



# Exploring Tumor Immune Microenvironment and Its Associations With Molecular Characteristics in Melanoma

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**Background:** The tumor microenvironment (TME), which involves infiltration of multiple immune cells into the tumor tissues, plays an essential role in clinical benefit to therapy. The chemokines and their receptors influence migration and functions of both tumor and immune cells. Also, molecular characteristics are associated with the efficacy of melanoma therapy. However, there lacked exploration of immune characteristics and the association with molecular characteristics.

**Methods:** We collected the currently available 569 melanoma samples that had both the genomic and transcriptional data from TCGA and SRA databases. We first identified TME subtypes based on the developed immune signatures, and then divided the samples into two immune cohorts based on the immune score. Next, we estimated the compositions of the immune cells of the two cohorts, and performed differential expression genes (DEGs) and functional enrichments. In addition, we investigated the interactions of chemokines and their receptors under immune cells. Finally, we explored the genomic characteristics under different immune subtypes.

**Results:** TME type D had a better prognosis among the four subtypes. The high-immunity cohort had significantly high 16 immune cells. The 63 upregulated and 384 downregulated genes in the high-immunity cohort were enriched in immune-related biological processes, and keratin, pigmentation and epithelial cells, respectively. The correlations of chemokines and their receptors with immune cell infiltration, such as CCR5-CCL4/CCL5 and CXCR3-CXCL9/CXCL10/CXCL11/CXCL13 axis, showed that the recruitments of 11 immune cells, such as CD4T cells and CD8T cells, were modulated by chemokines and their receptors. The proportions of the four TME subtypes in each

molecular subtype were comparable. The two driver genes, CDKN2A and PRB2, had significantly different MAFs between the high-immunity and low-immunity.

**Conclusion:** We dissected the characteristics of immune infiltration, the interactions of chemokines and their receptors under immune cells, and the correlation of molecular and immune characteristics. Our work will enable the reasonable selection of anti-melanoma treatments and accelerate the development of new therapeutic strategies for melanoma.

**Keywords:** melanoma, tumor microenvironment, molecular characteristics, survival outcome, chemokines and their receptors

## INTRODUCTION

Melanoma is the most deadly kind of skin cancer in which cancer cells form in melanocytes (1). It is also a highly heterogeneous cancer, which has been putting a heavy burden on human health with a higher risk of metastasis and a high death rate (2). The number of cases of melanoma has been increasing over the past 30 years. It was reported that there were more than 324,600 diagnosed cases and 57,000 deaths worldwide in 2020 (2).

The tumor microenvironment (TME), which involves infiltration of multiple immune cells into the tumor tissues, plays an essential role in prognosis and clinical benefit to therapy. Some studies have reported the infiltration of immune cells in the melanoma microenvironment (3, 4). Immune cells play a dualistic role, including promoting tumor development or anti-tumor development, and they create different microenvironments depending on their types and interactions (5). The chemokines and their receptors influence migration and functions of both tumor and immune cells, which significantly impacts tumor fate and is critical in melanoma control and progression (6). Therefore, it is important to understand the characteristics of immune infiltration and the interactions of chemokines and their receptors under immune cells.

A study reported a widely suitable immune subtyping method, which identified six immune subtypes among more than 10,000 tumors comprising 33 diverse cancer types, based on the five representative signatures including IFN- $\gamma$ , TGF- $\beta$ , Macrophage, Lymphocyte and Wound healing based on 160 immune expression signatures (7). Then, a pan-cancer study extended to 29 functional gene expression signatures (Fges) for immune subtyping (8). It selected Fges representing the major functional components and immune, stromal, and other cellular populations of the tumor by integrating knowledge from multiple publications. Then, the '29 Fges' were chosen by using tSNE projections and Mann-Whitney tests, and were divided into four groups according to their functions, processes or cell types. Finally, the tumor samples were classified into four subtypes using unsupervised clustering based on the ssGSEA scores of the expression patterns of 29 Fges. The two studies focused on the pan-cancer, but lacked exploration of immune characteristics for melanoma. A recent study revealed three immune cell infiltration (ICI) clusters associated with different immune subtypes, and assessed the predictive values of the ICI scores for the immune therapy benefit in melanoma (4). The ICI clusters were identified using limited features, and this study provided little information for the association of TME and molecular characteristics for melanoma.

The Cancer Genome Atlas (TCGA) developed a molecular subtyping method based on the mutations for melanoma (9). The resulting four molecular subtypes were BRAF subtype characterized by BRAF hot-spot mutations, RAS subtype defined by the presence of RAS hot-spot mutations in all three RAS family members (N/K/HRAS), NF1 subtype featured by NF1 loss-of-function mutations, and Triple-Wild-Type defined by the absence of hot-spots BRAF, N/H/K/-RAS or NF1 mutations. A study showed the whole genome mutation landscape of 183 samples of three melanoma (cutaneous, acral and mucosal), and revealed diverse carcinogenic processes across the three subtypes (10). These two studies depicted molecular characteristics only.

