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Original Article

Identification of immune-related biomarkers associated with tumorigenesis and prognosis in skin cutaneous melanoma

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Abstract: Skin cutaneous melanoma (SKCM) is one of the most malignant and aggressive forms of cancer. Investigating the mechanisms of carcinogenesis further could lead to the discovery of prognostic biomarkers that could be used to guide cancer treatment. In this study, we conducted integrative bioinformatics analyses of TCGA database, STRING, cBioPortal, TRRUST, The Human Protein Atlas, and DGIdb to determine which hub genes contributed to tumor progression and the cancer-associated immunology of SKCM. The results show that immune-related 873 differential genes grouped SKCM samples into subtypes. The initial results showed that the optimal number of clusters was two subgroups. Further analysis showed that there were significant differences in survival rate and immune infiltration level between the two subgroups. Subsequently, obtaining the different genes between groups, construct PPI to screen 6 hub genes (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, HLA-DRB5). In total, 6 MHC class II molecules were significantly related to overall survival. We then analyzed the expression of these genes along with their mutation landscapes, transcription factor regulation, and drug regulatory networks. In summary, our study identified 6 MHC class II molecules (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, HLA-DRB5) as important biomarkers in the occurrence and progression of SKCM tumors. Their expression levels are closely related to prognosis and immune infiltration and can help us better understand the tumorigenesis of SKCM.

Keywords: Cutaneous melanoma, biomarker, prognosis, HLA, immune, infiltration

Introduction

Skin cutaneous melanoma (SKCM) is one of the most malignant and aggressive cancers and is responsible for approximately 55,500 deaths per year, worldwide [1, 2]. Advanced melanoma is very aggressive and is not sensitive to radiotherapy or chemotherapy. Surgical resection is usually the first choice of treatment for patients with primary melanoma [3]. Previous studies have shown that high levels of immune cell infiltration are associated with a good prognosis [4], and targeted therapies for immune checkpoint molecules (such as anti-PD-1, anti-PDL-1, anti-CTLA4, and MAGE-A3) can significantly improve the prognosis for patients with metastatic melanoma [5]. However, 50-60% of patients still show no significant improvement, even with these therapies [6]. Therefore, discovering new, highly specific and sensitive diagnostic and prognostic markers is vital for improving SKCM treatment.

In this study, the mRNA expression data of 470 skin melanoma patients were downloaded from The Cancer Genome Atlas (TCGA) database for analysis in order to obtain differentially expressed genes (DEGs). Normal tissue data from the GTEx database was used as a control. After the intersection of DEGs and immune-related genes, the skin melanoma samples were sub-typed. The results showed that the best cluster number was two groups, and there were significant differences in the survival rate

and immune infiltration level between the two groups. After further analysis, the differential genes between the two subgroups were obtained, and the hub genes were screened by constructing a protein-protein interaction network. In total, 6 HLA family genes were found to be significantly related to overall survival (OS) (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, HLA-DRB5). We then analyzed the expression of these genes along with their mutation landscapes, transcription factor regulation, and drug regulatory networks. Our study identified 6 immune-related genes associated with skin melanoma, clarified their diagnostic and prognostic value, and revealed their underlying gene regulatory network.

Materials and methods

Gene expression and clinical correlation analysis

The mRNA expression data and clinical information of 470 skin melanoma patients were downloaded from TCGA database [7]. The corresponding normal tissue data of 812 cases were obtained from the GTEx database as a control. We used R-3.6.3 for differential gene analysis (|LogFC|>1, Adjusted P<0.05) and 6 genes (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, and HLA-DRB5) expression level and clinical correlation analysis in skin melanoma [8]. The pROC software package was used to draw the ROC curve, with the area under the ROC curve shown to be between 0.5 and 1. The Survival software package was used to statistically analyze the survival data, with the prognosis type being OS. The log-ranch test was used to compare survival differences, with a *p*-value of <0.05 indicating statistical significance. In the univariate Cox regression analysis, indexes with a *p*-value of <0.1 were included in the multivariate Cox regression analysis. When p is <0.05, we can speculate that this variable may be an independent prognostic factor.

Immune infiltration analysis

We used the CIBERSORT algorithm in the Immundeconv package to analyze the differences between the immune cells in the two sets of samples and used a heat map to display the results [9]. The horizontal axis is the subgroup type based on the differentially expressed immune-related genes, while the vertical axis is the immune cell type, which is where the difference is. The color represents the trend of expression in the different samples. We analyzed the expression values of the 8 immune checkpoint-related genes (SIGLEC15, TIGIT, CD274, HAVCR2, PDCD1, CTLA4, LAG3, and PDCD1LG2) using ggplot2 and pheatmap and observed the expression of the immune checkpoint-related genes. The significance of the two sample groups was determined by the Wilcoxon signed-rank test.

Differential gene screening and enrichment analysis between groups

We used the Limma software package (version 3.40.2) to study the differential expression between the two subgroups (Tumor-G2/Tumor-G1). |LogFC|>1, adjusted P<0.05 is defined as the threshold for mRNA differential expression screening. We used volcano maps to display differential genes. The results of the feature enrichment were determined by the ClusterProfiler package (version: 3.18.0). In the GO and KEGG enrichment analysis results, the color and size of the dots represent the enrichment significance and enrichment degree of the corresponding items.

