Bioinformatics analysis identifies *PSMB8* as a key gene in the cutaneous malignant melanoma tumor microenvironment

Lin Yan[1,2], Zhiyu Yu[3], Huakang Wang[3], Caijie Qu[2], Yuyang Wang[3], Han Yao[4], Tongxin Shi[2], Yang Li[2]

[1]Graduate School of Dalian Medical University, Dalian, China; [2]Qingdao Municipal Hospital Group, Qingdao, China; [3]Graduate School of Inner Mongolia Medical University, Hohhot, China; [4]Qingdao Municipal Hospital Affiliated to Qingdao University, Qingdao, China

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Correspondence to: Tongxin Shi; Yang Li. Qingdao Municipal Hospital Group, 1 Jiaozhou Road, Shibei District, Qingdao 266000, China. Email: shitx2006@163.com; kacacpc@163.com.

Background: Cutaneous tumors are commonly seen in clinical practice, and malignant melanoma (MM) is the leading cause of cutaneous tumor-induced death. The tumor microenvironment (TME), a critical part of tumorigenesis, has been a research hotspot in recent years. However, the effects of the MM microenvironment components remain elusive. This study aimed to analyze the various components in the TME of MM to identify factors affecting the tumorigenesis, progression, and metastasis of MM and the survival of MM patients. We also aimed to identify biomarkers related to TME rehabilitation to provide a new direction for MM treatment.

Methods: We used bioinformatics to analyze the RNA-seq and somatic mutation data of 473 MM patients from The Cancer Genome Atlas database. Firstly, the patients' immunity and stroma were separately scored by the Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) method. According to the median score, the participants were split into high- and low-score groups. Then, Gene Set Enrichment Analysis (GSEA) was performed, showing that high-expression genes were highly abundant in biological and metabolic activities associated with the immune system.

Results: Differentially expressed genes (DEGs) and differentially mutated genes (DMGs) were identified and intersected to obtain the key immune-related genes *PSMB8*, *FAM216B*, *DYSF*, and *FAM131C*. *PSMB8* was finally selected as the preferred immune-related prognostic marker; it was positively associated with overall survival and therefore considered a protective gene for MM patients. The GSEA analysis showed that *PSMB8* with high expression had greater gene abundance in biological and metabolic processes related to immune system. In addition, CIBERSORT analysis showed an association between the proportion of tumor-infiltrating immune cells and *PSMB8* expression.

Conclusions: Our results suggest that *PSMB8* might be associated with tumorigenesis and MM progression and could serve as a biomarker for the TME rehabilitation of MM. Our findings provide a new perspective and direction for the treatment of MM.

Keywords: *PSMB8*; tumor microenvironment (TME); malignant melanoma (MM)

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Introduction

Cutaneous malignant melanoma (MM) is the fifth most common malignancy globally and has the highest malignancy among cutaneous tumors. It accounts for only 1% of cutaneous tumors but has the highest mortality. In 2022, there have been approximately 99,780 patients diagnosed with aggressive MM, and 7,650 cases have died of this disease in the United States. Dependent on the tumor stage, primary treatments for MM include surgery, immunotherapy, targeted therapy, radiotherapy, and chemotherapy (1). Some of these treatments achieve tumor control via immunoregulation with *CTLA-4* and *PD-1* antibodies, and some focus on targeting the proliferation of specific mutations in MM using *BRAF* and *MEK* inhibitors. Despite these new treatment options, the proliferation and recurrence of MM are not well controlled, so the therapeutic effects of these options are limited for patients with advanced MM (2). Therefore, there is an urgent need for a new treatment strategy to improve patients` prognoses. Immunotherapy based on immune checkpoint inhibitors is a novel treatment strategy and has achieved great success in treating various cancers. The effects of *PD-1* inhibitors and *CTLA-4* inhibitors have been validated in the treatment of multiple tumors, such as non-small cell lung cancer (3). However, *PD-1* inhibitors suppress tumors by enhancing the immune response, so they can also disrupt inherent immune tolerance causing immune-related adverse reactions such as the common dermatologic condition of bullous pemphigoid (4). Moreover, the clinical response of this agent in MM patients remains unsatisfactory.

The tumor microenvironment (TME) plays a key role in tumor generation and progression and has become a research hotspot. It refers to the internal environment on which tumor cells depend for survival and consists of tumor cells, immune cells, vascular endothelial cells, fibroblasts, cytokines, chemokines, and cellular metabolic products (5). It has been demonstrated that the cellular constituents of the TME are closely associated with the generation, proliferation, and metastasis of tumors. TME-targeting has become a new focus of antitumor treatment (6). MM has a high metastatic potential, Within the melanoma microenvironment, there are numerous immune cells, including T lymphocyte subpopulations, B lymphocytes, natural killer cells (NK), dendritic cells (DC), M1 and M2 type macrophages, and immature cells of myeloid origin called myeloid-derived suppressor cells (MDSC) (7). They exert anti-cancer effects by inducing apoptosis of transformed cells, producing anti-tumor cytokines or cytotoxic reactions, and participating in the recruitment of antigen-presenting cells. At the same time, melanoma cells can evade T cell recognition by (I) β down-regulation, (II) defects/deletions of antigen processing mechanisms, which may include proteasome subunits or transporters (TAPs) associated with antigen processing and/or (III) MHC molecules. By reducing antigen presentation, melanoma cells can evade immune surveillance to a certain extent, forming the characteristics of high malignancy and rapid metastasis, so the high metastasis potential of CMM is inseparable from the components and functions of its tumor microenvironment (8-11).

However, a comprehensive understanding of the biological characteristics and effects of the TME in MM is yet to be elucidated. Understanding this from the perspective of transcription and somatic mutations may help interpret the potential mechanisms of MM generation and progression.

This study used bioinformatics to analyze the transcriptome RNA-seq and somatic mutation data of 473 MM patients from The Cancer Genome Atlas (TCGA) database. Firstly, the patients' immunity and stroma were separately scored by the Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) method. Further analysis showed that the immune scores were significantly better than the stromal

Highlight box

Key findings

- The expression of *PSMB8* had a positive correlation with the overall survival in malignant melanoma patients and the proportion of most infiltrating immune cells in the tumor microenvironment.

What is known and what is new?

- The activity of tumor cell intrinsic signaling pathways is correlated with dynamic changes in the immune patterns within the tumor microenvironment and may greatly influence patients' responses to immunotherapy strategies.
- With the changes in tumor stage, both the expression of *PSMB8* and immune infiltrating cells gradually decreased in malignant melanoma patients, suggesting that the expression of *PSMB8* was a protective factor in malignant melanoma patients.

What is the implication, and what should change now?

