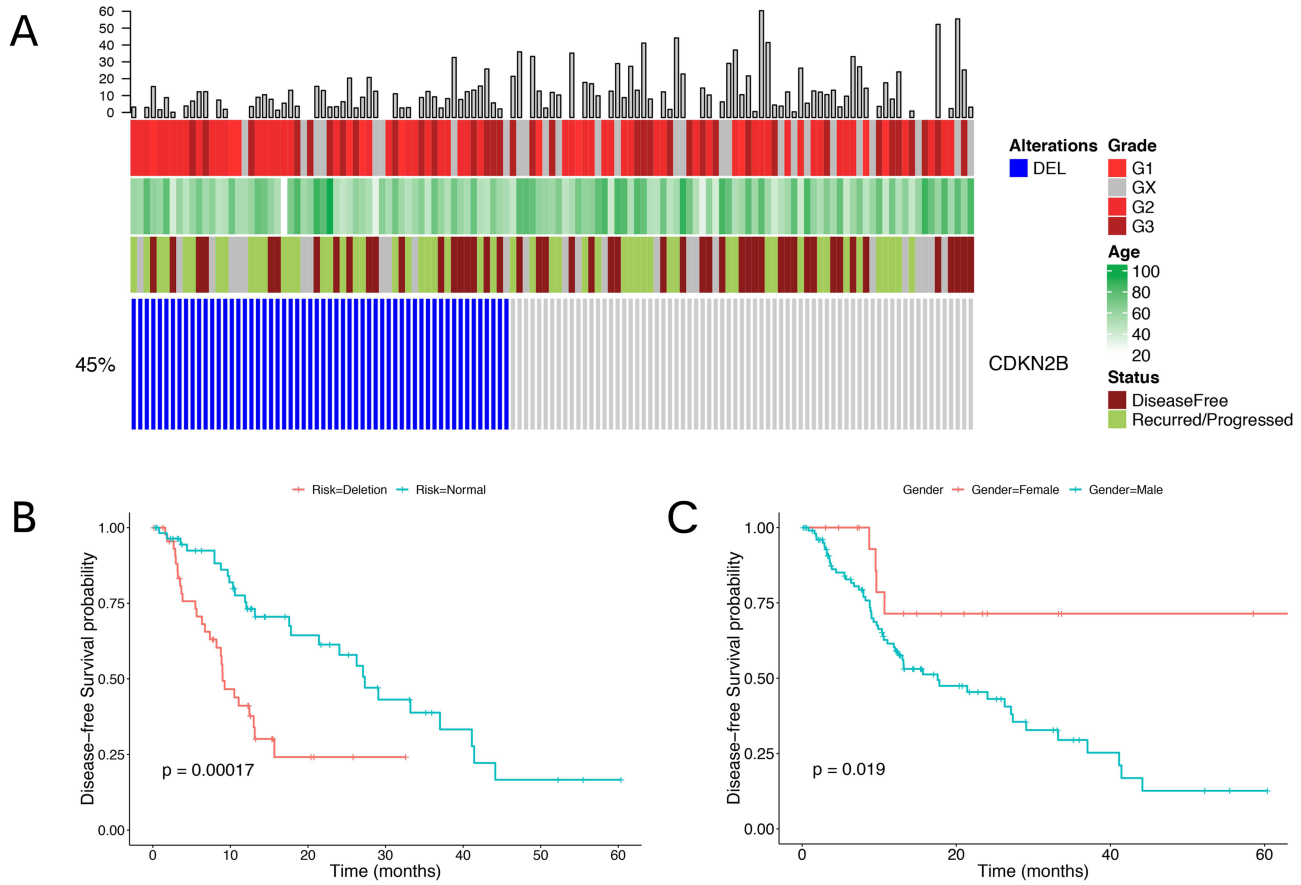


**Figure 1** Copy number states and survival analysis of CDKN2B in esophageal carcinoma. (**A**) The landscape of somatic mutations in TCGA EC dataset; (**B** and **C**) DFS was significantly higher in the low-risk score group than in the high-risk score group (P = 0.00017).

197) or with (n = 134) CDKN2B deletions. The results indicated that CDKN2B$^{WT}$ patients were highly enriched in 99 biological processes, of which nine were immune-related biological processes. These included adaptive immune responses based on somatic recombination of immune receptors, built from immunoglobulin superfamily domains, B cell-mediated immunity, and humoral immune responses mediated by circulating immunoglobulins ($p < 0.05$). CDKN2B$^{deletion}$ patients were significantly enriched in 53 biological processes, but no immune-related biological processes were found. Considering that CDKN2B is merely a bystander in the 9p21.3 deletion (often co-deleted with CDKN2A), it may suggests that CDKN2B may have a more direct role in immune regulation than previously thought.[37]

## Identification of Differentially Expressed IRGs Between CDKN2B$^{WT}$ and CDKN2B$^{deletion}$ EC Samples

We used the edgeR package to perform differential expression analysis with CDKN2B$^{WT}$ and CDKN2B$^{deletion}$ EC sample,s to identify differentially expressed immune-related genes associated with CDKN2B status, and 1247 genes were differentially expressed between CDKN2B$^{WT}$ and CDKN2B$^{deletion}$ EC samples(|log2-fold change (FC)|>1.0, FDR<0.05) (Figure 2 and Supplementary Table 3). Among the 1247 genes investigated, 136 were present in the biological pathways that were enriched by GSEA analysis (Figure 2 and Supplementary Table 4).

## Construction of an IPM and Evaluation of Its Predictive Ability in the TCGA EC Cohort

Considering the differences in immune status between patients with CDKN2B$^{WT}$ and CDKN2B$^{deletion}$ EC, we performed univariate Cox regression analysis of the 136 differentially expressed genes, to evaluate the predictive ability of the DEGs. The results showed that 7 of the 136 differentially expressed genes were significantly associated with disease-free survival (DFS) ($p<0.05$) (Supplementary Table 5). To explore the most significant prognostic genes, we employed Cox proportional hazards analysis based on L1-penalized (LASSO) estimation and identified six genes (Supplementary Table 6). Finally, three genes (Growth hormone-releasing hormone, Histone H2B type 1E, and mucin6) were selected for model construction after the expression of the six genes, and the survival time and status of the samples were analyzed using multivariate Cox Regression analysis ($p<0.05$, Supplementary Table 7). We also found that the cytoplasmic expression of HIST1H2BE was significantly higher in EC tissues than in non-cancerous esophageal tissues (Figure 3A–D). As shown in Figure 3E, positive HIST1H2BE protein staining was significantly higher in EC tissues than in the adjacent normal esophageal tissues ($P < 0.001$). This findings are consistent with the trend of using immune biomarkers to stratify patients and predict responses to immunotherapy. The IPM score was established by weighting the expression of the three genes (GHRH, HIST1H2BE, and MUC6) to the regression coefficient of the multivariate Cox regression analysis. The IPM risk score = (1.32 × GHRH expression) + (2.56 ×HIST1H2BE expression) + (0.88 ×MUC6 expression). We then calculated the risk score for each sample and classified