To fill in the gaps of these previous studies, we employed the currently available 569 melanoma samples that had both the genomic and transcriptional data, and we took the advantages of the developed immune and molecular subtyping methods to dissect the characteristics of immune infiltration, the chemokine-receptor pairs under immune cells, and the correlation of molecular and immune characteristics. We identified candidate biomarkers that can be used to predict clinical benefits for melanoma, which enables the reasonable selection of anti-melanoma treatments. Our work contributes to the understanding of melanoma immune microenvironment and the association with molecular characteristics, which will facilitate the development of new therapeutic strategies for melanoma.

## MATERIALS AND METHODS

### Melanoma Data Resources

We collected 569 melanoma samples from four datasets from the Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>) and SRA databases, of which 567 samples had both transcriptional and genomic data and 2 samples had the transcriptional data only (**Supplementary Table S1**). Accessions for these 569 samples are the TCGA skin cutaneous melanoma project (TCGA-SKCM), phs001036 (11), phs000452 (12) and SRP070710 (13).

### RNA-Seq Processing

99 of the 569 samples had no normalized counts, so we aligned their RNA-seq reads to the human reference genome GRCh37 using STAR (14) v2.7.5b with default parameters, and quantified the resulting gene expression count matrix with RSEM (15) v1.3.1. Then we integrated with the count matrix of the 470 samples, and

did quantile normalization. The batch effect was eliminated by Combat\_seq (16).

## Detection of CNV

The CNVs of the 469 samples were predicted by GISTIC (17) with 0.95 GISTIC significance threshold. The CNV levels were extracted from the output files for all genes using a cutoff of  $\pm 2$ . The CNVs of the 98 samples were downloaded from cBioportal (<https://www.cbioportal.org/datasets>).

## TME Subtyping

29 Fges representing four immune signature modules, including anti-tumor microenvironment, pro-tumor microenvironment, angiogenesis fibrosis and malignant cell properties, were selected to identify TME subtypes. The detailed information about the 29 Fges was showed in **Supplementary Table S2**. 29 Fges signature scores were calculated using Single-sample gene set enrichment analysis (ssGSEA) in python3 and median-scaled for all the samples. Then we classified the samples using louvain community detection algorithm to identify immune subtypes based on the signature scores. The immune and stromal cell scores were estimated by the ESTIMATE method (18). According to the immune scores, we divided the patients into two cohorts, high-immunity and low-immunity for the following analysis.

## Evaluation of Overall Survival

To evaluate the impact of immune features on patients' outcome, the R package survival and survminer were used to analyze the Overall Survival (OS) using the Kaplan-Meier method. P-value of less than 0.05 was considered to be statistically significant.

## Depiction of Immune Cell Population and the Relationship With Chemokines and Their Receptors

To identify the differences of the fractions of immune cell types between the high-immunity and low-immunity cohorts, we used ImmuneCellAI (19) to estimate the compositions of the immune cells. For each immune cell, the statistical difference in the compositions between the high-immunity and low-immunity cohorts was evaluated by Mann-Whitney U test. The relationships of immune cells and the interaction between chemokines and their receptors were evaluated by Spearman's correlation.  $Abs(r) > 0.5$  and  $p < 0.05$  were considered to be related and statistically significant, respectively. The interaction network of Chemokines and their receptors for immune infiltration in melanoma was constructed by Gephi v0.9.2.

## Detection of Significantly Mutated Genes and Their CNVs

To identify driver genes, MutSigCV (20) was used based on 568 samples. Genes with  $q < 0.1$  were deemed significant, and the genes whose MAFs were less than 1% were removed. The statistical differences of the identified driver genes between the high-immunity and low-immunity cohorts were performed by Fisher's exact test. The distributions of the driver genes in the high-immunity and low-immunity cohorts were performed by R

package Maftools (21) and ComplexHeatmap (22). For the CNVs of these significantly mutated genes, the statistical differences between the high-immunity and low-immunity cohorts were evaluated by Fisher's exact test.

## Identification of Differentially Expressed Genes and Functional Enrichment Analysis

The differential gene expression analyses between the high-immunity and the low-immunity cohorts were performed by DESeq2 (23), and the estimated significance level (P-value) for the differential expression analysis was adjusted by Benjamini and Hochberg False Discovery Rate (FDR) correction. The Gene Ontology (GO) enrichment analyses of differential expression genes were performed by the R package clusterProfiler (24, 25).