- The *PSMB8* gene is a potential indicator for predicting the prognosis of malignant melanoma patients and their responses to immunotherapy. Further research should focus on the association between the expression of *PSMB8* and immune infiltrating cells as well as known immune checkpoints.

scores for predicting the overall survival (OS) of MM patients; therefore, according to the median immune score, the participants were split into high- and low-immune score groups. In this study, we innovatively analyzed somatic mutation data to determine the correlation between gene mutations and immune components in the cutaneous melanoma tumor microenvironment. Somatic mutation-related data of the two groups were downloaded and analyzed. We found a significant difference in the genetic mutations between the two groups; thus, the differentially expressed genes (DEGs) and differentially mutated genes (DMGs) were identified and intersected to obtain the key immune-related genes *PSMB8*, *FAM216B*, *DYSF*, and *FAM131C*. *PSMB8* was positively correlated with patients' OS and therefore considered a protective gene for MM patients. Hence, Gene Set Enrichment Analysis (GSEA) and CIBERSORT were performed to analyze the immune-related biological characteristics of *PSMB8*. Finally, we analyzed the correlation between *PSMB8* and conventional heterogeneity checkpoint molecules to evaluate the response of MM patients to immune checkpoint inhibitors. The above conclusions suggest that *PSMB8* might play a critical role in TME.

The results of this study indicate that *PSMB8* plays a critical role in the TME of MM patients at different stages of the disease and has the potential to be a predictive biomarker for immune-related prognosis or an indicator of TME recovery. It is of great benefit to reveal the potential mechanisms of MM generation and progression to improve MM treatment. We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5761/rc).

Methods

Original data

The transcriptome data, corresponding clinical data, and somatic mutation data of 473 MM patients were downloaded from the TCGA database (2022-04-07), and a dataset consisting of one normal sample and 472 tumor samples was obtained, which contained information such as patient' survival, tumor TNM stage, and time of the last follow-up. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

Calculation of immune score, stromal score, and ESTIMATE score

The "ESTIMATE" R software package (Ver 4.2.0) was used to analyze each MM sample and calculate the immune score (proportion of immune components), stromal score (proportion of stromal components), and ESTIMATE score (the sum of the above two scores). A higher score indicated more abundant corresponding components (immune, stromal, and non-tumor components) in the TME.

Survival analysis for transcriptome data

A survival analysis was performed using the "Survival" R software package for patients in the high- and low-score groups. A survival curve was plotted with the Kaplan-Meier method. Statistical significance was tested using the Log-Rank test.

Correlation between the scores and clinical information

Significant data were identified (including 405 patients at TNM stage I-IV, 385 at stage Tis-T_4, 403 at N_0-N_3, and 430 at M_0-M_1). Scores and clinical information of different components were pooled according to each patient's immune, stromal, and ESTIMATE scores. Correlation analysis was performed using the "ggpubr" R software package for the scores and clinical information. Wilcoxon and Kruskal Wallis rank-sum tests were adopted for the statistical analyses.

DEG identification

Patients were divided into a high immune-score group and a low immune-score group using the median immune score as the cutoff. A divergence analysis was conducted with the "limma" R software package. By comparing the differences between the high and low immune-score groups, the DEGs were counted, a total of 406 DEGs in the different groups were obtained, and a heat map was drawn. We set the significance threshold for DEGs according to the following criteria: (I) false discovery rate (FDR) was less than 0.05; (II) |log2 fold change (FC)| >1 (high- or low-immunity cohorts).

DMG identification

The somatic mutation information of MM patients was accessed via the TCGA database and saved in a mutation

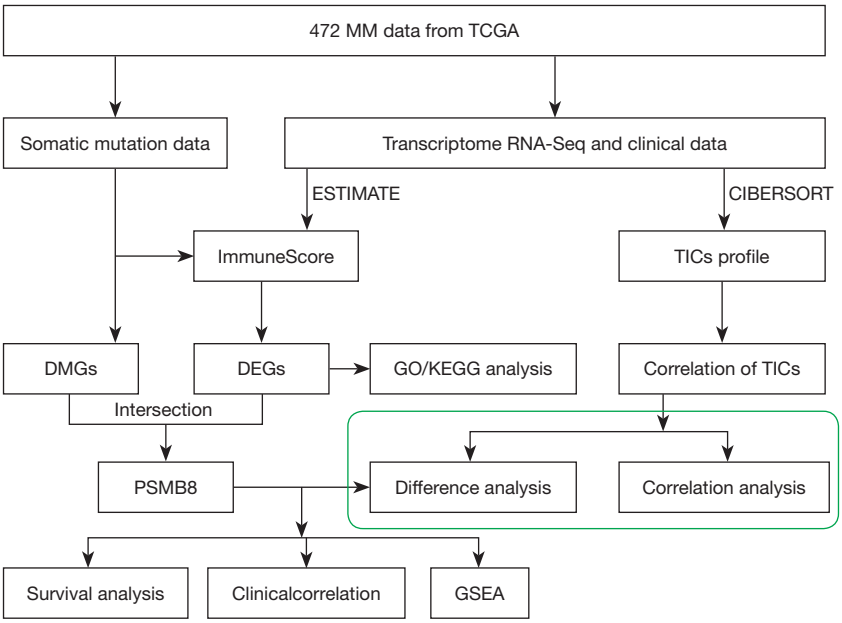


Figure 1 The analysis flow chart. MM, malignant melanoma; TCGA, The Cancer Genome Atlas; DMGs, differentially mutated genes; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TICs, tumor-infiltrating immune cells; GSEA, Gene Set Enrichment Analysis.

annotation format. According to the median immune score, 456 tumor samples were evenly divided into a high-immunity cohort and a low-immunity cohort. The two cohorts were compared using the "mafTools" R software package to identify DMGs, where a P value less than 0.05 indicated statistical significance.

GO and KEGG enrichment analysis

Immune scoring software was used to perform the intersection analysis for DEGs and DMGs, and four key genes were identified: *PSMB8*, *FAM216B*, *DYSF*, and *FAM131C*. *PSMB8* was finally selected as the target gene and was analyzed along with the patients' clinical data, including OS and tumor stage.

Results

Study design and procedure

This study was conducted using the analysis procedure shown in *Figure 1*. The RNA-seq map and relevant clinical data were downloaded from the TCGA database. The CIBERSORT and ESTIMATE programs were used to compute the ratio of tumor-infiltrating immune cells (TICs) to immune and stromal components in 472 MM patients. We also downloaded the somatic mutation data and determined the DMGs in the high and low immune-score groups according to the median immune score and GO and KEGG analyses were performed for these genes. An intersection analysis was conducted for the DEGs and DMGs, and *PSMB8*, *FAM216B*, *DYSF*, and *FAM131C* were obtained. Further analyses for *PSMB8* were performed, including correlation analysis for OS with the tumor-node-metastasis (TNM)-based staging, GSEA (Gene Set Enrichment Analysis), and TICs, as shown in *Figure 1*.