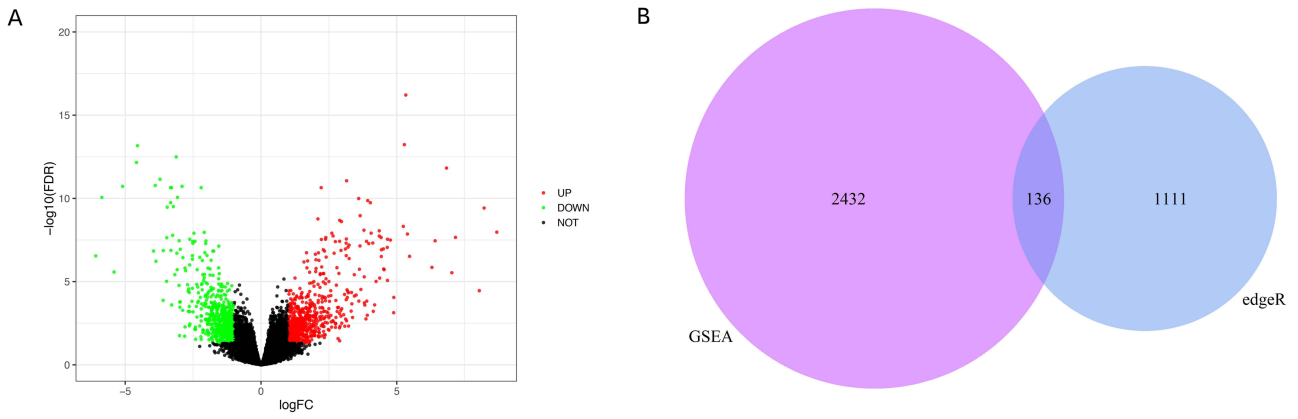


**Figure 2** Differentially expressed genes. (**A**) Differentially expressed genes between CDKN2B$^{WT}$and CDKN2B$^{deletion}$ cohorts. (**B**) Differentially expressed genes from intersections of GSEA and edgeR.


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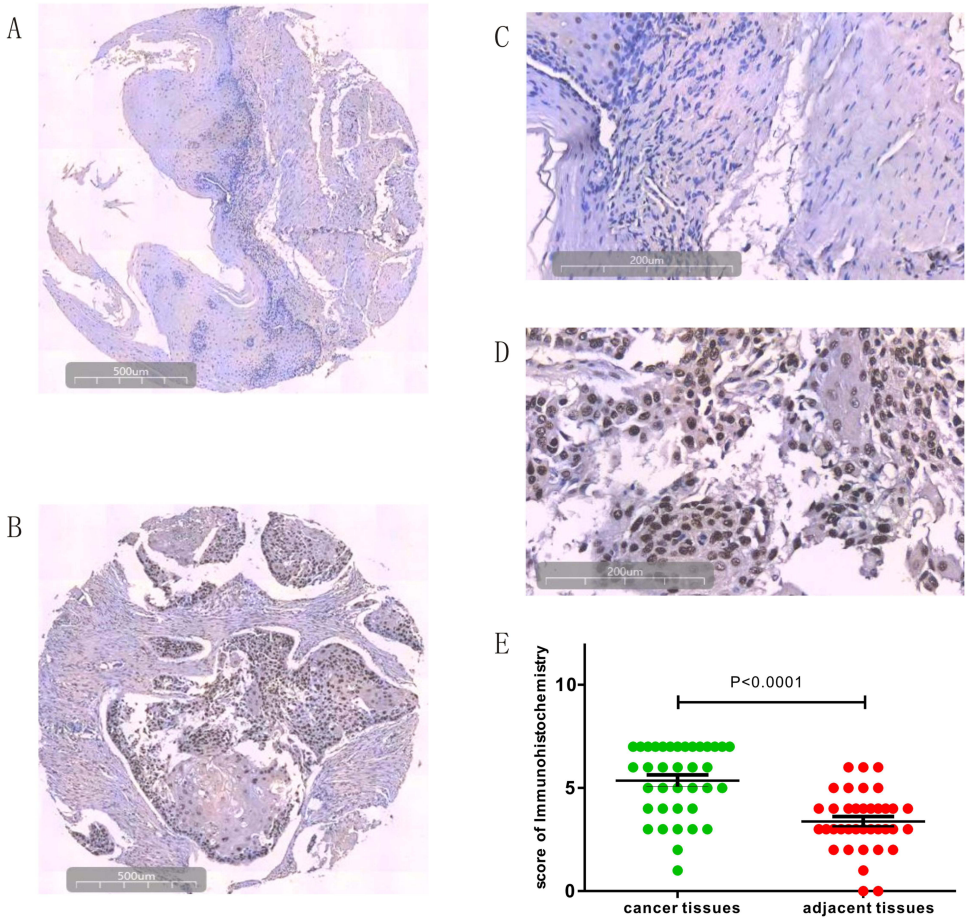


**Figure 3** Immunohistochemical analysis of HIST1H2BE expression in human EC tissues. (**A** and **B**) Representative IHC staining images of HIST1H2BE protein in non-cancerous esophageal tissues and (**C** and **D**) EC tissues at X5 and X20 magnification, respectively. (**E**) Comparison of the HIST1H2BE expression score indicate that HIST1H2BE protein levels are significantly higher ($P < 0.001$) in EC tissues than in non-cancerous esophageal tissues (N = 37).

the patients into high- or low-risk groups according to the optimal cut-off point (0.188) using the maxstat R package (Supplementary Table 8). The results indicated that the high-risk group had a shorter DFS than the low-risk group in the TCGA cohort (Figure 4A). Risk scores and gene expression distributions are shown in Figure 4B. The predictive ability of IPM is shown in Figure 4C, and the area under the ROC curve (AUC) for DFS was 0.86 at 1 month, 0.78 at 0.5 years, 0.72 at 1 year, 0.73 at 3 years. This is one of the first studies to propose a model specifically linked to CDKN2B deletion in EC.

## Stratification Analysis of DFS for IPM Based on CDKN2B Status in the TCGA EC Cohort

CAKN2B status is significantly associated with the prognosis of patients with EC. Stratification analysis was performed to verify the relationship between prognostic value and CDKN2B status. We divided patients in the TCGA EC cohort into two groups based on CDKN2B status. Stratification analyses indicated that IPM was significantly correlated with DFS in patients with $CDKN2B^{WT}$ and $CDKN2B^{deletion}$ EC (Figure 5A and B). Moreover, correlation analyses showed that the risk score of IPM was negatively associated with survival time in these patients (Figure 5C). Univariate and multivariate Cox regression analyses suggested that the predictive ability of IPM for DFS in patients is independent of the CDKN2B status (Figure 5D and Supplementary Table 9).