PPI network construction and hub gene screening

Taking the different genes between the two subgroups as the research object, we constructed the protein interaction network (PPI) using the STRING database [10], and the interaction score threshold was set to 0.70 (high confidence). The Cytohubba [11] plugin for Cytoscape [12] was used to screen the hub genes. Finally, we determined the top 8 key genes in the ranking based on the MCC algorithm and analyzed the expression correlation of the hub genes.

cBioPortal database

A comprehensive web resource, the cBioPortal database can visualize and interpret multimodal cancer genomics data [13]. cBioPortal provided the genetic changes of predictive genes.

TRRUST database

TRRUST is a useful tool for predicting transcriptional regulatory networks in humans and mice [14]. The TRRUST database, which con-

tains 8444 transcription factor (TF)-target regulatory linkages for 800 human TFs, can help researchers figure out how these interactions are regulated.

The drug-gene interaction database

DGIdb (version 2.0) is an open-source initiative that allows users to mine existing information and generate hypotheses about how therapeutically targeted or prioritized genes are for drug development [15].

The human protein atlas

The Human Pathology Atlas Project incorporates immunohistochemistry (IHC) data obtained through tissue microarray research, including proteome analysis of 17 cancer types and 44 normal tissue types [16]. Patient information, staining intensity, location, and amount were available online along with staining intensity, location, and quantity in various cancer types. We utilized the Human Pathology Atlas in this study to analyze the protein expression of hub genes in SKCM and normal skin tissue.

Statistical analysis

We utilized R-3.6.3 for the statistical analysis. Wilcoxon's signed-rank sum test was used to analyze the expression of the hub gene in unpaired samples, the Kaplan-Meier method was used for survival analysis, and the log-ranch test was used to compare survival differences. Additionally, Spearman's correlation analysis was used to describe the correlation between quantitative variables that do not have a normal distribution. A *p*-value of <0.05 indicates statistical significance (*P<0.05, **P<0.01, ***P<0.001).

Results

Differential gene screening and cluster analysis

Using normal tissue data in the GTEx database as a control, the mRNA expression data of 470 skin melanoma patients were downloaded from TCGA database for analysis in order to obtain the DEGs. It was found that there were 2124 up-regulated genes and 2376 down-regulated genes (**Figure 1A**), and the screening threshold was |LogFC|>1 (adjusted P<0.05). The GO and KEGG enrichment analyses showed that the functions of differential genes are mainly related to the activation, proliferation, and differentiation of immune cells (**Figure 1B**). The overlap among DEGs and immune-related genes included 873 genes as shown in the Venn diagram (**Figure 1C** and Supplementary Table 1).

Construction of immune subgroups and analysis of survival and immune infiltration between groups

The 873 immune-related differential genes were used to cluster the skin melanoma samples. The CDF curve and CDF Delta area curve (Supplementary Figure 1) showed that the best cluster was two groups (**Figure 2A**), and the survival analysis results showed a better prognosis for the G2 group (P<0.001, **Figure 2B**). The level of immune infiltration is an important factor affecting the prognosis of patients with skin melanoma [17]. The results of the immune infiltration analysis of the two subgroups showed that there were significant differences in the infiltration levels of multiple immune cells in the two subgroups. Among them, the infiltration level of activated CD4+ memory T cells, γδ T cells, Th cells, M1 macrophages, CD8+ T cells, activated NK cells, mast cells and memory B cells in the G2 subgroup was significantly higher than those in the G1 group, while the infiltration level of M2 macrophages, M0 macrophages and resting NK cells in the G2 subgroup was significantly lower than those in the G1 group (P<0.01, **Figure 2C**). The mRNA expression levels of the 8 immune checkpoint molecules (SIGLEC15, TIGIT, CD274, HAVCR2, PDCD1, CTLA4, LAG3, and PDCD1LG2) in the G2 group were significantly lower than those in the G1 group (P<0.001, **Figure 2D**).

Analysis of differential genes between immune subgroups and selection of hub genes after PPI network construction

Knowing that immune infiltration was better in the G2 subgroup, we screened the differential genes between the two groups and performed interaction analysis to reveal the key genes. **Figure 3A** shows the volcano map with the differential genes obtained from the G2 subgroup/G1 subgroup comparison, including 61 up-regulated genes and 829 down-regulated genes (|LogFC|>1, Adjusted P<0.05). STRING data construct a PPI network for differential genes and utilized Cytoscape to visualize and