DEG identification in MM patients

Correlation between the scores and OS of MM patients

The TME immune, stromal, and ESTIMATE scores were calculated using the ESTIMATE algorithm. A high immune or stromal score indicated a large proportion of the component in the TME of MM patients. The ESTIMATE score represented the results of adding immune and stromal scores, indicating tumor purity. The results showed that the ratio of immune components to stromal components was positively correlated with OS (*Figure 2*, A: ESTIMATE, P=0.001; B: Immune, P=0; C: Stromal, P=0.047). The immune components showed the most

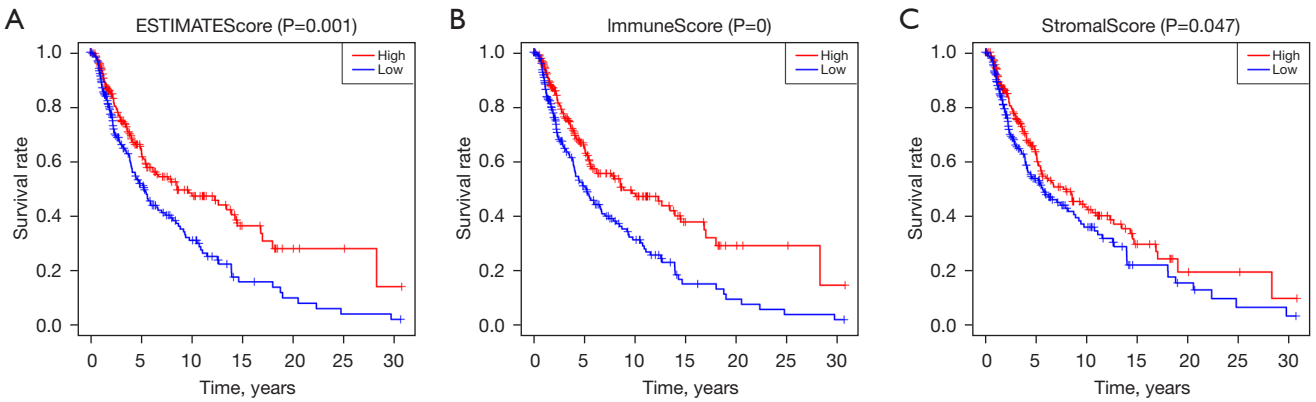


Figure 2 The Kaplan-Meier survival analysis for malignant melanoma patients. A comparison of overall survival between the high- and low-score groups determined by the median immune score for (A) ESTIMATE Score, (B) Immune Score, and (C) Stromal Score (P=0.001, 0.000, and 0.047, respectively, according to the log-rank test).

significant difference (P=0). Based on the above results, we considered that the immune components were more significant for MM generation and progression and would be more likely to reveal patients' prognosis. On this basis, we analyzed the clinical characteristics of MM patients to identify the correlation between these three scoring systems and the clinical data (see *Figure 3*). It was observed that the immune, ESTIMATE, and stromal scores were significantly correlated with tumor (T) stage. These findings elucidated the crucial role of immune components in MM generation and progression, especially in tumor infiltration and invasion.

DEG identification based on the immune score

Given the significant impact of immune components on MM progression, we further conducted a comparative analysis of the high and low immune-score groups. Patients were allocated to a high or low immune-score group according to the median immune score, and 406 DEGs were identified, of which 364 were upregulated, and 42 were downregulated. The heat map is provided in *Figure 4*.

GO and KEGG enrichment analysis of DEGs

The GO analysis showed that the DEGs were mainly correlated with immunity, such as leukocyte-mediated immunity, adaptive immune response based on somatic recombination of immune receptors, and regulation of the immune effector process (*Figure 5A*). According to the KEGG analysis, DEGs were highly enriched in biological processes associated with immune system, such as the B cell receptor signaling pathway, viral protein interaction with cytokine and cytokine receptors, and cell adhesion molecules (*Figure 5B*). It was evident that immune-related biological processes reflected the main functions of DEGs in MM patients, indicating that immune components might play a major role in the TME of MM patients.

Somatic mutations in MM patients

The specific mutation of tumor cells could produce new antigens that could induce an immune response to kill the tumor cells (12). We analyzed and visualized the somatic mutation data of MM patients and found 151 somatic mutant genes. Patients were divided into two groups according to the median immune score. The somatic mutation data in both the high and low immune-score groups were analyzed, and the top 30 mutant genes in each group were identified through the absolute values of Log2Fc (*Figure 6A,6B*) to reveal the different gene mutations in MM patients. The commonly mutated genes in both groups were analyzed, and the results showed that the high immune-score group had a higher proportion of mutated genes than the low immune-score group (*Figure 6C,6D*), indicating that patients with more mutated genes typically had a higher immune concentration.

Subsequently, we performed an intersection analysis for the 406 DEGs and 147 DMGs (*Figure 6E*), and the following four genes were identified: *PSMB8*, *DYSF*, *FAM216B*, and *FAM131C*.

PSMB8 encodes a catalytic subunit of the 20S immunoproteasomes called b5i. Immunoproteasome-mediated proteolysis generates immunogenic epitopes