## Immune Landscape of the Low and High Risk EC Patients

The CIBERSORT method combined with the LM22 characteristic matrix was used to perform immune infiltration analysis to assess the differences in the 22 immune-infiltrated cell types between patients with low- and high-risk EC. As

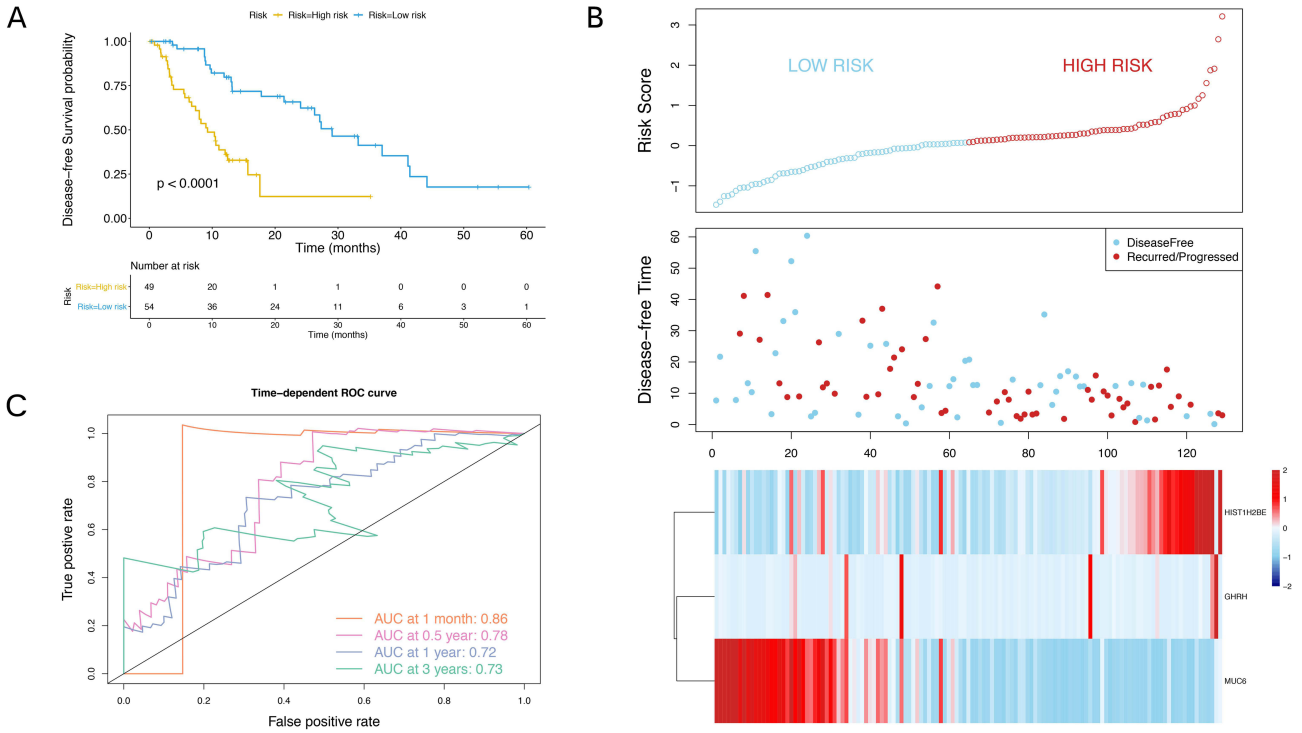


**Figure 4** Construction and validation of the IPM. (**A**) Kaplan-Meier survival of the IPM (P < 0.0001). (**B**) The relationship between Expression of the three immune-associated genes and risk score distribution. (**C**) ROC curve of the IPM.

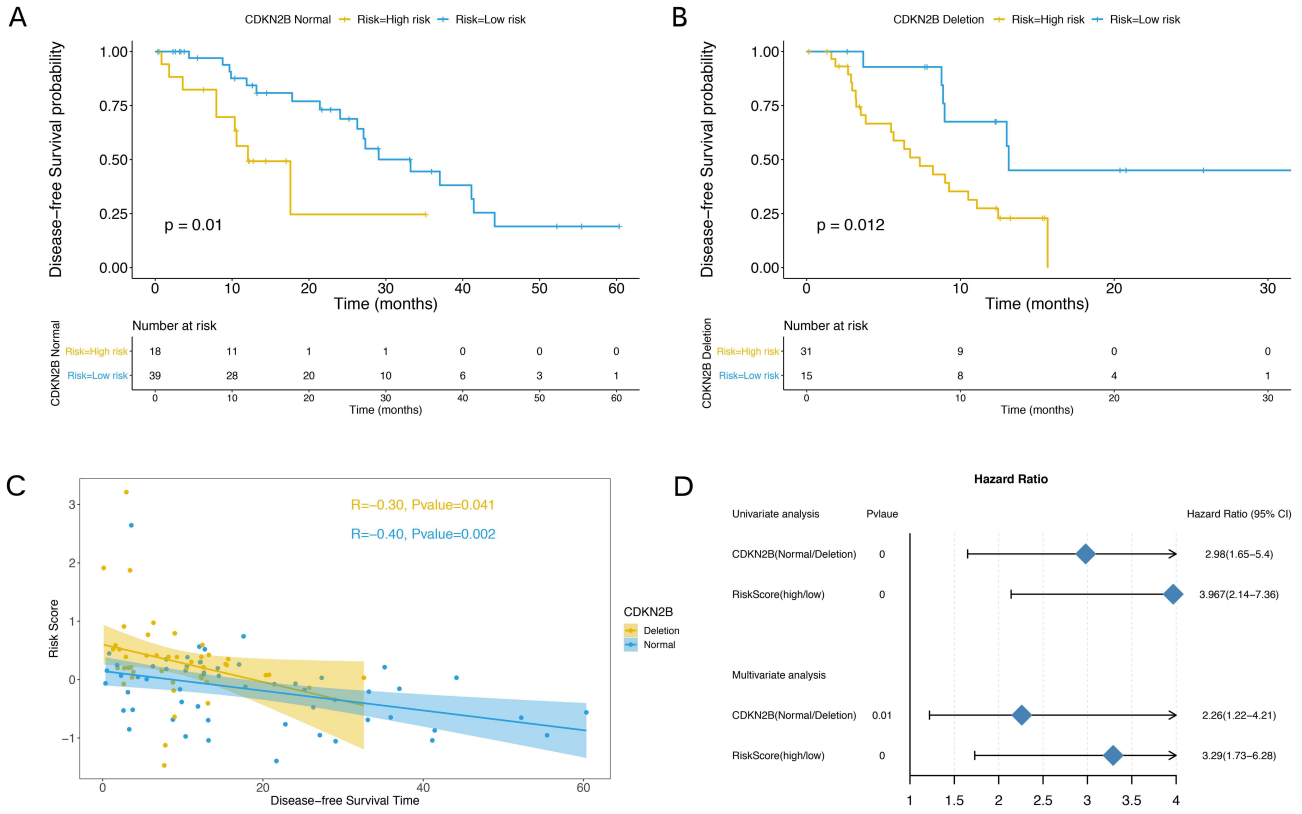


**Figure 5** Prognostic analysis of the CDKN2B$^{deletion}$. (**A**) Kaplan–Meier survival analysis of CDKN2B$^{WT}$ EC patients (P =0.01). (**B**) Kaplan–Meier survival analysis of CDKN2B$^{deletion}$ EC patients (P =0.012). (**C**) The relationship between the risk score and the survival time according to CDKN2B status in EC patients (R=−0.3, P=0.041; R=−0.4, P=0.002). (**D**) Univariate and multivariate regression analyses between the IPM and CDKN2B status regarding the prognostic value.