## Molecular Subtyping

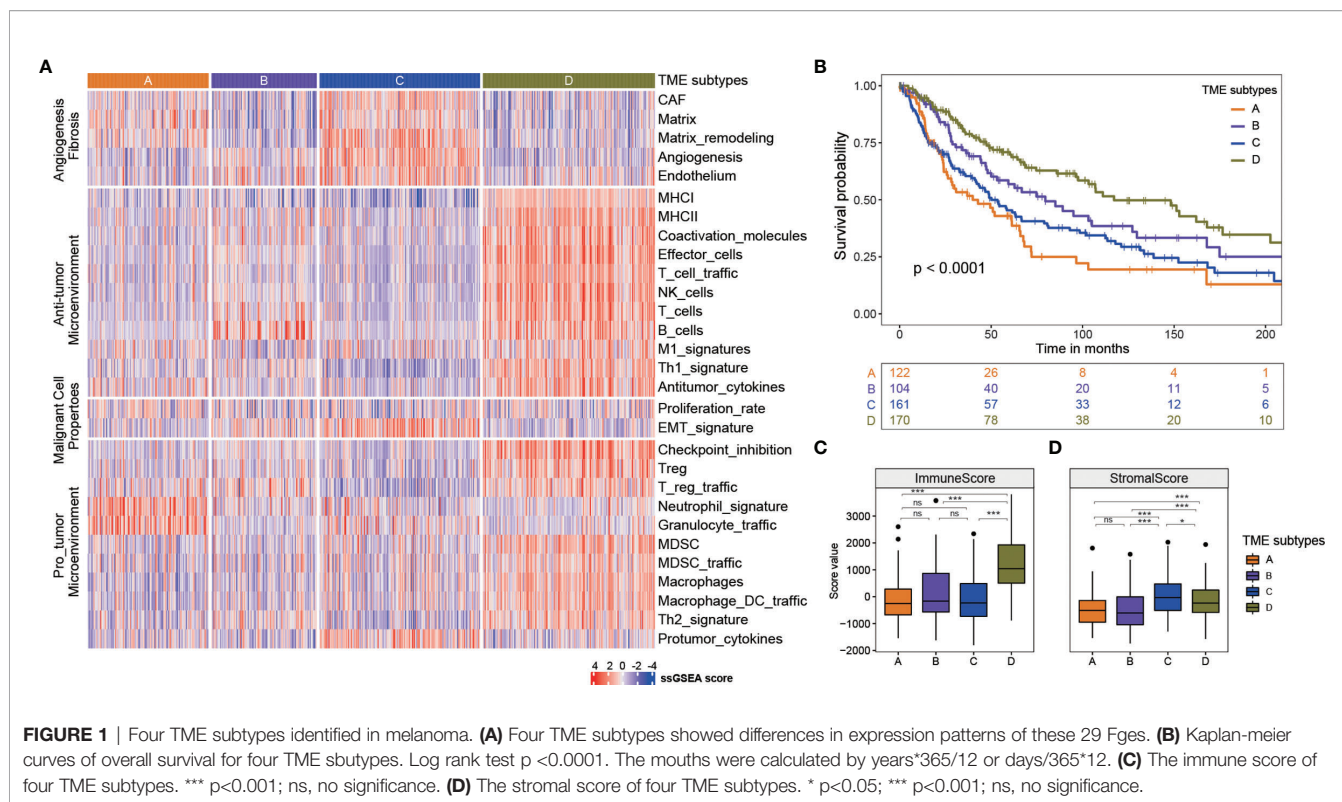
We classified the samples into four molecular subtypes: BRAF subtype characterized by BRAF hot-spot mutations, RAS subtype defined by the presence of RAS hot-spot mutations in the three RAS families (N-, K-, and H-RAS), NF1 subtype characterized by the NF1 loss-of-function mutations, and Triple-Wild-Type defined by the absence of hot-spots BRAF, N/H/K/-RAS, or NF1 mutations. Hot-spot mutations with p-values less than  $1e-8$  for the genes were chosen from HotSpotsAnnotations.

## RESULTS

### Different Survival Outcomes of the TME Subtypes in Melanoma

To understand how the interaction of the tumor cells and immune and stromal cells influences the prognosis, we did the TME subtyping and the prognostic analysis of the 569 melanoma samples. The 29 Fges constitute four groups, including anti-tumor, pro-tumor microenvironment, angiogenesis fibrosis and malignant cell properties (8). The expression patterns of these 29 Fges were used to identify the immune subtype. The 569 samples were classified into four immune subtypes (**Figure 1A**). Compared with the other immune subtypes, TME type D had higher expressions in genes involved in anti-tumor immune and tumor-promoting processes. TME types A and C had higher expressions in genes involved in angiogenesis fibrosis. Furthermore, TME type B displayed higher gene expression levels in B\_cells Fges than types A and C, and TME type A had the highest gene expression levels in neutrophil\_signature and granulocyte\_traffic.

To assess the prognostic values of TME subtypes, the Kaplan-Meier method was used to analyze the overall survival (the log-rank test, A: B: C: D,  $p < 0.0001$ , **Figure 1B**). The overall survival analysis showed that TME subtype D was characterized by a better overall survival, whereas TME subtypes A and C had the worse prognosis. TME subtype B had an intermediate survival outcome. Then, the stromal score and immune score of these four TME subtypes were calculated by ESTIMATE algorithm to measure the presence of tumor-associated stroma and the immune score. As shown in **Figure 1C**, the immune score of TME subtype D was significantly higher than that of the other



subtypes, indicating subtype D had an immune-hot phenotype with a better prognosis (Mann-Whitney  $U$  test,  $p < 0.05$ ). In terms of the stromal score, subtype C represented a higher score, while the subtype B had the lowest score (Mann-Whitney  $U$  test,  $p < 0.05$ , **Figure 1D**).