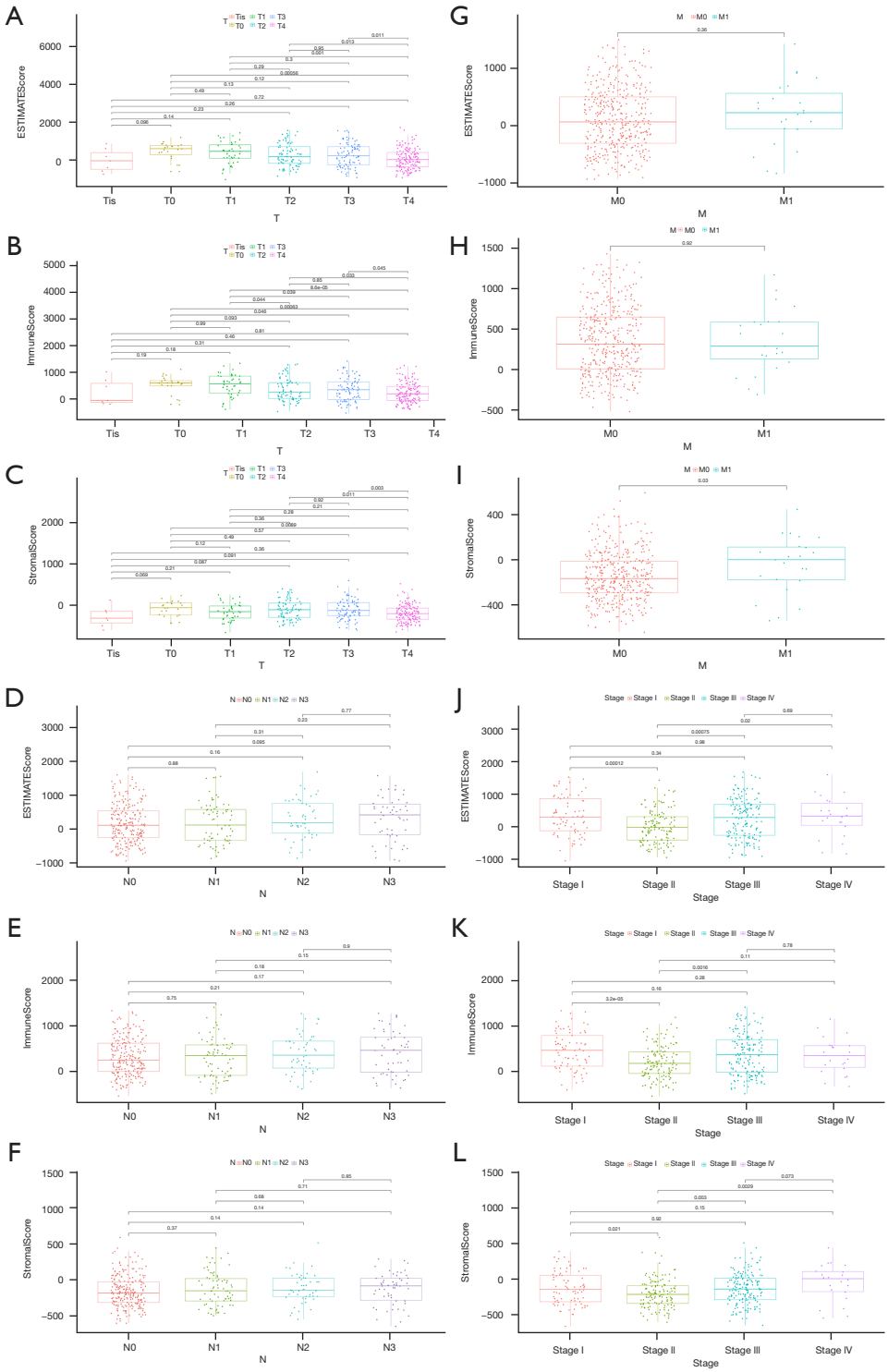


Figure 3 Correlation between scores and malignant melanoma's clinical features. (A-C) Distribution of scores in the T classification for the ESTIMATE, Immune, and Stromal Scores, respectively, based on the Kruskal-Wallis test. (D-F) Distribution of scores in the N classification for the ESTIMATE, Immune, and Stromal scores, respectively, based on the Kruskal-Wallis test. (G-I) Distribution of scores in the M classification for the ESTIMATE, Immune, and Stromal Scores, respectively, based on the Wilcoxon rank sum test. (J-L) Distribution of scores in the Stage classification for the ESTIMATE, Immune, and Stromal Scores, respectively, based on the Kruskal-Wallis test.

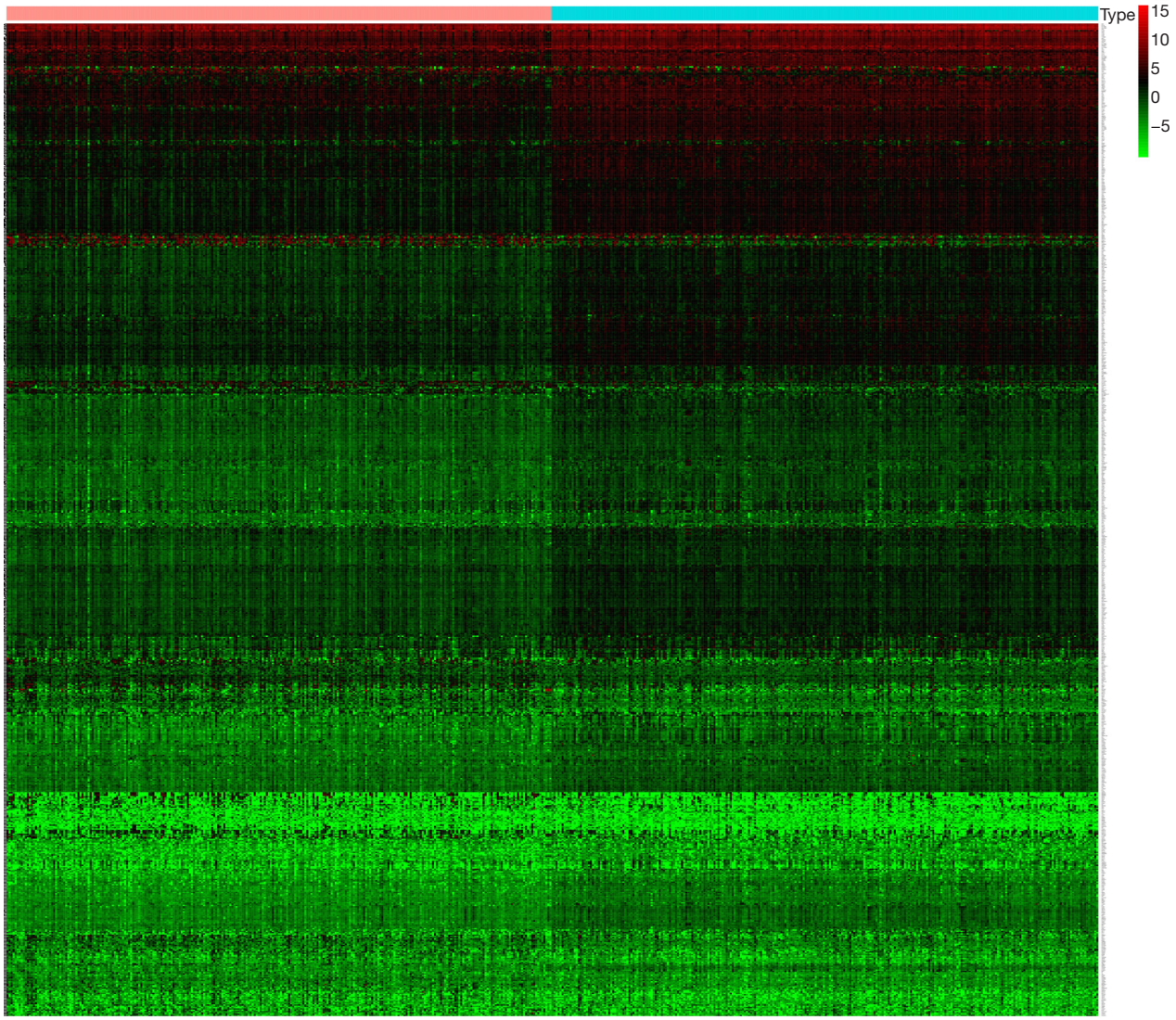


Figure 4 Heat map of DEGs. Heat map for differentially expressed genes generated by comparison of the high *vs.* low score groups in Immune Scores. Rows reflect the gene's name, and columns represent sample identifications (not shown in the plot). DEGs were identified using the Wilcoxon rank sum test, with FDR <0.05 and $|\log_2 FC| > 1$ as the cut-off of significance. DEGs, differentially expressed genes; FDR, false discovery rate.

presented by major histocompatibility complex (MHC) class I molecules. we have already confirmed the role of *PSMB8* in the evolution of cutaneous squamous cell carcinoma, papillary thyroid carcinoma, and prostate adenocarcinoma, (13-15), In addition to the previously mentioned tumors, *PSMB8* plays an inhibiting neovascularization role in glioma by regulating ERK1/2 and PI3K/AKT signaling pathways (16,17). These findings elucidate that the expression of PSMB8 is associated with multiple immune-related pathways in vivo, and that the gene may act as a powerful biomarker to determine the prognosis of a variety of cancers.

In summary, we selected *PSMB8* as the target gene.

PSMB8 expression in MM patients

We divided the MM samples into *PSMB8* high- and *PSMB8* low-expression groups. A survival analysis revealed that MM patients in the *PSMB8* high-expression group had a higher survival rate than those in the low-expression group within 15 years after tumorigenesis.