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shown in Figure 5A, the proportion of immune-infiltrating cell types varied within and between the EC groups (Figure 6A and Supplementary Table 10). The diverse proportions of tumor-infiltrating immune cells may represent the intrinsic characteristic phenotype of individual patients with EC. The proportion of immune cells in the low- and high-risk groups was estimated using a *t*-test. The results showed that there were significantly higher proportions of

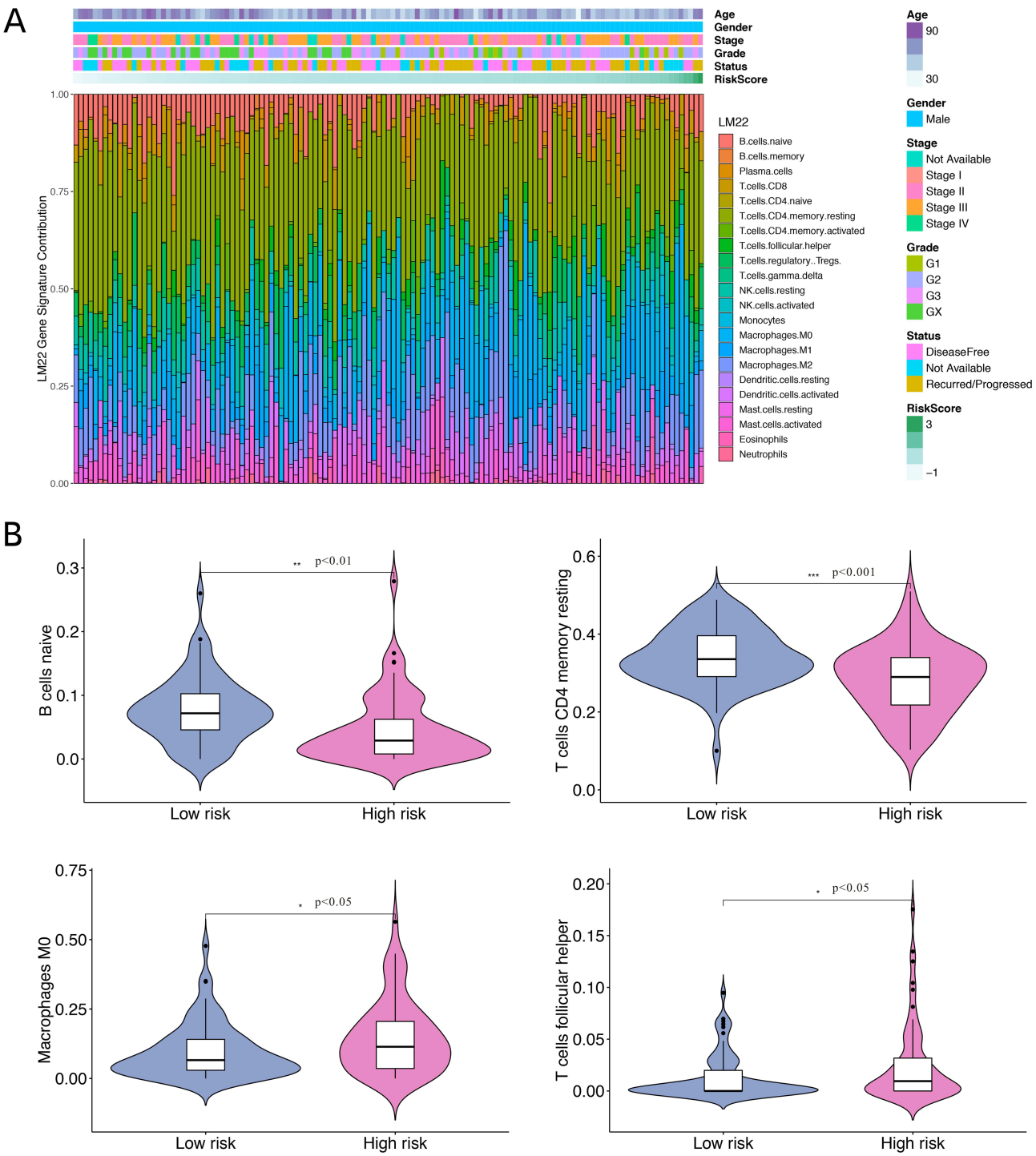


**Figure 6** The Immune infiltrate landscape in high- and low-risk EC patients. (**A**) Relative proportion of immune cell infiltrates in high- and low-risk patients. (**B**) Violin plots visualizing significantly different immune infiltrating cells between high- and low-risk patients (*P<0.05, ** P<0.01, ***P<0.001).

follicular helper T cells and macrophages M0, while significantly lower proportions of resting CD4 memory T cells and naïve B cells in patients with high-risk EC patients than in those with low-risk (Figure 6B and Supplementary Table 11). This results are consistent with previous studies that have linked immune cell composition to tumor behavior and patient outcomes in EC and other cancers. Therefore, the present results indicate that abnormal and heterogeneous immune cell infiltration in EC may be used as prognostic indicators and targets for immunotherapy and have significant clinical significance.

## Biological Processes and Pathways Analysis in Low and High Risk Group Patients

We performed differential expression analysis between the high- and low-risk groups of patients and identified 2845 differentially expression immune-associated genes (|log2-fold change (FC)|>1.0 and FDR<0.05) (Supplementary Table 12). We then investigated the correlation between these differentially expressed genes and risk scores using correlation analysis and identified 726 differentially expressed genes associated with risk scores (Pearson correlation coefficient>0.4 and $p < 0.05$) (Supplementary Table 13). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to explore the biological functions and pathways based on these genes, and 15 significantly enriched pathways were identified (Figure 7A and B). The results suggested that the immune-associated genes related to the risk score in the TCGA EC dataset were mostly enriched in immune-related pathways, including