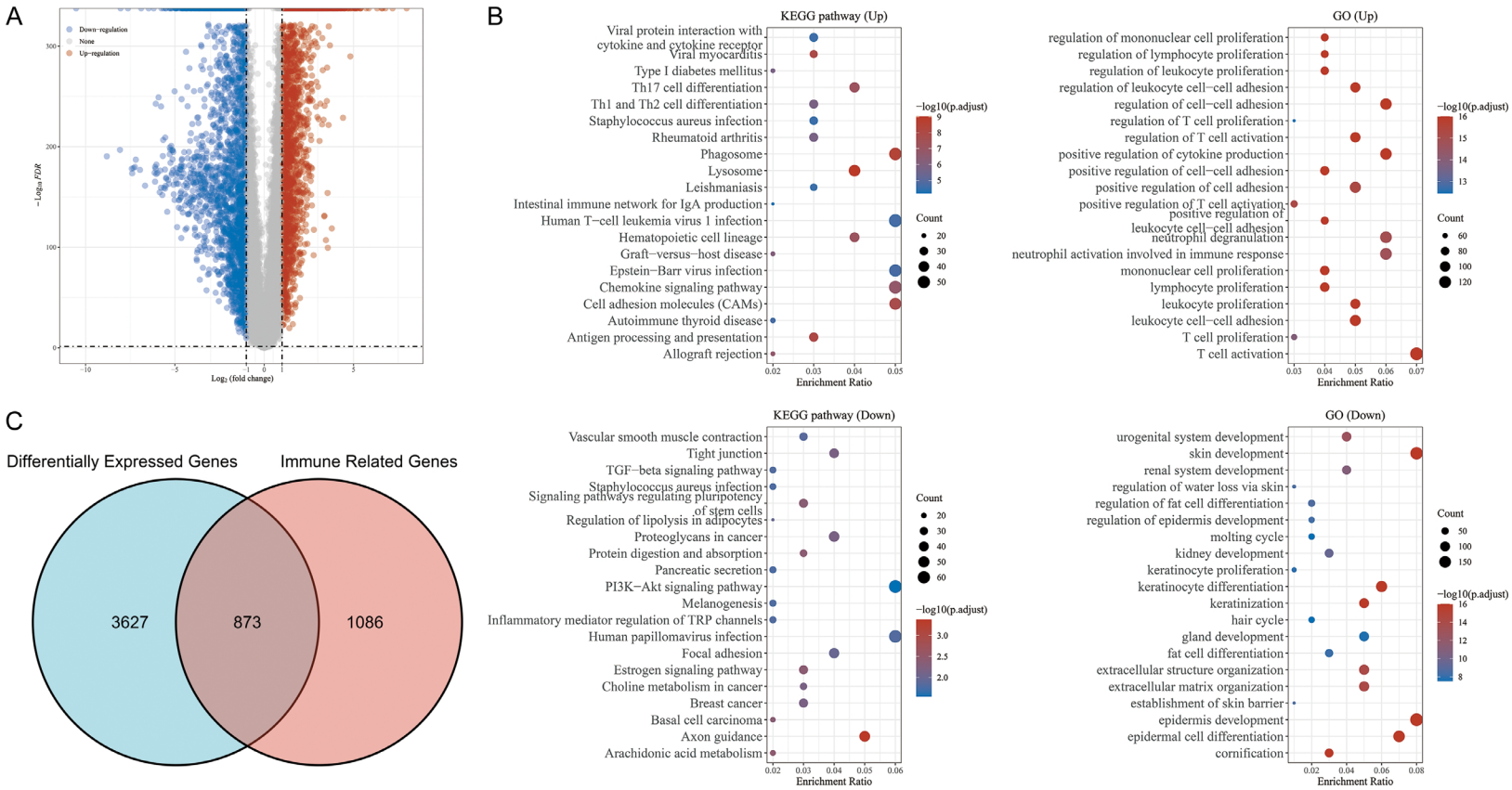

Figure 1. GO and KEGG analysis of the DEGs in SKCM. A. Volcano plot of differential gene expression analysis. B. GO and KEGG enrichment analysis. C. VN map showing the overlap of differential genes and immune-related genes.