On the other hand, we found that *PSMB8* expression

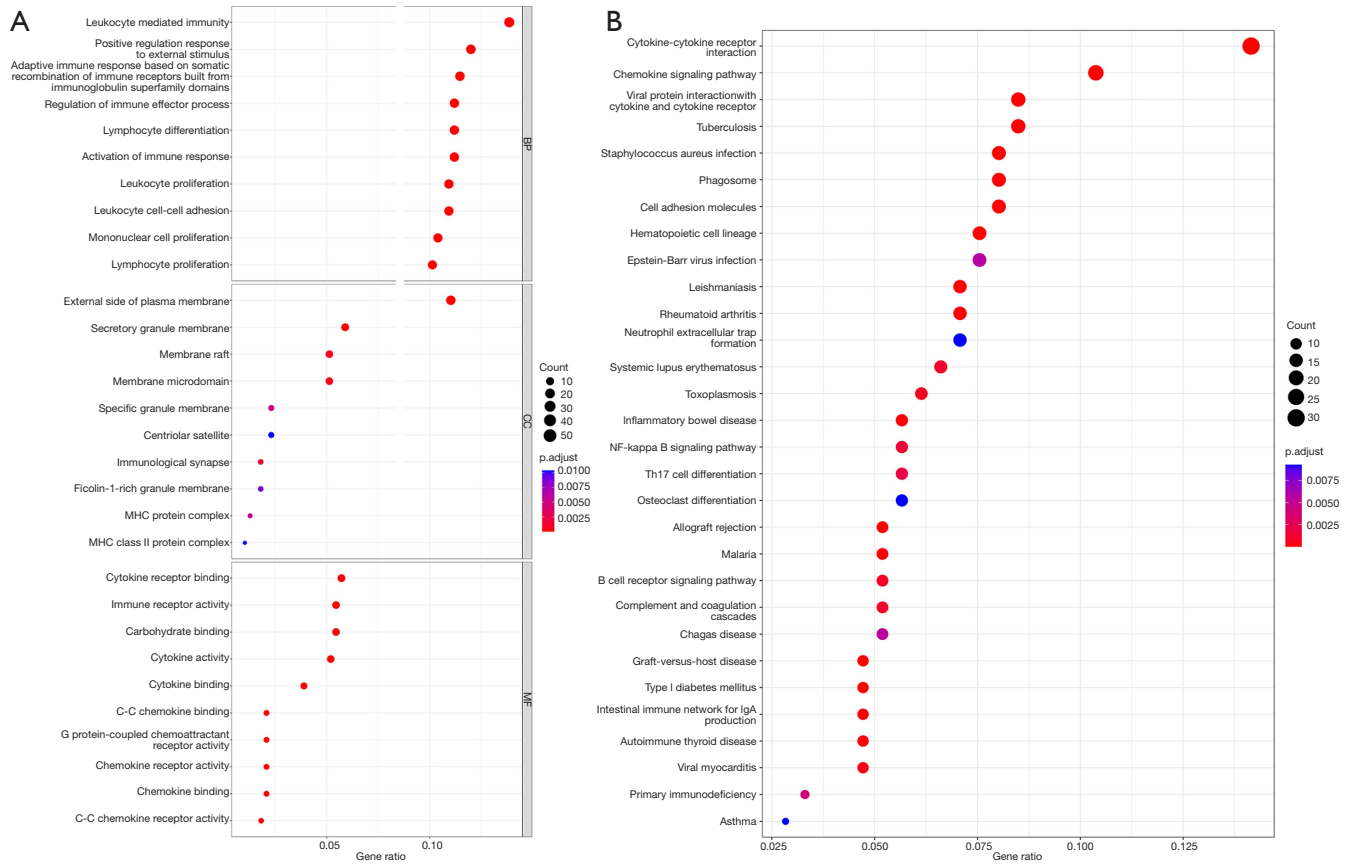


Figure 5 Enrichment analysis of GO and KEGG for differentially expressed genes. GO and KEGG enrichment analysis for 406 differentially expressed genes, where terms with P and q<0.05 were considered to be enriched significantly. The figure on the left shows the three ontologies in GO enrichment analysis, describing the BP, CC, and MF of genes involved. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological processes; CC, cellular components; MF, molecular functions.

varied among MM patients according to the tumor stage. The expression became downregulated as the tumor stage progressed. This finding suggested that *PSMB8* is a protective factor for MM patients and may be closely associated with prognostic factors, such as tumor infiltration and OS (*Figure 7*).

Correlation of PSMB8 expression with tumor-infiltrating immune cells (TICs)

We further validated the correlation of *PSMB8* expression with immune components using the CIBERSORT method. We downloaded 22 common TIC maps and analyzed the correlation of *PSMB8* expression with the TICs (*Figure 8*). The correlation and variation analysis showed significant differences in the contents of 14 TICs between the *PSMB8* high- and low-expression groups (*Figure 8C*). We also observed that in the TME of MM patients, the contents of 15 common TICs were correlated with *PSMB8* expression. We performed an intersection analysis for these two variables and found ten types of TICs (*Figure 8E*). *PSMB8* expression was positively correlated with the contents of M1 macrophages, CD8 T cells, $CD4^+$ T cells, follicular helper T cells, γδ T cells, regulatory (Tregs) T cells, and activated NK cells, and was negatively correlated with M0 macrophages, resting mast cells, and CD4 resting memory T cells (*Figure 8D*). This finding indicated that *PSMB8* expression significantly affected the immunocompetence of TME.

Correlation of PSMB8 with common immune checkpoints (ICPs)