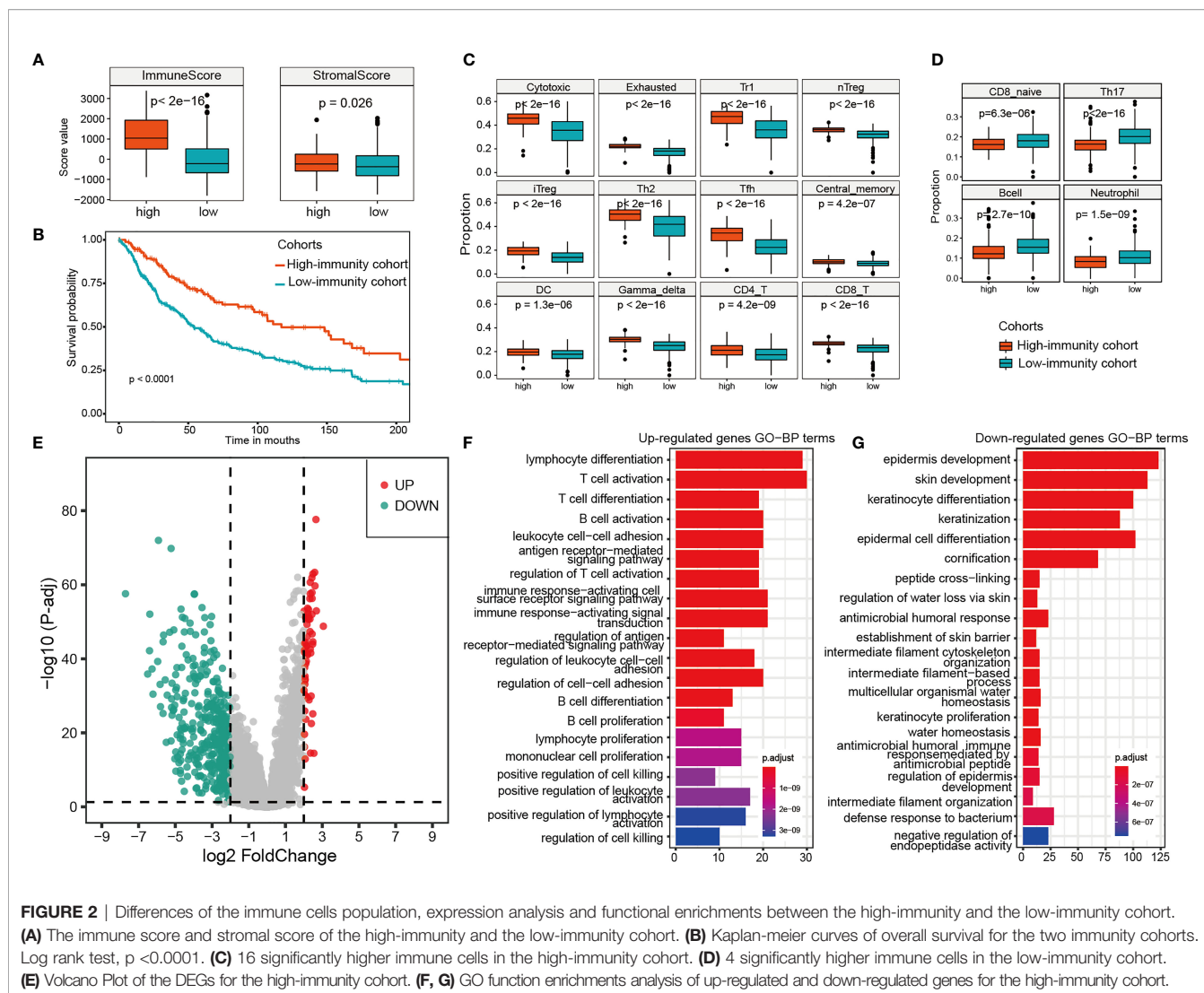
## Differences of the Immune Cells Population Between Immune Subtypes

Tumor-infiltrating immune cells are able to deeply influence tumor progression and patient outcome. So based on the immune score, we divided the samples into two cohorts, a high-immunity cohort including 175 samples from TME subtype D, and a low-immunity cohort including 393 samples from TME subtypes A, B and C (**Figures 2A, B**). The overall survival analysis showed that the high-immunity cohort had a better prognosis, whereas the low-immunity had a worse outcome (**Figure 2B**). Furthermore, ImmuneCellAI method was employed to measure the immune cell compositions. As shown in **Figures 2C, D**, the compositions of the immune cells varied between the two cohorts. The high-immunity cohort had significantly higher cytotoxic T cells, exhausted T cells, T regulatory type 1 (Tr1) cells, natural regulatory T (nTreg) cells and induced regulatory T (iTreg) cells, T helper type 2 (Th2) cells, T follicular helper (Tfh) cells, central memory T (TCM) cells, as well as dendritic cells (DC), gamma delta T cells and T lymphocytes (both CD4 T cell and CD8 T cell) (Mann-Whitney  $U$  test,  $p < 0.001$ , **Figure 2C**), while the low-immunity had significantly higher naive CD8 T cells, helper 17 (Th17) cells, B cells and neutrophil (Mann-Whitney  $U$  test,  $p < 0.001$ , **Figure 2D**). On the contrary, macrophage, mucosal associated invariant T (MAIT) cells, natural

killer (NK) cells showed decreased differences between the two cohorts (Mann-Whitney  $U$  test,  $0.001 < p < 0.05$ ), and there were no statistically significant differences in naive CD4 T cells, T helper type 1 (Th1) cells, effector memory T (TEM) cells, natural killer T (NKT) cells and monocyte cells between the two cohorts (**Supplementary Figure S1**).