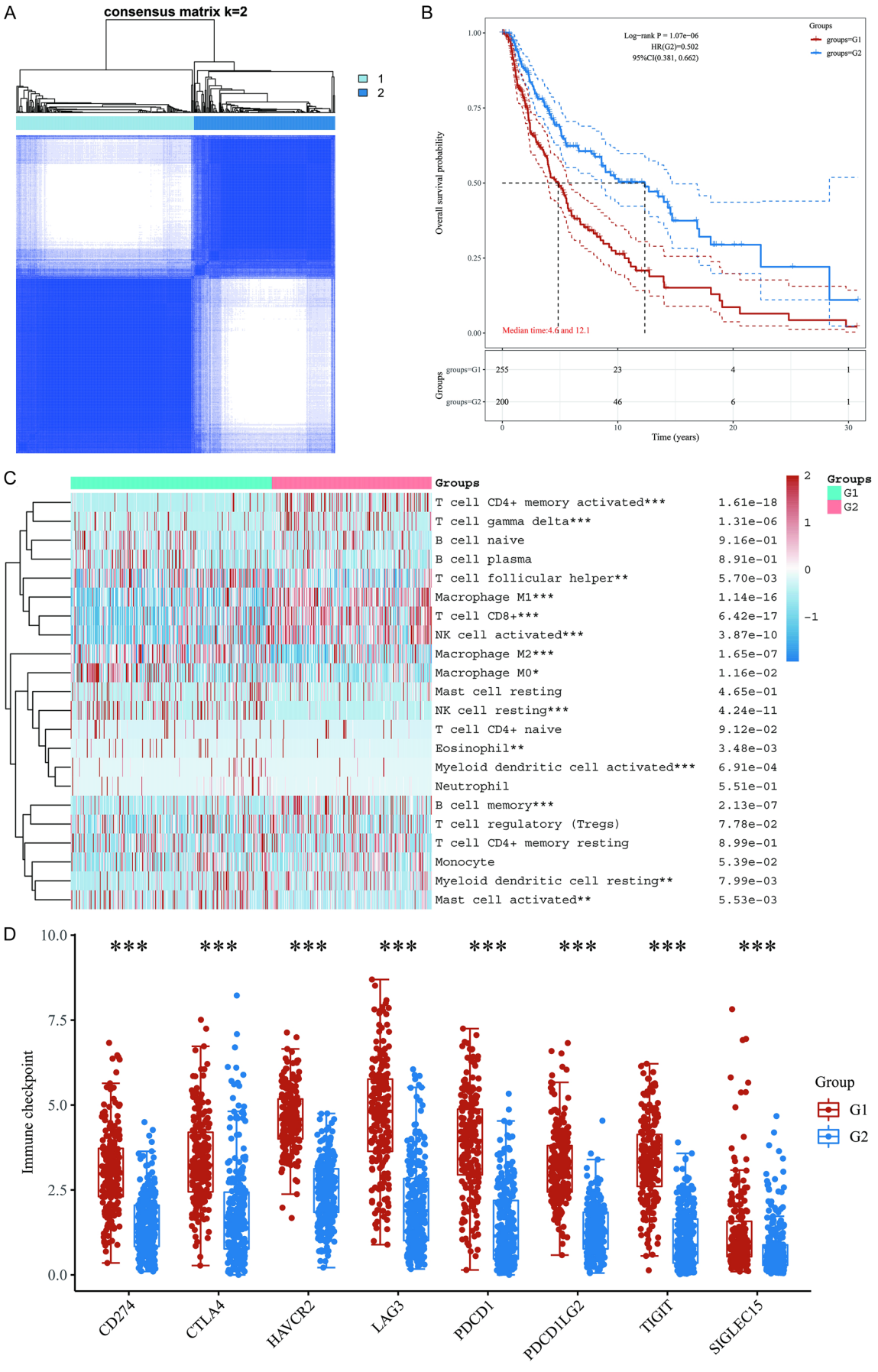
A
consensus matrix k=2
1
2
B
Log−rank P = 1.07e−06
HR(G2)=0.502
95%CI(0.381, 0.662)
Groups
groups=G1
groups=G2
Overall survival probability
Median time:4.6 and 12.1
Groups
groups=G1 255 23 4 1
groups=G2 200 46 6 1
Time (years)
C
Groups
T cell CD4+ memory activated*** 1.61e-18
T cell gamma delta*** 1.31e-06
B cell naive 9.16e-01
B cell plasma 8.91e-01
T cell follicular helper** 5.70e-03
Macrophage M1*** 1.14e-16
T cell CD8+*** 6.42e-17
NK cell activated*** 3.87e-10
Macrophage M2*** 1.65e-07
Macrophage M0* 1.16e-02
Mast cell resting 4.65e-01
NK cell resting*** 4.24e-11
T cell CD4+ naive 9.12e-02
Eosinophil** 3.48e-03
Myeloid dendritic cell activated*** 6.91e-04
Neutrophil 5.51e-01
B cell memory*** 2.13e-07
T cell regulatory (Tregs) 7.78e-02
T cell CD4+ memory resting 8.99e-01
Monocyte 5.39e-02
Myeloid dendritic cell resting** 7.99e-03
Mast cell activated** 5.53e-03
Groups
G1
G2
D
Immune checkpoint
Group
G1
G2
CD274
CTLA4
HAVCR2
LAG3
PDCD1
PDCD1LG2
TIGIT
SIGLEC15

Figure 2. Immune subtypes of SKCM samples and immune infiltration analysis. A. Two cluster subgroups. B. Overall Survival (OS) subgroup analysis. C. Immune infiltration analyses of two subgroups. D. Correlation of two subgroups with immune checkpoint genes expression. *P<0.05, **P<0.01, ***P<0.001.

screen hub genes. The top 8 genes in the ranking were HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, HLA-DRB5, HLA-DQB1, and HLA-DQB2 (**Figure 3B**).

Hub gene expression analysis and ROC curve drawing

Using the data from TCGA database, we constructed box plots to display the mRNA expression of the selected 8 hub genes in the skin melanoma patients and the controls. The results showed that 8 genes were significantly expressed in tumors (P<0.001, **Figure 4A**), and the ROC curve of each hub gene is shown in **Figure 4B**. Taking AUC-0.8 as the threshold, the hub genes were further determined as HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, and HLA-DRB5. Based on the data from the HPA database, the protein expression levels of these 6 hub genes in SKCM are consistent with their mRNAs, all showing high levels of expression (**Figure 5**).

The prognostic and diagnostic value of hub genes in SKCM patients

Using the Kaplan-Meier method, we determined the prognostic value of the hub genes in SKCM patients, with **Figure 6** showing that 6 hub genes are significantly related to OS. High expressions of HLA-DPA1 (HR=0.45, P<0.001), HLA-DPB1 (HR=0.50, P<0.001), HLA-DQA1 (HR=0.48, P<0.001), HLA-DRQ (HR=0.49, P< 0.001), HLA-DRB1 (HR=0.51, P<0.001), and HLA-DRB5 (HR=0.54, P<0.001) were significantly associated with better OS rates. Univariate and multivariate Cox regression analyses were performed in order to identify the prognostic independence of the 6-gene signature (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, and HLA-DRB5). The results showed that the 6 genes are associated with higher OS rates but do not have independent diagnostic value (Supplementary Table 2).

Genetic alteration and correlation analysis in patients with SKCM

Using the data from the cBioPortal database, we analyzed the genome mutation characteristics of the hub genes. The results showed that nearly 3% of the HLA-DPB1 SKCM samples, 4% of the HLA-DPA1, HLA-DRB1, and HLA-DRB5 SKCM samples, 6% of the HLA-DQA1 samples, and 7% of the HLA-DRA samples had genetic alteration. The most common genetic changes were mainly related to amplification (**Figure 7A**). The expression correlation of these 6 prognostic-related genes was further analyzed and was found to be positively correlated, which was consistent with expectations (**Figure 7B**). Among them, HLA-DPB1 and HLA-DRA had the highest positive correlation, with a Spearman's rank correlation coefficient of 0.979.