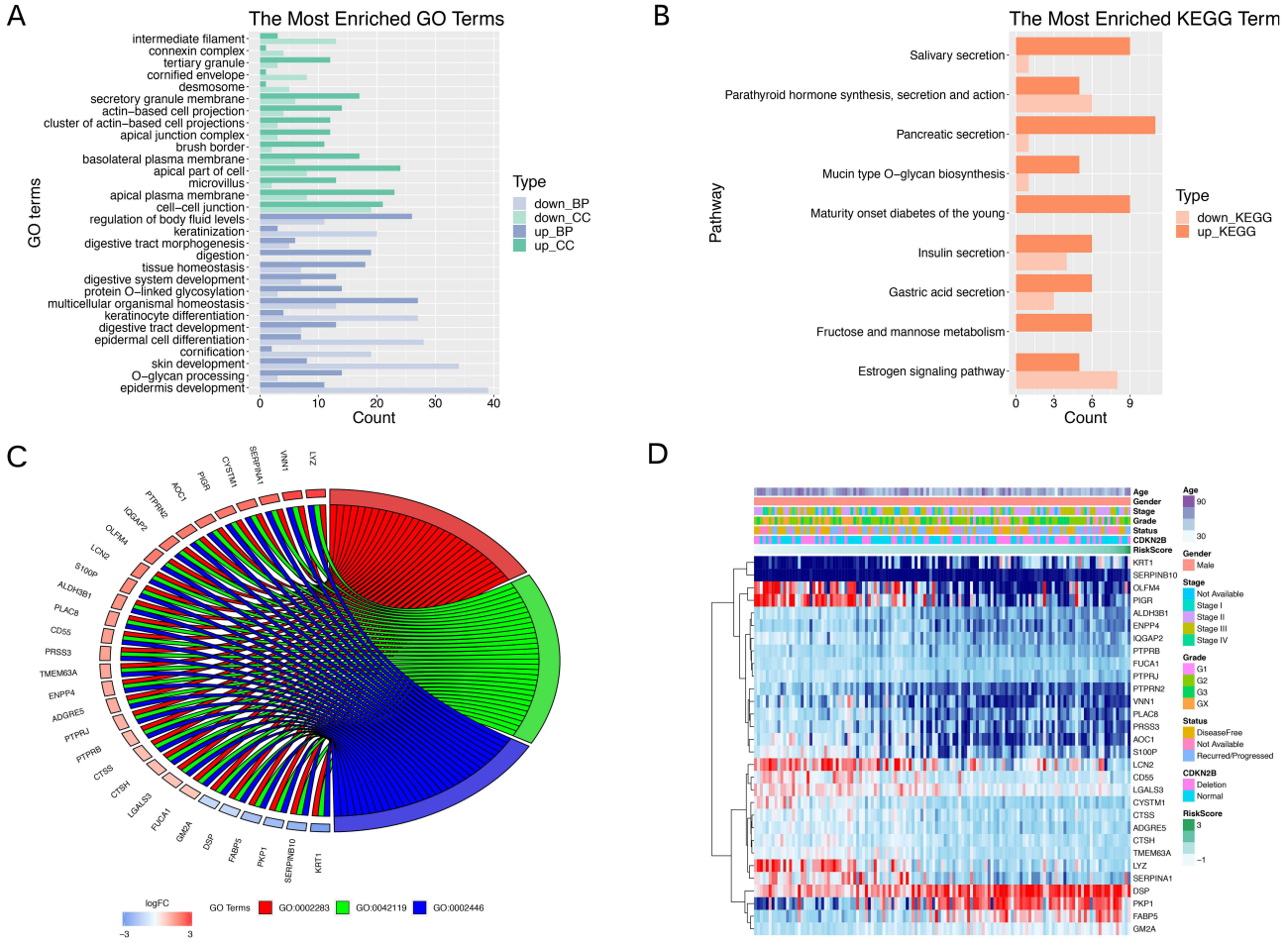


**Figure 7** Enrichment analysis of the IPM. (**A**) The most enrichment of GO pathways for immune-associated genes. (**B**) The most enrichment of KEGG pathways for immune-associated genes. (**C**) Enrichment of biological processes for immune associated differentially expressed immune-associated genes. (**D**) Heatmap of differentially expressed immune-associated genes in high- and low-risk patients.


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immune response involving activated neutrophils (GO:0002283), neutrophil activation (GO:0042119), and neutrophil-mediated immunity (GO:0002446) (Figure 6C, D and Supplementary Table 14).

## The IPM Is Better Than Conventional Clinical Characteristics

We conducted univariate and multivariate Cox regression analyses to investigate the prognostic value of IPM compared with conventional clinical characteristics in the TCGA EC cohort. Univariate Cox regression analysis involving clinical features (age, pathological stage, and grade) and the prognostic model risk score suggested that IPM was an independent prognostic factor. (Figure 8A and Supplementary Table 15). Multivariate Cox regression analysis showed that IPM was significantly associated with survival and the highest median risk score (hazard ratio [HR] = 2.95, 95% CI = 1.96–4.43) (Supplementary Table 15). We also evaluated the C-index between IPM and conventional clinical factors to determine the predictive ability of IPM (Supplementary Table 16). Among 7 survival-predictive factors, the c-index value of IPM (0.70) was higher than the conventional clinical characteristics (0.48–0.60). Taken together, these findings suggest that the IPM is independent of conventional clinical features and can improve survival predictions in patients with EC. To provide a quantitative method for predicting the prognosis of patients with EC, we constructed a nomogram by integrating the IPM and independent clinical risk parameters (stage) (Figure 8B and Supplementary Table 17). The calibration curve showed good performance between prediction and observation for the 0.5-, 1, and 3 year survival (Figure 8C). The AUC was also largest for the nomogram (Figure 8D). These results suggest that the nomogram is a better model than individual prognostic factors for predicting survival in patients with EC than individual prognostic factors.