## Differential Expression Analysis and Functional Enrichments Between Immune Subtypes

The differential expression analysis between the high-immunity and low-immunity cohorts was performed based on the expression profiles of the 569 melanoma samples. The genes with the FDR  $< 0.05$  and abs (log<sub>2</sub> FoldChange)  $> 2$  were selected as differentially expressed between the two cohorts. As a result, there were 63 upregulated and 384 downregulated genes in the high-immunity cohort (**Figure 2E**). PLA2G2D, IFNG, CD8B and NKG7 were found to be significantly upregulated, which are associated with tumor immunity (**Supplementary Table S3**). LAG3 is also significantly upregulated, which has primarily been shown to inhibit activity of T-cells (26). However, it was reported that LAG3 expression was associated with an improved overall survival (OS) compared to patients without LAG3 expression in oesophageal adenocarcinoma (27). LAG3 may serve as a biomarker for a strong immune response, and the two opposite immune functions (positively or negatively) of LAG3 can be attributed to the complexity of tumor immune microenvironment and tumor heterogeneity (28). The function of LAG3 in melanoma needs further experiments to verify. The downregulated genes were



mainly keratin, pigmentation, and epithelial genes such as *KLK7* and *IVL* (Supplementary Table S3).

Furthermore, functional enrichments by clusterProfiler found that the upregulated genes in high-immunity cohort were enriched in immune-related biological processes, such as T cell activation, cell killing and leukocyte cell-cell adhesion (Figure 2F), indicating a favorable lymphocyte activation related to the immune system. Some upregulated genes, such as *ADORA2A* (29) and *SLAMF6* (30), have been experimentally verified to be associated with tumor infiltration and T cell activation. In addition, *TIGIT*, which is a T-cell immune receptor with Ig and ITIM domains (31) and was upregulated in the high-immunity, has been recently identified as an attractive cancer immunotherapy target, because of its key role in interactions between the cells within the tumor microenvironment. The down-regulated genes were enriched in cornification, epidermis development, and keratin-related process (Figure 2G), all of which are associated with forming the outermost skin barrier (32). For example, human tissue kallikreins 7 (*hKLK7*) takes part

in skin desquamation, which has been involved in keratinocyte cell shedding to catalyze the degradation of intercellular cohesive structures at the skin surface (33).

## Different Chemokine-Receptor Pairs in Immune Cells

Patient prognosis is related to the variety of immune cells. Particular chemokine and their receptors that are expressed on tumor and immune cells are strongly associated with patient prognosis as well. The immune cells are recruited from the circulation to the tumor microenvironment through the chemokine-receptor pairs, all of which play the essential roles in cell migration, proliferation and survival (34). We examined the differences in gene expressions of the chemokines and their receptors between the high-immunity and low-immunity cohort. The chemokines, *XC11*, *XC12*, *CCL4*, *CCL5*, *CXCL13*, *CXCL11*, *CXCL10* and *CXCL9* were found to be upregulated in the high-immunity cohort, which recruit different subsets of immune cells into the tumor microenvironment. Most of the chemokine