Key transcription factors analyses of prognostic genes and drug-gene interaction prediction

Using the TRRUST database, we explored potential transcription factor targets of the 6 MHC class II molecules. The results showed that RFXANK, RFXAP (FDR=2.39E-19), RFX5 (FDR=5.91E-19), CIITA (FDR=1.34E-10), ILF3 (FDR=7.50E-10), and RFX1 (FDR=8.83E-06) were found to be the key transcription factors for HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, and HLA-DRB5 (Supplementary Table 3). Using the data from TCGA database, we verified the mRNA expression levels of these 6 transcription factors. Among them, RFXANK, RFX5, CIITA, and RFX1 were highly expressed in tumors (P<0.001), RFXAP is also expressed in tumors (P<0.001), and the expression level of ILF3 showed no significant difference between tumors and normal tissue (**Figure 8A**). The correlation analysis results of the transcription factors and hub genes show that CIITA is highly positively correlated with the expression of the 6 hub genes (**Figure 8B**). Based on the data from the HPA database, we confirmed that CIITA is highly expressed in SKCM (**Figure 8C**). Finally, using the DGIdb database, we obtained drug-gene interaction pairs (**Figure 9** and Supplementary Table 4), which may help in developing new targets for SKCM therapy.

Discussion

Cutaneous melanoma is the most common form of skin cancer, and its incidence has increased rapidly in the past few decades.

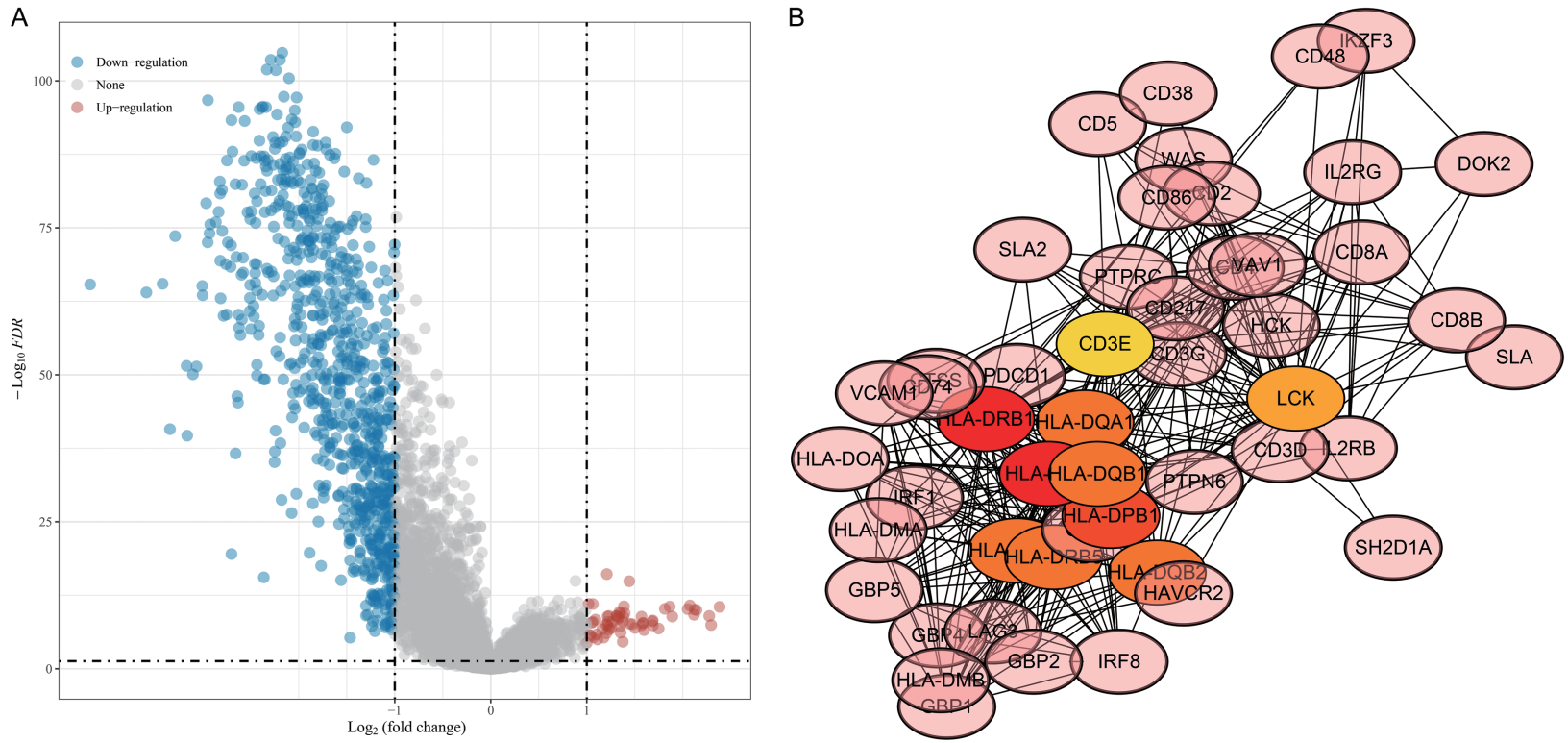

Figure 3. Analysis of differential genes among two subgroups and screening of hub genes. A. Volcano plot of differential gene expression. B. PPI network and hub genes network of the DEGs using Cytoscape. (DEGs, differentially expressed genes; PPI, protein-protein interaction).