We also investigated whether *PSMB8* expression could

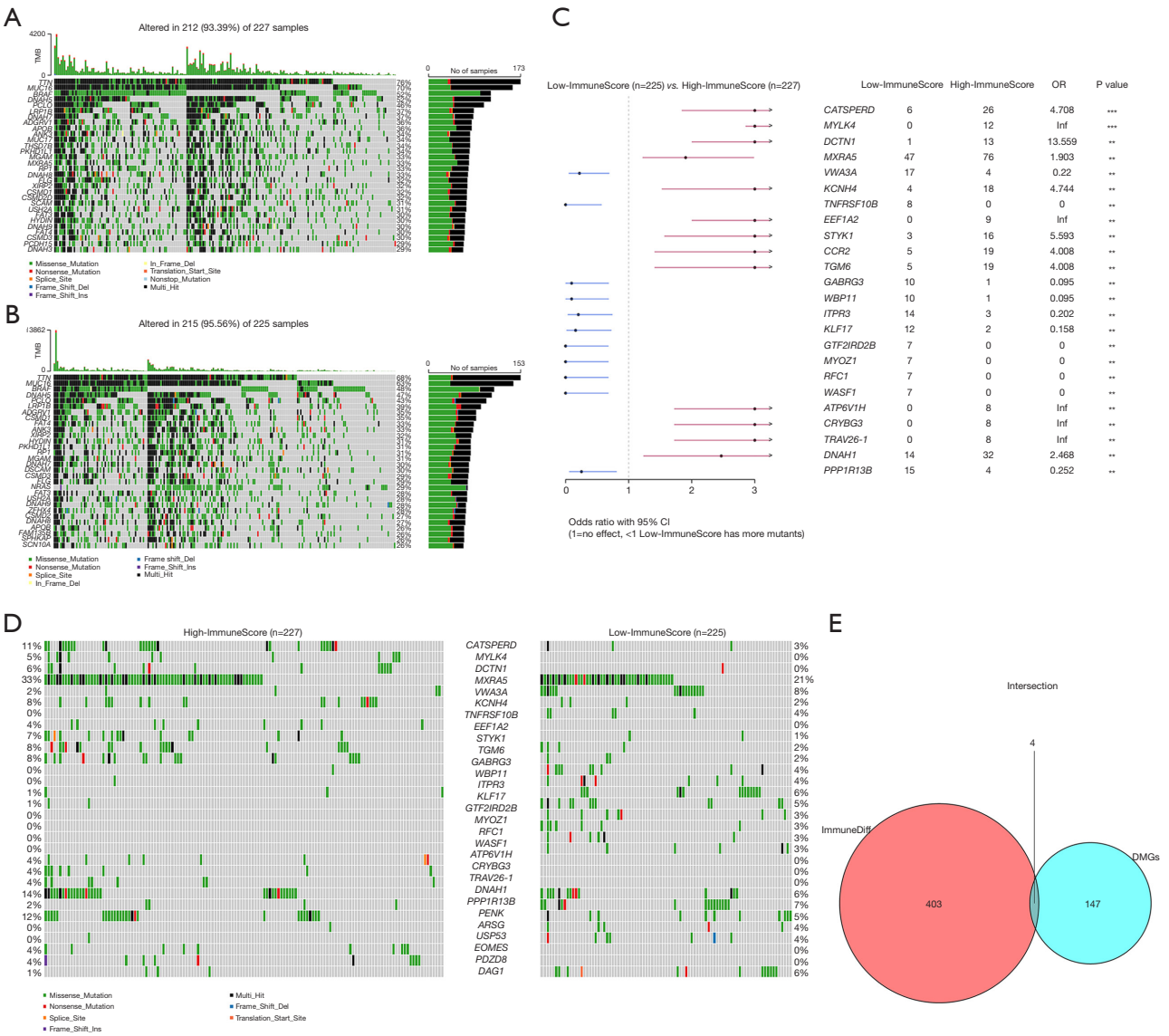


Figure 6 Somatic mutation analyses and identification of shared genes in DEGs and DMGs. (A,B) Oncoplots show the top 30 frequently mutated genes. The types of mutations in each MM (malignant melanoma) sample are displayed in the central panel, and the mutation frequency is presented in the upper panel. The frequency and type of mutation in the high- and low-immunity cohorts are depicted in the right-hand bar graphs, respectively. The legend for the mutation types is shown at the bottom. (C) Forest plot displays the significant DMGs between the two cohorts. The confidence intervals for each included study were described with multiple line segments parallel to the horizontal axis centered on a vertical invalid vertical line with a abscissa scale of 1, and when the 95% C.I horizontal line intersected the invalid vertical line, we considered that the mutation did not differ significantly in the grouping of high and low immunity. When the horizontal line is to the right of the invalid vertical line, the gene has more mutations in the high immune score group, and when the horizontal line is to the left of the invalid vertical line, it means that the gene has more mutations in the low immune score group. ***P<0.001, **P<0.01. (D) Oncoplot shows the 30 differentially mutated genes (P<0.03) between the high- and low-immunity groups. The types of mutations in each malignant melanoma sample are presented in the central panel, whereas the legend for the mutation types is located at the bottom. (E) Venn plot presents the shared genes of DEGs and DMGs, ImmuneDiff in the figure represents differentially expressed genes in the high and low immune groups. DEGs, differentially expressed genes; DMGs, differentially mutated genes.

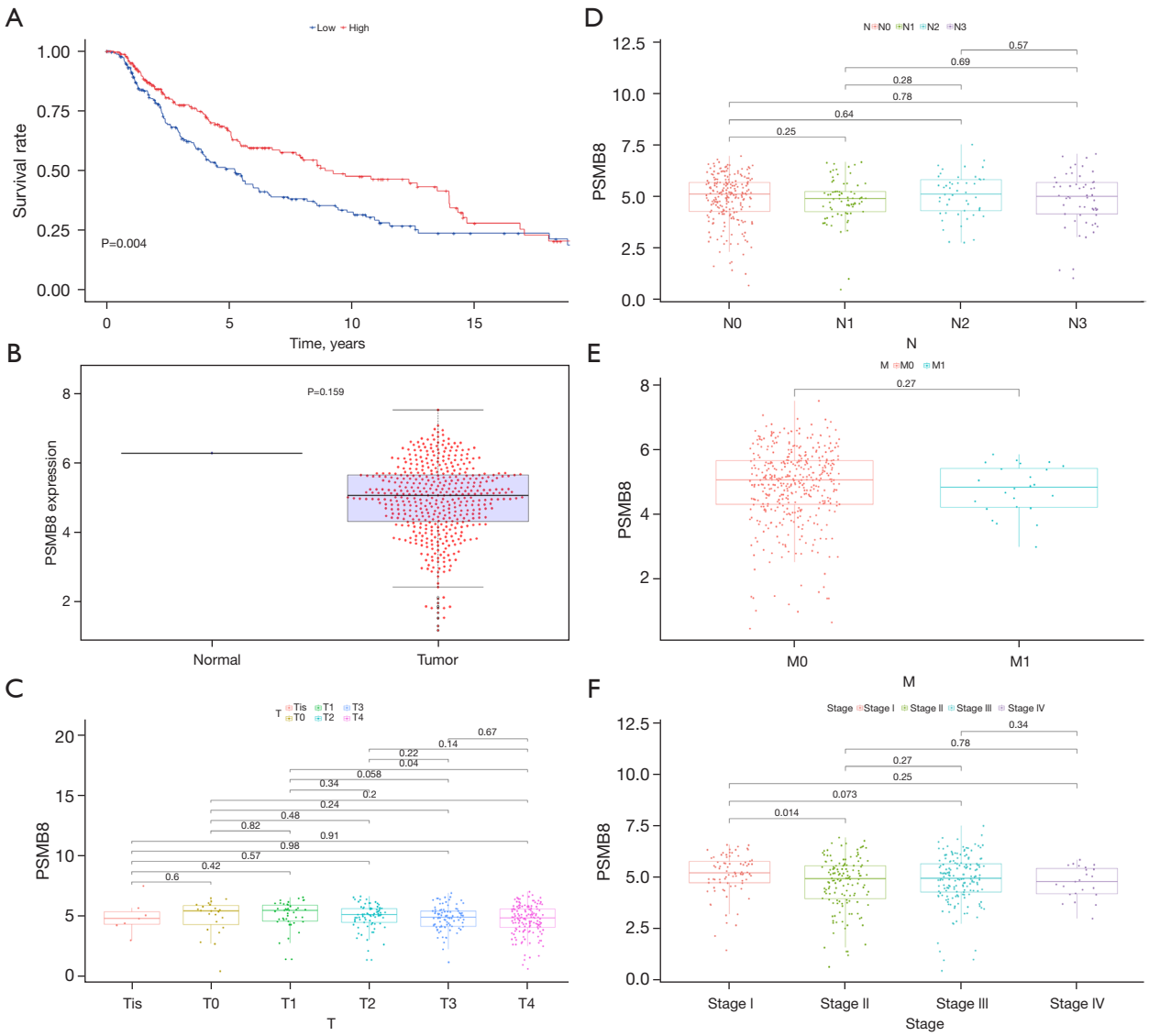
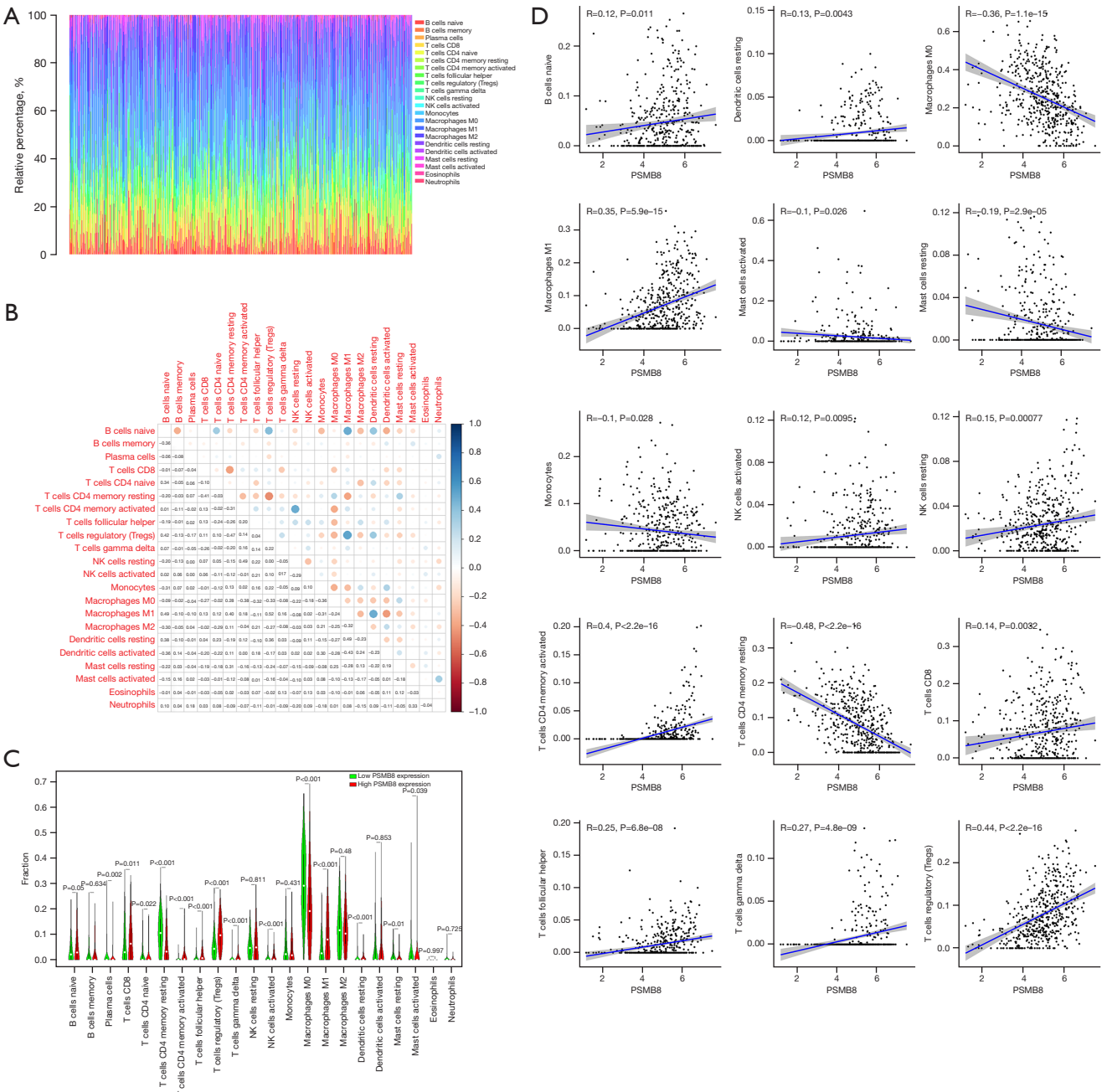