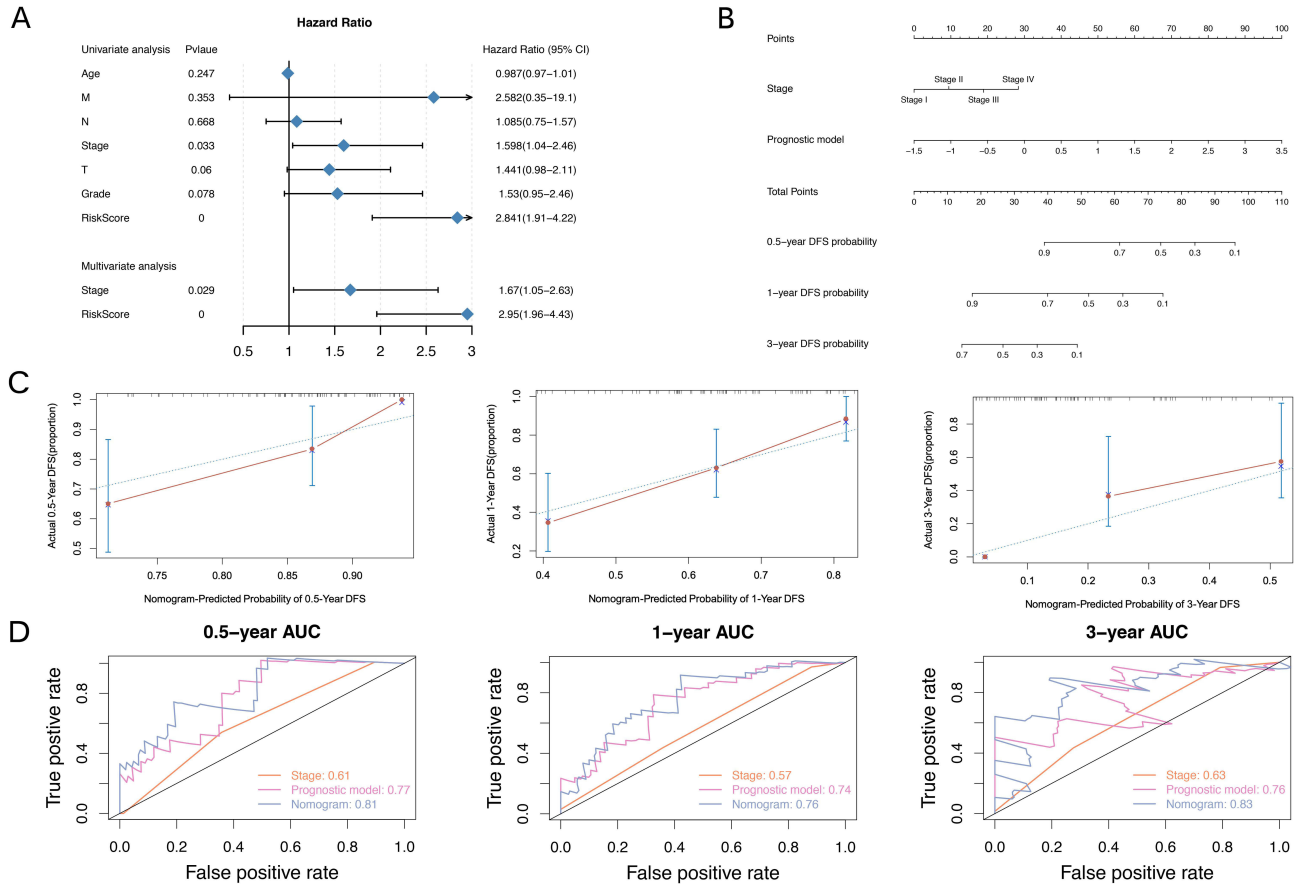


**Figure 8** Relationship between the IPM and clinical factors. (**A**) Univariate and multivariate regression analysis of the Correlation between the IPM and clinicopathological features. (**B**) Nomogram for predicting the 0.3-, 1-, and 3-year survival in patients with EC. (**C**) Calibration curve of the nomogram for predicting the 0.3-, 1-, and 3-year survival. (**E**) ROC curve analyses of nomogram.


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## Discussion

Growing evidence has demonstrated that the immune microenvironment plays a crucial role in the development, progression, and therapy of tumors. Studies on the relationship between CDKN2B deletion and the tumor immune microenvironment have been conducted in recent years. In gliomas, CDKN2B deletions are associated with cytolytic activity, poor survival, immune response, and immune checkpoint expression.[22,24] CDKN2B deletion is correlated with immune cytolytic activity and the expression of multiple immune checkpoint genes in pancreatic cancer.[23] CDKN2B deletion is one of the most common genomic alterations associated with tumor development and clinical outcomes in EC, as confirmed in the present study.[15,38,39] Studies have demonstrated that EC patients benefit from ICIs therapy. However, the mechanism by which CDKN2B deletion affects the immunophenotype and prognosis of EC remains unknown. CDKN2B deletions are common in various cancers, and the insights gained from this study could be applicable to understanding the immune microenvironment and prognosis in these cancers as well. Therefore, it is necessary to explore the immune-associated effects of the CDKN2B deletion in EC.

In recent years, immune-related prediction models have shown great potential for identifying prognostic biomarkers and evaluating the effectiveness of immunotherapy. Several gene signatures of the immune status have been established and reported to be clinically significant in solid tumors.[40–42] Although studies have sought to identify the immune signature of EC, the role of the immune infiltration landscape in the prognosis and treatment prediction of EC has not yet been fully investigated.[10–12] In this study, we focused on the relationship between CDKN2B deletion and the modulation of the immune phenotype in EC. GSEA analysis suggested that $CDKN2B^{WT}$ EC patients were enriched in immune-related biological processes than $CDKN2B^{deletion}$ patients, suggesting that $CDKN2B^{deletion}$ may be correlated with immunomodulatory effects in EC. We identified the three most significant genes (GHRH, HIST1H2BE, and MUC6) associated with CDKN2B deletion. We constructed an immune prognostic model (IPM) that could predict the prognosis of patients based on $CDKN2B^{deletion}$-linked genes. The survival analysis indicated that IPM was a good predictor of the prognosis of patients with EC patients, and the predictive performance was independent of CAKN2B status.

These three most valuable prognostic genes are involved in the development, progression, and treatment of tumors, and play a pivotal role in mediating inflammation and tumor immunity. GHRH is a peptide that regulates the secretion of growth hormone (GH) to stimulate the growth of various cells, including cancer cells.[43] GHRH promotes the aggressiveness of lung cancer by regulated the expression of cyclin D1/D2, CDK4/6, E-cadherin, β-catenin, as well as cAMP/CREB and PAK-STAT3 signaling pathways.[44] Xiong et al reported that GHRH is associated with malignant properties and poor survival in patients with EC.[45] Cai et al demonstrated an association between GHRH and multiple intracellular signaling pathways involved in cellular proliferation, metastasis, apoptosis, and inflammation in various cancers.[46] HIST1H2BE gene locus on chromosome 6p21-22 encodes histone H2B. Kohei et al identified HIST1H2BE somatic mutations in intravascular NK/T-cell lymphoma (IVNKTCL).[47] The regulation of HIST1H2BE expression has significant effects on cell growth and estrogen response in resistant breast cancer cells, and HIST1H2BE is highly expressed in AI-resistant breast tumors.[48] We also found high expression of HIST1H2BE in EC compared with non-cancerous esophageal tissues. MUC6 is a secretory mucin involved in various biological activities including malignant transformation.[49] MUC6 is aberrantly expressed in invasive mucinous adenocarcinoma (IMA), and diffuse MUC6 expression is associated with clinicopathological features and favorable survival.[50] The expression of MUC6 was significantly lower in GC and was related to poor stage and prognosis, and the regulation of MUC6 expression could modulate the migration and invasive abilities of gastric cancer cells.[51]

Recent studies have demonstrated that immune cells and immune cell infiltrates play vital roles in the TME and are related to the prognosis of patients with EC.[52,53] In the present study, we investigated the TME and immune cell infiltration in patients with low- and high-risk EC. The analyses indicated that high-risk patients had higher levels of T follicular helper cells (Tfh) and M0 macrophages and lower levels of resting CD4 memory T cells and naive B cells. Activation of Tfh could provide help for increasing levels of interferon IFN-γ production, $CD8^+$ T cells, B cells.[54] Tfh interact with $CD8^+$ T cells to improve tumor immune cell infiltration and promote $CD8^+$ effector functions, and are also essential for the efficacy of anti-PD-1 therapy.[55] M0 macrophages differentiate into M1 or M2 macrophages, which exert immunosuppressive effects and promote angiogenesis.[56] Resting CD4 memory T cells are involved in various immune responses, including the regulation of CD8+ T and NK cells.[57] A previous study confirmed that resting memory CD4

memory T cells are associated with better survival and could be an independent prognostic factors in cancer.[58] Our results suggest that high-risk patients have an immunosuppressive microenvironment that may be responsible for poor prognosis, indicating that there may be a better benefit from immunotherapy for high-risk patients. These results reveal that IPM may serve as a predictor of prognosis, immune cell infiltration, and immunotherapy in patients with EC. By predicting immune cell infiltration and prognosis, the IPM could guide immunotherapy strategies and improve patient outcomes in multiple tumor types. Future studies should explore the application of this model in other cancers, particularly those with high frequencies of CDKN2B deletions, to validate its utility and refine its predictive capabilities.

Additionally, GO and KEGG analyses demonstrated that IPM was correlated with immune-related pathways, validating the predictive role of IPM in immune cell infiltration and the TME. Our prognostic model was constructed and validated using three candidate genes (GHRH, HIST1H2BE, and MUC6) to investigate the local immune status and predict the survival of patients with EC. The analysis demonstrated that IPM acts as an independent prognostic factor after adjusting for clinical features, and we conducted a comprehensive evaluation by integrating IPM and clinical factors (age and pathological stage). The calibration curves indicate satisfactory agreement between the predicted and practical values for 0.5, 1, and 3 years. Most importantly, our IPM provides a complementary perspective on individual tumors and provides a personalized scoring method for patients with EC. Taken together, these results indicate that the IPM may be a powerful tool for clinicians in the future.