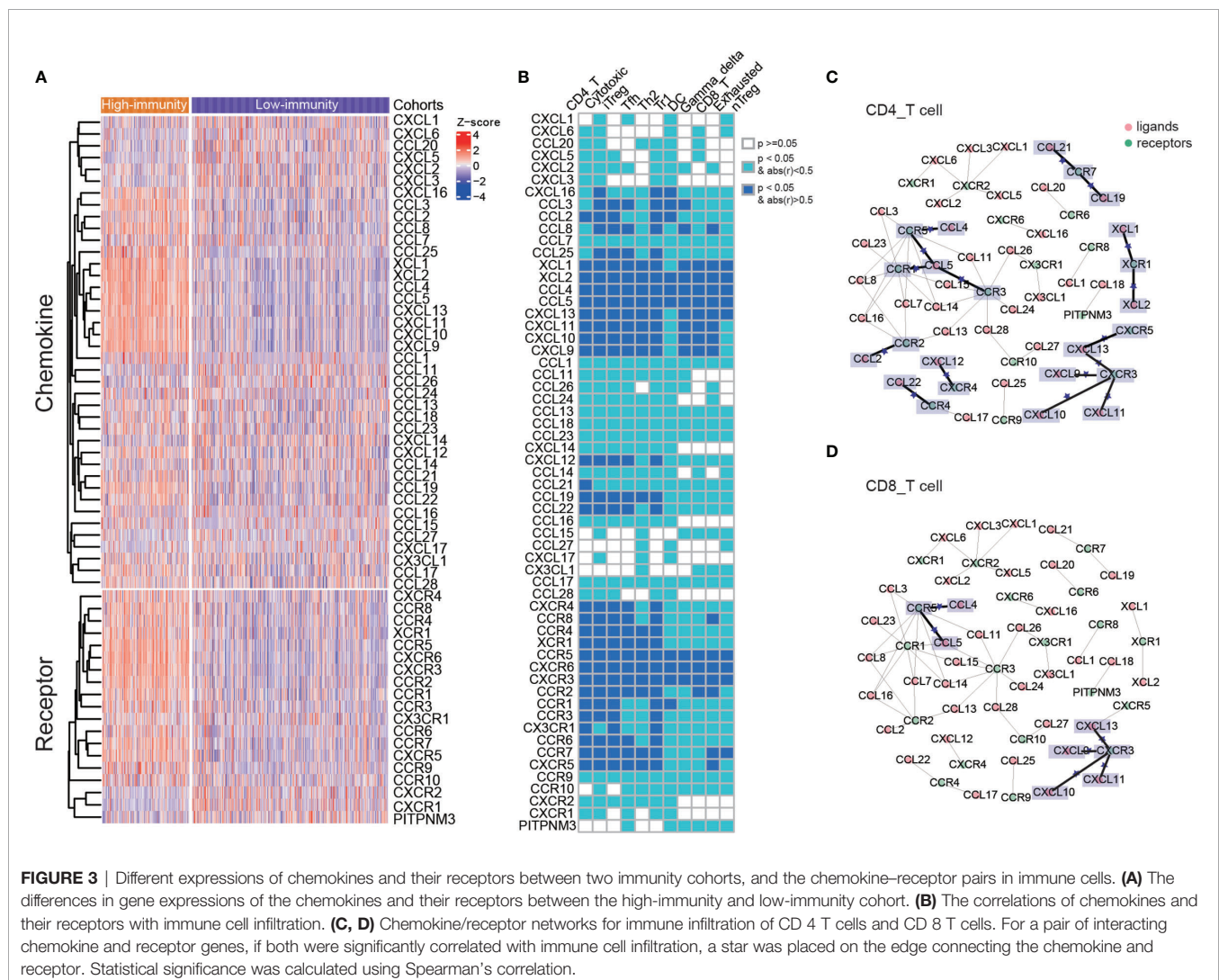
receptors were overexpressed in the high-immunity cohort as well (**Figure 3A**).

The chemokine–receptor pairs with immune cell infiltration showed that the recruitments of CD4 T cells, cytotoxic T cells, iTreg, Tfh, Th2, Tr1 cells, DC cells, gamma delta T cells, CD8 T cells, exhausted T cells and nTreg cells were modulated by chemokines and their receptors (**Supplementary Figures 2A, B**). The latter 5 cells had less significantly related chemokine receptors (Spearman's correlation,  $\text{abs}(r) > 0.5$ ,  $p < 0.05$ , **Figure 3B**). The chemokine–receptor pairs in all of the above immune cells were displayed in **Supplementary Table S4**. CCR5-CCL4/CCL5 and CXCR3-CXCL9/CXCL10/CXCL11/CXCL13 axis were significantly correlated with CD 4 T and CD 8 T cells, indicating that these chemokine–receptor pairs may recruit the T lymphocytes into tumor (**Figures 3C, D**). The role for CCR5 has been documented in T-cell recruitment to the tumor site, and local production of CCL5 or CCL3 induces selective recruitment of CD8 T cells and CTL-dependent tumor suppression in mouse models (35). It showed that high levels of CXCL9 were associated with strong infiltration of malignant melanoma by CD8 T cells and

improvement in patient survival (36). In addition, CD 4 T cell was significantly associated with other chemokine–receptor pairs, including XCR1-XCL1/XCL2, CCR7-CCL19/CCL21, CCR3/CCR1-CCL5, CCR2-CCL2, CXCR4-CXCL12 and CCR4-CCL22 (**Figure 3C**). Overexpressed chemokines and their receptors were likely to be involved in the anti-tumor immune response by promoting the migration of immune cells.

## The Genomic Characteristics Under Different Immune Subtypes

The differences of somatic mutations, including single nucleotide polymorphism (SNP), insertion (INS), deletion (DEL), missense mutations and nonsense mutations, between the high-immunity and low-immunity cohort were investigated. The high-immunity cohort held a significantly larger number of SNP, missense and nonsense mutations than that in the low-immunity (Mann-Whitney U test,  $p < 0.05$ , **Figure 4A**). Then, we identified the significantly mutated genes, which were CDKN2A, NRAS, PPP6C, BRAF and TP53, PTEN, NBPFF1, NF1, as well as PRB2, RAC1, B2M, PARM1, FLT3. Considering that HRAS and KRAS belong to the