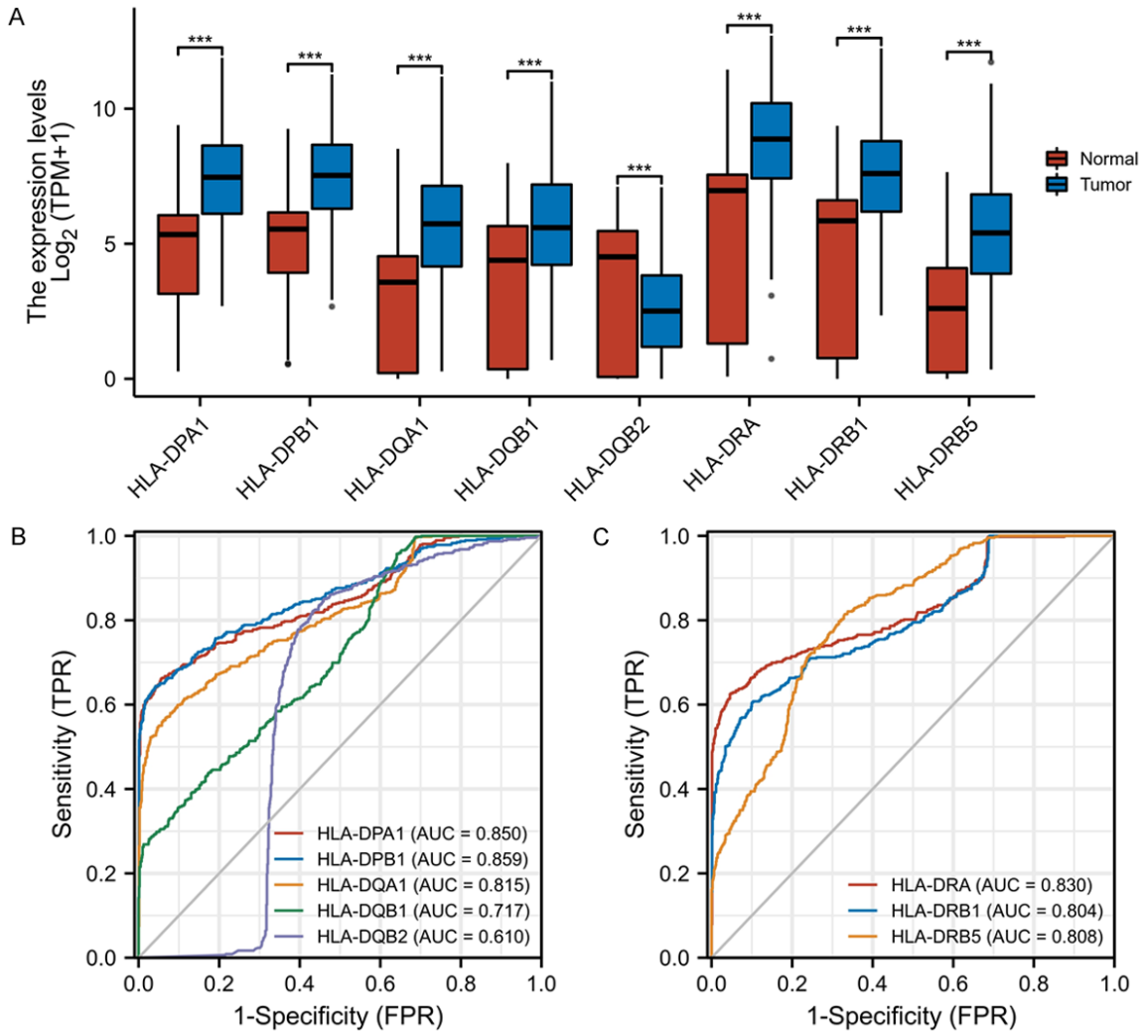

Figure 4. Six hub genes were identified (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, and HLA-DRB5). A. The mRNA expression levels of 8 hub genes in SKCM. B, C. ROC curve of 8 hub genes in SKCM.

Melanoma is one of the most immunogenic tumors, so it is most likely to respond positively to immunotherapy. Researchers have expounded great effort in using immunotherapy to treat SKCM [18]. Immune cell infiltration into the tumor is an important factor affecting the survival and prognosis of patients with skin melanoma. MHC-I molecules are expressed by most nucleated cells and mainly present endogenous peptide antigens to CD8+ T cells. MHC class II (MHC II) molecules are mainly expressed by professional antigen-presenting cells (pAPC), such as dendritic cells (DC), B cells, and macrophages, and mainly present exogenous peptide antigens to CD4+ T cells [19]. The exact role of MHC II molecules in melanoma progression is unknown. However, it has been suggested that they may assist melanoma cells in escaping immune surveillance by presenting tumor Ags that activate regulatory T cells (Tregs) and/or transmitting signals in melanoma cells that protect against apoptosis [20].