Figure 7 The differential expression of *PSMB8* in samples and its correlation with survival and clinicopathological staging characteristics in malignant melanoma patients. (A) Survival analysis for malignant melanoma patients with different *PSMB8* expressions. Based on the median expression level, patients were classified into the high- and low-expression groups (P=0.004, log-rank test). (B) Differential expression of *PSMB8* in the normal and tumor samples (P=0.159, Wilcoxon rank sum test). (C-F) The association between *PSMB8* expression and clinicopathological characteristics. Wilcoxon or Kruskal-Wallis rank sum tests was performed to test the statistical significance.

assess the effects of immunotherapy by analyzing the correlation of *PSMB8* with common ICPS. The results showed that *PSMB8* expression was correlated with *CTLA4*, *LMTK3*, *CD28*, *CD40*, *TNFRSF9*, *ICOS*, *TNFRSF18*, and *TNFSF18* (*Figure 8F*).

Discussion

Based on MM patient data in the TCGA database, this study aimed to identify differentially expressed and mutated immune-related genes in the TME of



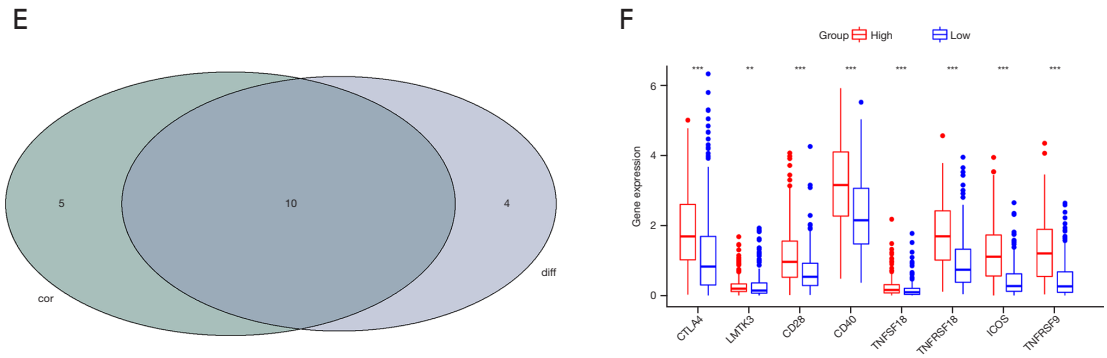


Figure 8 TIC profile in malignant melanoma samples, correlation analysis, and the correlation of the proportion of tumor-infiltrating immune cells and common immune checkpoints with *PSMB8* expression. (A) Bar plot shows the ratio of 21 types of TICs in tumor samples, and the columns represent sample identifications. (B) Heat map describes the association between 21 types of tumor-infiltrating immune cells with numeric in each box representing the P value of the correlation. The shadows in color boxes indicate correlation values. The significance test was conducted using Pearson coefficient. (C) Violin plot depicts the ratio differentiation of 21 immune cells in malignant melanoma samples with low or high *PSMB8* expression. The Wilcoxon rank sum test was used to test the significance. (D) Scatter plot shows the correlation between 15 Tumor-infiltrating immune cells and *PSMB8* expression (P<0.05). Blue line is a fitted linear model that represents the proportional tropism of the immune cell along with *PSMB8* expression. The correlation test was conducted using Pearson coefficient. (E) Venn plot displays ten types of tumor-infiltrating immune cells correlated with *PSMB8* expression, which are codetermined by the difference and correlation analyses. (F) The results show that the high *PSMB8* expression group had significantly higher expression of immune checkpoints than the low *PSMB8* expression group (***P<0.001, **P<0.01). TIC, Tumor-infiltrating immune cell.