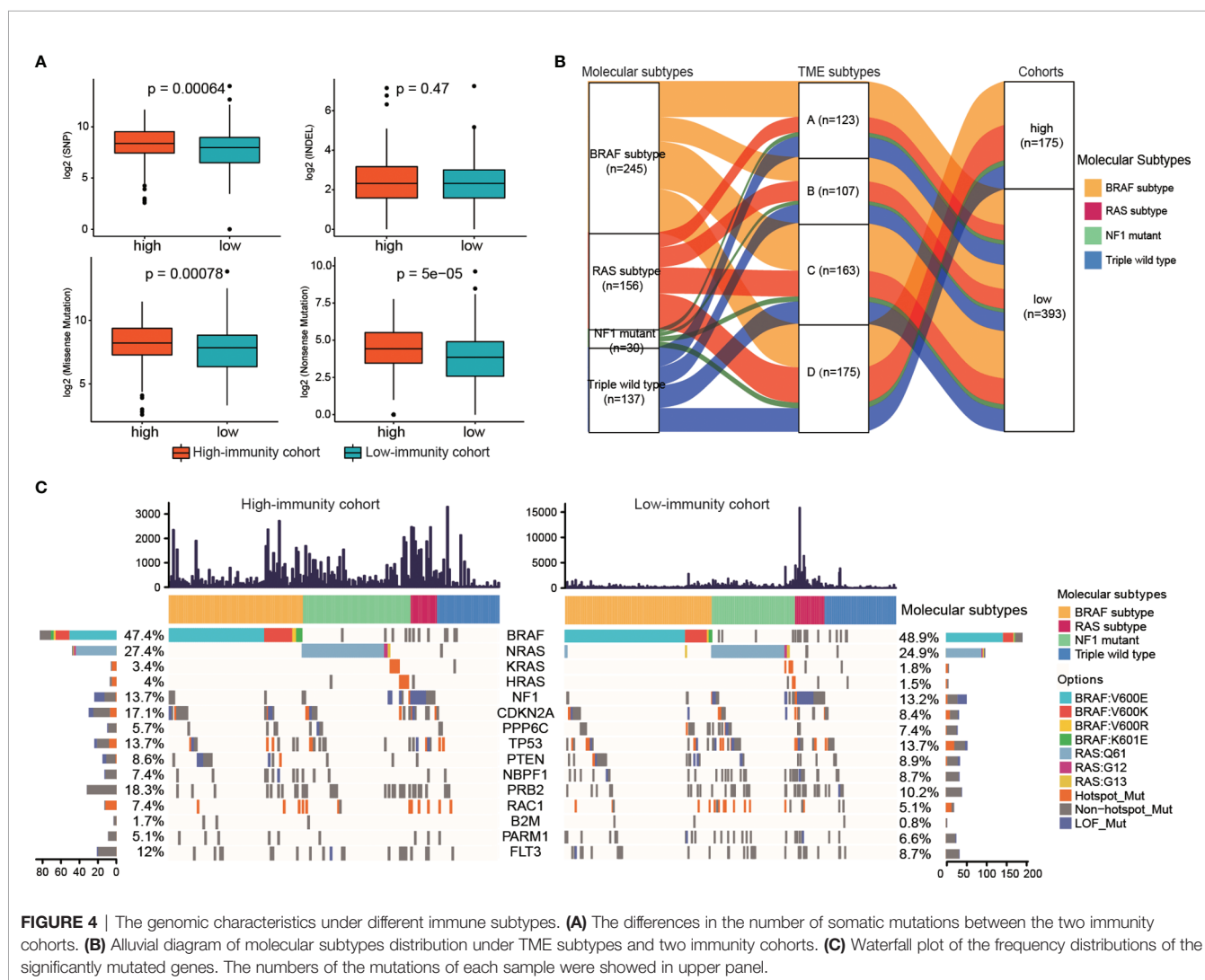


RAS family, the two genes were thought to be significantly mutated genes (9). We observed that two genes (CDKN2A and PRB2) had different MAFs between the high-immunity and low-immunity cohort (Fisher's exact test,  $p < 0.05$ , **Supplementary Table S5**). The CDKN2A and PTEN genes were significantly deleted in the low-immunity cohort, whereas RAC1 was significantly amplified in the low-immunity cohort (Fisher's exact test,  $p < 0.05$ , **Supplementary Table S5**).

Furthermore, to explore the relationship of the immune subtypes and the molecular subtypes, we did molecular subtyping based on the mutations (9). As shown in **Figure 4B**, the alluvial diagram displayed the distribution of the TME subtypes under different molecular subtypes and immune-cohorts. The proportions of the four TME subtypes in each molecular subtype were different. BRAF subtype showed the largest representations in all the four TME subtypes, while NF1 mutant subtype displayed the smallest representations (**Supplementary Figures S3A, B**). The proportion of RAS subtype in TME subtype A was lower than that in Triple wild type (20.33% for RAS-subtype, 26.83% for Triple wild type), while the proportions of RAS subtype in the other three TME

subtypes were higher than that in Triple wild type (29.91%, 25.77%, 32.57% for RAS-subtype in TME B, C and D, and 28.04%, 22.09%, 21.71% for Triple wild type in TME B, C and D).