Using normal tissue as a control, the enrichment analysis results of DEGs in SKCM samples performed in this study suggest that its mediated function may be closely related to the immune system. When considering the impact of immune heterogeneity on the immunotherapy of patients with skin melanoma, the results of the intersection of DEGs and immune-related genes were used to group the subtypes of patients with skin melanoma and showed that the optimal number of clusters is two groups. Further analysis showed that there

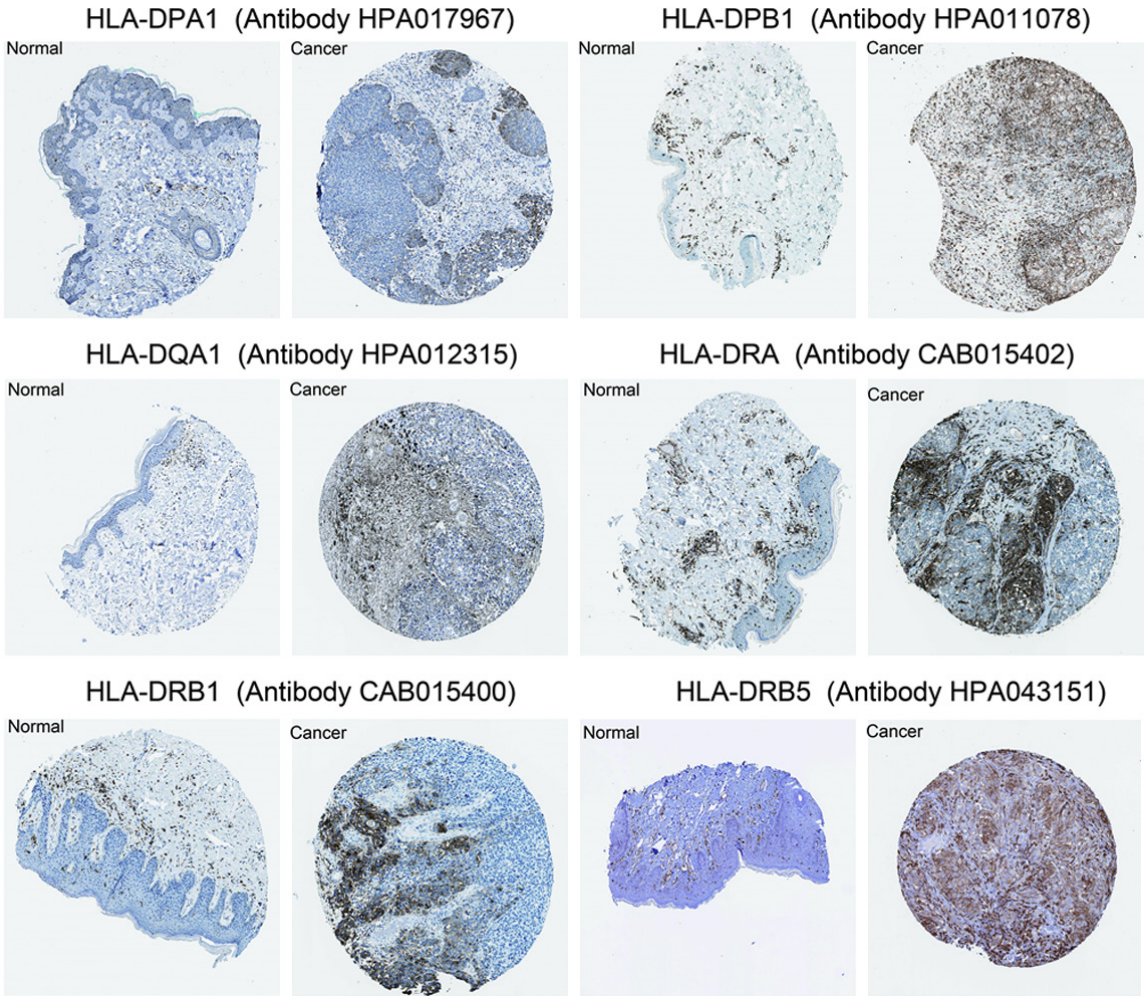

Figure 5. Validation of protein expression levels of hub genes in the HPA database.

were significant differences in survival rate and immune infiltration level between the two subgroups. Subsequently, the differential genes between the two subgroups were obtained, the hub gene was screened by constructing a PPI network, and the gene range was reduced by setting an ROC diagnostic efficiency threshold. Finally, it was determined that 6 MHC class II molecules (HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, and HLA-DRB5) were significantly related to OS rates.

Studies have shown that HLA-DPA1 is one of the key genes associated with hypoxia in multiple myeloma, and the down-regulation of HLA-DPA1 in patients is associated with poor prognosis [21]. The pseudogene HLA-DPB2 promotes the expression of the parent gene HLA-DPB1 through the ceRNA mechanism, promoting tumor immune infiltration. A high rate of expression of HLA-DPB1 also leads to a better prognosis [22]. The HLA-DQA1 locus may be a potential risk factor for the onset of primary melanoma in the Spanish population [23]. In non-muscle-invasive bladder cancer (NMIBC), patients with a low expression of HLA-DRA have better progression-free survival rates than patients with high expression [24]. A study from China showed that the polymorphism of the HLA-DRB1 gene is associated with dermatomyositis [25]. These research results show that MHC II molecules have important functions in tumors. However, due to the general heterogeneity of tumors, their roles may be diverse or even contradictory. Therefore, in order to clarify the function and potential clinical significance of these 6 MHC II molecules in skin melanoma, based on TCGA database and its accompanying clinical information, we analyzed the expression characteristics of HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, HLA-DRB5 and the correlations bet-