However, this study has several limitations. First, it was retrospective, the reliance on a single dataset introduces potential biases. Second, the IPM was moderate for predicting the DFS of patients with EC. Third, the sample size and there has a severe gender imbalance between male and female samples, which could lead to biases. In addition, while the IPM represents a significant advancement in understanding the relationship between CDKN2B deletion, immune cell infiltration, and prognosis in EC, prospective studies, functional analyses, and clinical trials are needed to translate this promising model into a practical tool that can improve patient outcomes and guide personalized treatment strategies in esophageal carcinoma in the future.

In conclusion, we constructed and validated an IPM based on CDKN2B deletion and three immune-related genes. To the best of our knowledge, this is the first study to propose a predictive model of CDKN2B deletion and cancer immunity. The IPM can predict survival and reflect immune status in the EC microenvironment, which may guide immunotherapy in patients with EC. The retrospective nature of the study limits direct observation of outcomes, and prospective clinical trials are needed to confirm our results to guide personalized treatment strategies in EC.

## Ethics Statement

The study was ethically approved by The Second Affiliated Hospital of Jianghan University (No.KY2022004). All patients-related information in the database is anonymous, so there is no need to obtain the informed consent of the patients.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

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

## References


1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Uhlenhopp DJ, Then EO, Sunkara T, et al. Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. *Clin J Gastroenterol*. 2020;13(6):1010–1021. doi:10.1007/s12328-020-01237-x
3. Huang FL, Yu SJ. Esophageal cancer: risk factors, genetic association, and treatment. *Asian J Surg*. 2018;41(3):210–215. doi:10.1016/j.asjsur.2016.10.005
4. Zeng H, Chen W, Zheng R, et al. Changing cancer survival in China during 2003–15: a pooled analysis of 17 population-based cancer registries. *Lancet Glob Health*. 2018;6(5):e555–67. doi:10.1016/S2214-109X(18)30127-X
5. Lagergren J, Smyth E, Cunningham D, et al. Oesophageal cancer. *Lancet*. 2017;390:2383e96.
6. Zhu R, Yang X, Guo W, et al. An eight-mRNA signature predicts the prognosis of patients with bladder urothelial carcinoma. *Peer J*. 2019;7:e7836. eCollection 2019. doi:10.7717/peerj.7836
7. Zheng Y, Chen Z, Han Y, et al. Immune suppressive landscape in the human esophageal squamous cell carcinoma microenvironment. *Nat Commun*. 2020;11(1):6268. doi:10.1038/s41467-020-20019-0
8. Xu B, Chen L, Li J, et al. Prognostic value of tumor infiltrating NK cells and macrophages in stage II+III esophageal cancer patients. *Oncotarget*. 2016;7(46):74904–74916. doi:10.18632/oncotarget.12484
9. George A, Sahin I, Carneiro BA, et al. Strategies to sensitize cancer cells to immunotherapy. *Hum Vaccin Immunother*. 2021;17(8):2595–2601. doi:10.1080/21645515.2021.1891817
10. Zhang H, Huang Z, Song Y, et al. The TP53-related signature predicts immune cell infiltration, therapeutic response, and prognosis in patients with esophageal carcinoma. *Front Genet*. 2021;12:607238. doi:10.3389/fgene.2021.607238
11. Guo X, Wang Y, Zhang H, et al. Identification of the prognostic value of immune-related genes in esophageal cancer. *Front Genet*. 2020;11:989. doi:10.3389/fgene.2020.00989
12. Zhang C, Luo Y, Zhang Z, et al. Identification of a prognostic immune signature for esophageal squamous cell carcinoma to predict survival and inflammatory landscapes. *Front Cell Dev Biol*. 2020;8:580005. doi:10.3389/fcell.2020.580005
13. Gil J, Peters G. Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all. *Nat Rev mol Cell Biol*. 2006;7(9):667–677. doi:10.1038/nrm1987
14. Fares J, Koller R, Humeniuk R, et al. The tumor suppressor p15Ink4b regulates the differentiation and maturation of conventional dendritic cells. *Blood*. 2012;119(21):5005–5015. doi:10.1182/blood-2011-10-387613
15. Lin X, Yan C, Gao Y, et al. Genetic variants at 9p21.3 are associated with risk of esophageal squamous cell carcinoma in a Chinese population. *Cancer Sci*. 2017;108(2):250–255. doi:10.1111/cas.13130
16. Zhang T, Ma K, Huang J, et al. CDKN2B is critical for verapamil-mediated reversal of doxorubicin resistance in hepatocellular carcinoma. *Oncotarget*. 2017;8(66):110052–110063. doi:10.18632/oncotarget.22123
17. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1. doi:10.1126/scisignal.2004088
18. McNeal AS, Liu K, Nakhate V, et al. CDKN2B loss promotes progression from benign melanocytic nevus to melanoma. *Cancer Discov*. 2015;5(10):1072–1085. doi:10.1158/2159-8290.CD-15-0196
19. Tu Q, Hao J, Zhou X, et al. CDKN2B deletion is essential for pancreatic cancer development instead of unmeaningful co-deletion due to juxtaposition to CDKN2A. *Oncogene*. 2018;37(1):128–138. doi:10.1038/onc.2017.316
20. Chen X, Yu X, Shen E. Overexpression of CDKN2B is involved in poor gastric cancer prognosis. *J Cell Biochem*. 2019;120(12):19825–19831. doi:10.1002/jcb.29287
21. Xia Y, Liu Y, Yang C, et al. Dominant role of CDKN2B/p15INK4B of 9p21.3 tumor suppressor hub in inhibition of cell-cycle and glycolysis. *Nat Commun*. 2021;12(1):2047. doi:10.1038/s41467-021-22327-5
22. Balli D, Rech AJ, Stanger BZ, et al. Immune cytolytic activity stratifies molecular subsets of human pancreatic cancer. *Clin Cancer Res*. 2017;23(12):3129–3138. doi:10.1158/1078-0432.CCR-16-2128
23. Wang ZL, Wang Z, Li GZ, et al. Immune cytolytic activity is associated with genetic and clinical properties of glioma. *Front Immunol*. 2019;10:1756. doi:10.3389/fimmu.2019.01756
24. Singh O, Pratt D, Aldape K. Immune cell deconvolution of bulk DNA methylation data reveals an association with methylation class, key somatic alterations, and cell state in glial/glioneuronal tumors. *Acta Neuropathol Commun*. 2021;9(1):148. doi:10.1186/s40478-021-01249-9
25. Jang HJ, Truong CY, Lo EM, et al. Inhibition of cyclin dependent kinase 4/6 overcomes primary resistance to programmed cell death 1 blockade in malignant mesothelioma. *Ann Thorac Surg*. 2021:S0003–4975(21)01670–2. doi:10.1016/j.athoracsur.2021.08.054
26. Ghobadi N, Mehramiz M, ShahidSales S, et al. A genetic variant in CDKN2A/2B locus was associated with poor prognosis in patients with esophageal squamous cell carcinoma. *J Cell Physiol*. 2019;234(4):5070–5076. doi:10.1002/jcp.27310
27. Reich M, Liefeld T, Gould J, et al. GenePattern 2.0. *Nat Genet*. 2006;38(5):500–501. doi:10.1038/ng0506-500
28. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005;102(43):15545–15550. doi:10.1073/pnas.0506580102
29. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26(1):139–140. doi:10.1093/bioinformatics/btp616
30. Li H, Lu H, Cui W, et al. A TP53-based immune prognostic model for muscle-invasive bladder cancer. *Aging*. 2020;13(2):1929–1946. doi:10.18632/aging.202150
31. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12(5):453–457. doi:10.1038/nmeth.3337
32. Yu G, Wang LG, Han Y, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284–287. doi:10.1089/omi.2011.0118
33. Walter W, Sánchez-Cabo F, Ricote M. GOplot: an R package for visually combining expression data with functional analysis. *Bioinformatics*. 2015;31(17):2912–2914. doi:10.1093/bioinformatics/btv300