The MAFs of the 15 significantly mutated genes, of which 5 genes were used to do molecular subtyping, in the high-immunity and low-immunity cohorts were illustrated in **Figure 4C**. The BRAF subtype represented the largest molecular classification, which constituted 47.4% and 48.9% of the high-immunity cohort and the low-immunity cohort, respectively. The most frequent BRAF mutation targeted the V600 amino acid residue. The second largest subtype was RAS family members (NRAS, 27.4% of the high-immunity cohort and 24.9% of the low-immunity; KRAS, 3.4% and 1.8% of the high-immunity cohort and the low-immunity cohort; HRAS, 4% and 1.5% of the high-immunity cohort and the low-immunity cohort). The RAS subtype included G12, G13 and Q61 hotspots mutations without the BRAF V600 and K601 mutations. The NF1 subtype contributed to 13.7% and 13.2% of the high-immunity and low-immunity cohort, respectively. NF1 is a significantly mutated gene in the MAPK pathway, which



downregulates RAS activity as a negative regulator of the RAS signal transduction pathway (37). The Triple-WT subtype was defined by a lack of hot-spot BRAF, N/H/K-RAS or NF1 mutations.

## DISCUSSION

Due to its high mutational load, melanoma represents one of the most immunogenic tumors with the potential to immune responses. To understand the immune microenvironment and molecular characteristics, we analyzed the transcriptional and genomic data of 569 melanoma samples from public databases. We identified four immune microenvironment subtypes. TME subtype D represented the overexpression of anti-tumor microenvironment, indicating that there were more immune features in this subtype. The TME subtypes A and C had the higher expressions of angiogenesis fibrosis comprising major stromal components. When it came to the prognostic benefit, TME subtype D had a better outcome, while TME subtype A represented a worse overall survival. Furthermore, TME subtype D had a significantly higher immune score than the other subtypes. The samples were divided into a high-immunity cohort and a low-immunity cohort based on their immune scores.

The high-immunity cohort had significantly higher T lymphocytes (CD 4 T and CD 8 T cells) and others immune cells such as Tr1, Th2, nTreg and iTreg, which explained the better overall survival of the high-immunity cohort. The differential expression analysis between the high-immunity and low-immunity cohorts showed that the high-immunity cohort had 63 upregulated and 384 downregulated genes. The 63 upregulated genes, including PLA2G2D, IFNG, LAG3, CD8B and NKG7, were associated with tumor immunity. The downregulated genes, such as KLK7 and IVL, were mainly associated with keratin, pigmentation, and epithelial genes. IVL may play an important role in the progression of melanoma (38). The upregulated genes were enriched in canonical immune biological processes, such as T cell activation and cell killing. As expected, the downregulated genes were enriched in cornification, epidermis development, and keratin-related process, all of which are likely able to modulate the tumor-development-related pathways (33).

Chemokines are a kind of chemotactic cytokines, which recruit lymphocytes through specific binding with their receptors in a specific way, and play an important role in the process of human inflammatory response and immune regulation (39). Chemokine receptors take part in the tumor proliferation, differentiation, invasion, and metastasis biological processes (34, 39). We found that the chemokines and their receptors, including XCL1, XCL2, CCL4, CCL5, CXCL13, CXCL11, CXCL10, CXCL9 and CCR5, CXCR3, CXCR6, significantly upregulated in the high-immunity cohort. Furthermore, CCR5-CCL4/CCL5 and CXCR3- CXCL9/CXCL10/CXCL11/CXCL13 axis were significantly correlated with CD 4 T and CD 8 T cells, indicating that these interactions may be essential for the recruitment of the T lymphocytes into tumor. These findings may provide a new clue for biomarkers in melanoma.

A recent study reported four molecular subtypes for melanoma that were proven to be beneficial for highlighting key potential subtype-specific drug targets (9). The proportions of the four TME subtypes in each molecular subtype were comparable. Next, we combined the genes used for molecular subtyping with the identified significantly mutated genes, and investigated their distributions in the immune subtypes. The two driver genes, CDKN2A and PRB2, had significantly different MAFs between the high-immunity and low-immunity. In terms of the CNVs, CDKN2A and PTEN were significantly deleted in the low-immunity cohort, whereas RAC1 was significantly amplified in the low-immunity cohort. These observations suggested that these mutations may be the potential biomarkers to predict clinical benefits in melanoma, and enable the reasonable selection of anti-melanoma treatments. Our study will provide a deep understanding of melanoma immune microenvironment and molecular characteristics, and facilitate the development of new therapeutic strategies for melanoma patients. There are some limitations of our study. The candidate biomarkers were achieved by computational methods. Experiments are needed to verify their roles in the clinical therapy of melanoma. Also, the biomarkers were identified based on the limited melanoma samples, and a larger cohort is needed to reveal biological signatures to improve the anti-melanoma strategies.

## DATA AVAILABILITY STATEMENT

The public datasets of TCGA-SKCM, phs001036, phs000452 and SRP070710 for this study can be found in the Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>) and SRA databases.

## AUTHOR CONTRIBUTIONS

TJ, JM and XC conceived the idea, designed the research concept of this manuscript, JW conducted the collection of data and did the analyses and drafted the manuscript. JM, CP, and WD helped in analysis. TJ and JM revised the manuscript. All authors read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2022.821578/full#supplementary-material>



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