MM that were significantly correlated with the clinical characteristics, survival, and prognosis of MM patients. Using bioinformatics analysis, we found that *PSMB8* was correlated with immune cell infiltration in MM generation and progression. It can be concluded that *PSMB8* plays a critical role in immune-related biological processes. PSMB8 also correlated with ICPs, indicating that *PSMB8* can be used as a biomarker for TME rehabilitation and may be a potential indicator for predicting the prognosis of MM patients and assessing the effects of immunotherapy.

Tumor biological behavior cannot be interpreted by the characteristics of a single specific tumor cell. Multiple components of the TME need to be taken into account. The TME is a dynamic network and has noticeable effects on tumor generation and progression, as well as complicated effects on tumor progression, immune regulation, and treatment (5). The biological function of tumor cells and activated T cells depends on glycolysis (18,19) so there is competition for nutrition among tumor cells, other immune cells, and stromal cells in the TME. Some metabolites produced in the TME might be able to inhibit an antitumor immune response. As such, a comprehensive understanding of the correlation of in-TME tumor cells with other cellular components would be of great therapeutic value. Increasing evidence indicates that the intracellular signaling pathway of tumor cells might be related to the dynamic change in immune patterns in the TME (20). In this study, bioinformatics analysis showed that the content of immune cells significantly influenced the OS rate and T stage of patients with MM. A variety of evidence has suggested that the density and diversity of invasive tumor immune cells are closely related to prognosis and the prediction of therapeutic effects. Therefore, immune cells in the TME may greatly affect the clinical response of MM patients to different immunotherapy strategies. The transformation of TME from a tumor-friendly to a tumor-suppressive type may be an effective treatment for MM patients to improve survival, delay tumor progression, and improve symptoms (21). In the past 10 years, antitumor treatments have been reformed many times. Conventionally, pharmacological agents (such as chemotherapy) have targeted a wide range of tumors, but current new treatment strategies target specific cells in the TME. Therefore, stimulating and activating the T-cell response in tumor cells and having an immune-promoting TME are critical. Immune checkpoint blockade (ICB) is an initial form of TME immunotherapy based on antibodies (22). ICB prevents receptor ligands, such as *CTLA4* and *PD1*, from interacting with T-cell

receptors, hence inhibiting T-cell activation and function. Immune checkpoint inhibitors (ICIs), particularly *CTLA4* and *PD-1* antibodies, have revolutionized the treatment of many cancers. For melanoma, many new immunotherapies are now used to treat melanoma, including *PD1*, *PD-L1*, and *CTLA-4* inhibitors. However, these regimens are only effective for a subset of patients, with others showing only a limited response or response failure, especially in the advanced stages (23,24). Furthermore, most patients with advanced melanoma do not respond to ICI therapy due to primary or acquired drug tolerance (22,25). Combining anti-*CTLA4* and anti-*PD-1* checkpoint inhibitors to enhance antitumor immune reactions, or ICIs combined with intratumoral oncolytic virotherapy, may trigger pro-inflammatory rebuilding of the TME to overcome anti-*PD-L1* resistance. It has been known that ICIs induce immunotoxicity while stimulating the body's immune system, inevitably resulting in immune-related adverse events. The therapies mentioned above also cause different degrees of immune-related adverse events (22,26). Unfortunately, no highly sensitive and specific biomarker for ICIs has been reported. As a result, more relevant biomarkers should be identified, and new TME-targeting agents are required to reduce the risk of immune-related adverse events. In this study, we conducted comprehensive bioinformatic analysis on transcriptome RNA-seq and somatic mutation data. The results showed a significant correlation between downregulated *PSMB8* expression and poor prognosis and advanced T stage. Moreover, patients in our high-immune group had more *PSMB8* mutations, indicating that *PSMB8* mutations induced the expression of new tumor-specific antigens to activate the immune response. Further, CIBERSORT analysis showed that a high *PSMB8* expression was closely correlated with the immune-related biological process. In addition, the expression of ICP was elevated in the high *PSMB8* expression group. Therefore, this study suggests that *PSMB8* is a possible prognostic marker, an indicator of immunotherapeutic response and TME recovery, and a potential therapeutic target of TME in patients with MM.

PSMB8 (proteasome 20S subunit beta 8) is a poly-catalytic protease complex possessing a highly ordered ring-shaped 20S core structure. It serves as a protein-coding gene, is distributed throughout eukaryotic cells at high concentration, and cleaves peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. Previous studies have shown that *PSMB8* inhibited angiogenesis in glioma by regulating the *ERK1/2* and *PI3K/AKT* pathways (16,17). Another study on the functions of *PSMB8* in the pathogenesis of mucinous ovarian cancer has confirmed that *PSMB8* is an intermediary between the presentation of foreign antigens by *MHC-I* molecules and the irregular nuclear factor-light chain enhancer (*Nik/NF-kB*) pathway of activated B cells (27). Additionally, *PSMB8* is involved in activating the *PI3K/AKT* pathway in acute myelogenous leukemia (28), and several studies have reported its contribution to the progression of cutaneous squamous cancer, papillary thyroid cancer, and prostate cancer (13-15). Several studies have proposed that *PSMB8* works by promoting immune cell concentration. In the T-cell-mediated antitumor immune response, *PSMB8* can reduce colony formation after radiation and increase the expression of apoptosis-inducing molecules, such as cleaved PARP and caspase-3 (29,30). Nonetheless, the role of *PSMB8* in the occurrence and progression of MM remains unclear, and the immunological and prognostic role of *PSMB8* in TME remains to be elucidated. The results of this study show that high *PSMB8* expression reflects the enhancement of immune components in MM patients, which may indicate an increase in the clinical response to immunotherapy and a more favorable clinical prognosis. *PSMB8* expression decreases with advancing tumor stage, suggesting that *PSMB8* may be a protective factor for patients with MM.

Further analysis demonstrated the effects of *PSMB8* on the TME, especially on immune components. *PSMB8* expression was positively correlated with M1 macrophages, CD8 T cells, $CD4^+$ T cells, follicular helper T cells, γδ T cells, regulatory (Tregs) T cells, and activated NK cells, and was negatively correlated with M0 macrophages, resting mast cells, and resting CD4 memory T cells. In addition, high ICP expression was common in the high *PSMB8*-expression group, suggesting *PSMB8* may be a potential predictor of the immunotherapeutic response in MM. In conclusion, *PSMB8* may be an antitumor biomarker for predicting patients' survival and immune response. Our comprehensive bioinformatics analysis revealed that MM patients in the high *PSMB8*-expression group demonstrated a higher OS rate, an earlier T stage, and increased TME immune components and common ICPs. As such, it can be determined that *PSMB8* is an indicator of TME rehabilitation, and has the potential to be used as an effective immunotherapy and clinical prognosis predictor.

Conclusions

Using comprehensive bioinformatics analysis, this study

is the first to show that *PSMB8* is a promising indicator of TME rehabilitation and can be used as a potential predictor of clinical prognosis, such as OS, tumor invasion, and immunotherapy response in MM patients. Further research on the correlation between *PSMB8* and ICPs and the potential mechanism of *PSMB8*-related immunobiological processes may facilitate the treatment of MM.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5761/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5761/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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