34. Harrell FE. rms: regression Modeling Strategies. *R package version 5.1-3.1*. 2019. Available from: https://CRAN.R-project.org/package=rms. Accessed April 15, 2025.
35. Peng X, Cai Z, Chen D, et al. Prognostic significance and immune characteristics of APOE in gastric cancer. *Aging*. 2023;15(23):13840–13853. doi:10.18632/aging.205265
36. Shen TY, Mei LL, Qiu YT, et al. Identification of candidate target genes of genomic aberrations in esophageal squamous cell carcinoma. *Oncol Lett*. 2016;12(4):2956–2961. doi:10.3892/ol.2016.4947
37. Su D, Zhang D, Jin J, et al. Identification of predictors of drug sensitivity using patient-derived models of esophageal squamous cell carcinoma. *Nat Commun*. 2019;10(1):5076. doi:10.1038/s41467-019-12846-7
38. Cassier PA, Peyramaure C, Attignon V, et al. Precision medicine for patients with gastro-oesophageal cancer: a subset analysis of the ProfiLER program. *Transl Oncol*. 2022;15(1):101266. doi:10.1016/j.tranon.2021.101266
39. Hoppe S, Jonas C, Wenzel MC, et al. Genomic and transcriptomic characteristics of esophageal adenocarcinoma. *Cancers*. 2021;13(17):4300. doi:10.3390/cancers13174300
40. Shen S, Wang G, Zhang R, et al. Development and validation of an immune gene-set based prognostic signature in ovarian cancer. *EBioMedicine*. 2019;40:318–326. doi:10.1016/j.ebiom.2018.12.054
41. Long J, Wang A, Bai Y, et al. Development and validation of a TP53-associated immune prognostic model for hepatocellular carcinoma. *EBioMedicine*. 2019;42:363–374. doi:10.1016/j.ebiom.2019.03.022
42. Chen JY, Yao CL, Qiao N, et al. Development and validation of a PBRM1-associated immune prognostic model for clear cell renal cell carcinoma. *Cancer Med*. 2021;10(19):6590–6609. doi:10.1002/cam4.4115
43. Schally AV, Varga JL, Engel JB. Antagonists of growth-hormone-releasing hormone: an emerging new therapy for cancer. *Nat Clin Pract Endocrinol Metab*. 2008;4(1):33–43. doi:10.1038/ncpendmet0677
44. Wang H, Zhang X, Vidaurre I, et al. Inhibition of experimental small-cell and non-small-cell lung cancers by novel antagonists of growth hormone-releasing hormone. *Int J Cancer*. 2018;142(11):2394–2404. doi:10.1002/ijc.31308
45. Xiong X, Ke X, Wang L, et al. Splice variant of growth hormone-releasing hormone receptor drives esophageal squamous cell carcinoma conferring a therapeutic target. *Proc Natl Acad Sci U S A*. 2020;117(12):6726–6732. doi:10.1073/pnas.1913433117
46. Cai R, Zhang X, Wang H, et al. Synthesis of potent antagonists of receptors for growth hormone-releasing hormone with antitumor and anti-inflammatory activity. *Peptides*. 2021;150:170716. doi:10.1016/j.peptides.2021.170716
47. Fujikura K, Yamashita D, Sakamoto R, et al. Intravascular NK/T-cell lymphoma: clinicopathological and integrated molecular analysis of two cases provides a clue to disease pathogenesis. *J Clin Pathol*. 2019;72(9):642–646. doi:10.1136/jclinpath-2019-205727
48. Nayak SR, Harrington E, Boone D, et al. A role for histone H2B variants in endocrine-resistant breast cancer. *Horm Cancer*. 2015;6(5–6):214–224. doi:10.1007/s12672-015-0230-5
49. Bhatia R, Gautam SK, Cannon A, et al. Cancer-associated mucins: role in immune modulation and metastasis. *Cancer Metastasis Rev*. 2019;38(1–2):223–236. doi:10.1007/s10555-018-09775-0
50. Kishikawa S, Hayashi T, Saito T, et al. Diffuse expression of MUC6 defines a distinct clinicopathological subset of pulmonary invasive mucinous adenocarcinoma. *Mod Pathol*. 2021;34(4):786–797. doi:10.1038/s41379-020-00690-w
51. Shi D, Xi XX. Regulation of MUC6 methylation correlates with progression of gastric cancer. *Yonsei Med J*. 2021;62(11):1005–1015. doi:10.3349/ymj.2021.62.11.1005
52. Lei X, Lei Y, Li JK, et al. Immune cells within the tumor microenvironment: biological functions and roles in cancer immunotherapy. *Cancer Lett*. 2020;470:126–133. doi:10.1016/j.canlet.2019.11.009
53. Yagi T, Baba Y, Ishimoto T, et al. PD-L1 expression, tumor-infiltrating lymphocytes, and clinical outcome in patients with surgically resected esophageal cancer. *Ann Surg*. 2019;269(3):471–478. doi:10.1097/SLA.0000000000002616
54. Noël G, Fontsa ML, Garaud S, et al. Functional Th1-oriented T follicular helper cells that infiltrate human breast cancer promote effective adaptive immunity. *J Clin Invest*. 2021;131(19):e139905. doi:10.1172/JCI139905
55. Niogret J, Berger H, Rebe C, et al. Follicular helper-T cells restore CD8(+)-dependent antitumor immunity and anti-PD-L1/PD-1 efficacy. *J Immunother Cancer*. 2021;9(6):e002157. doi:10.1136/jitc-2020-002157
56. Wang Y, Lyu Z, Qin Y, et al. FOXO1 promotes tumor progression by increased M2 macrophage infiltration in esophageal squamous cell carcinoma. *Theranostics*. 2020;10(25):11535–11548. doi:10.7150/thno.45261
57. Rosenberg J, Huang J. $CD8^+$ T cells and NK cells: parallel and complementary soldiers of immunotherapy. *Curr Opin Chem Eng*. 2018;19:9–20. doi:10.1016/j.coche.2017.11.006
58. Zhang X, Quan F, Xu J, et al. Combination of multiple tumor-infiltrating immune cells predicts clinical outcome in colon cancer. *Clin Immunol*. 2020;215:108412. doi:10.1016/j.clim.2020.108412