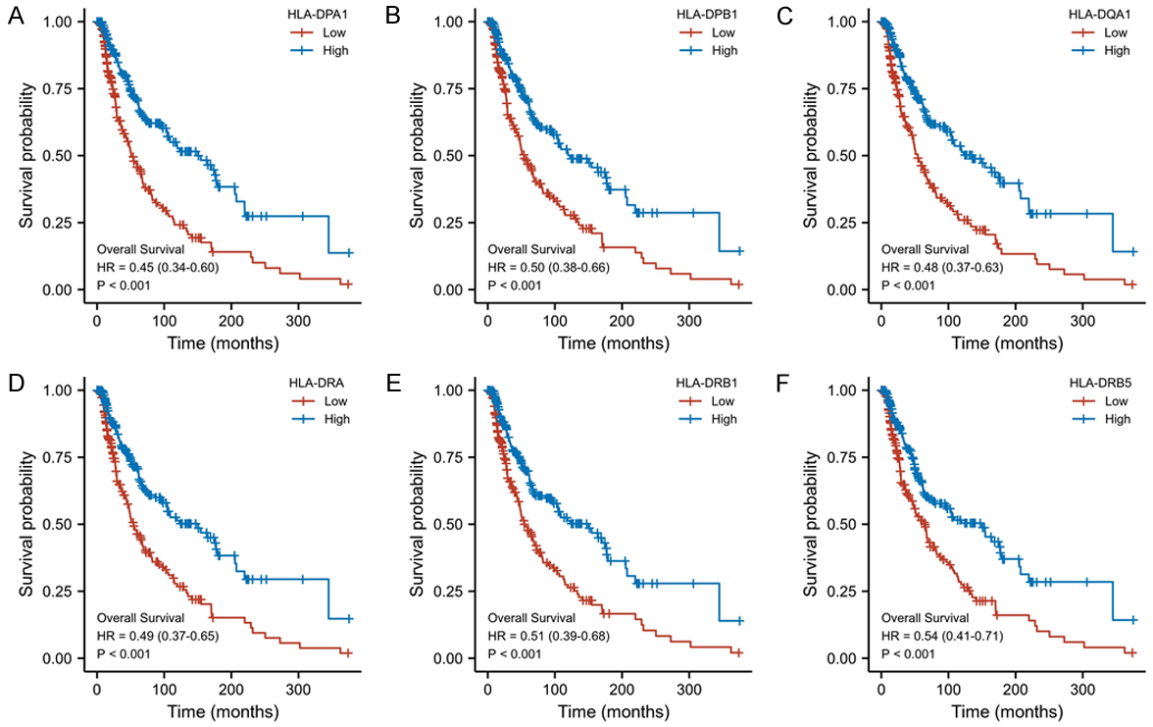

Figure 6. Kaplan-Meier overall survival analysis of patients in the TCGA cohort.

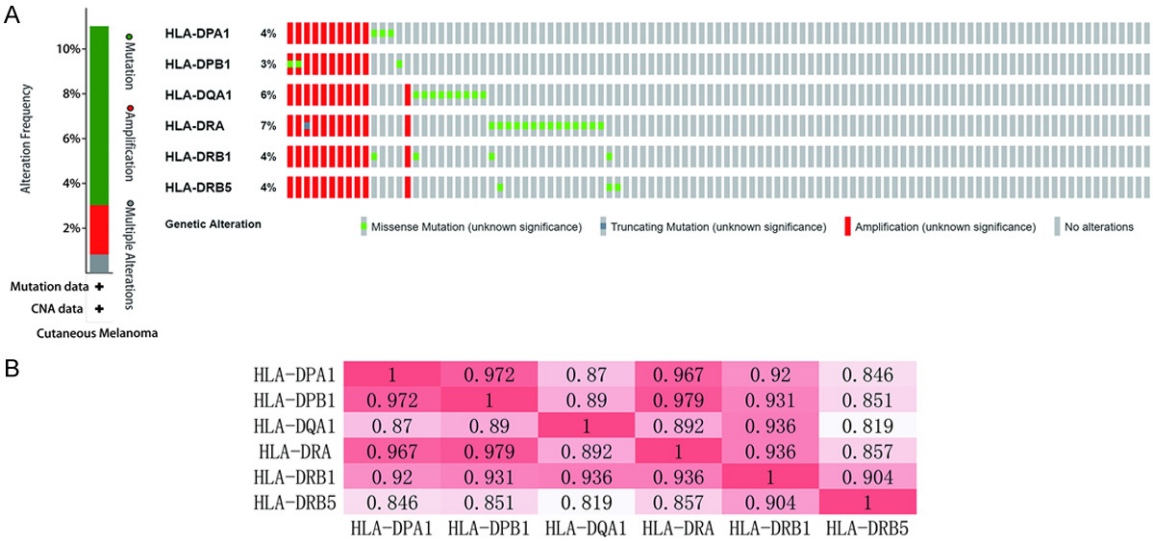

Figure 7. Hub genes expression correlation and mutation analysis in SKCM (cBioPortal). A. Hub genes mutation analysis. B. Correction between different hub genes.

ween diagnosis and prognosis. The HPA database confirmed its protein expression level.

Additionally, and in order to fully comprehend the upstream regulatory molecules and potential drugs of these 6 genes, we predicted the transcription factors that may regulate them, by using the TRRUST database, and verified the expression correlation between the molecules. Using data from the DGIdb database, we constructed a drug-gene interaction that we hope will provide a reference point for the immune-related treatment of SKCM patients.

In summary, our study showed that 6 MHC class II molecules (HLA-DPA1, HLA-DPB1, HLA-

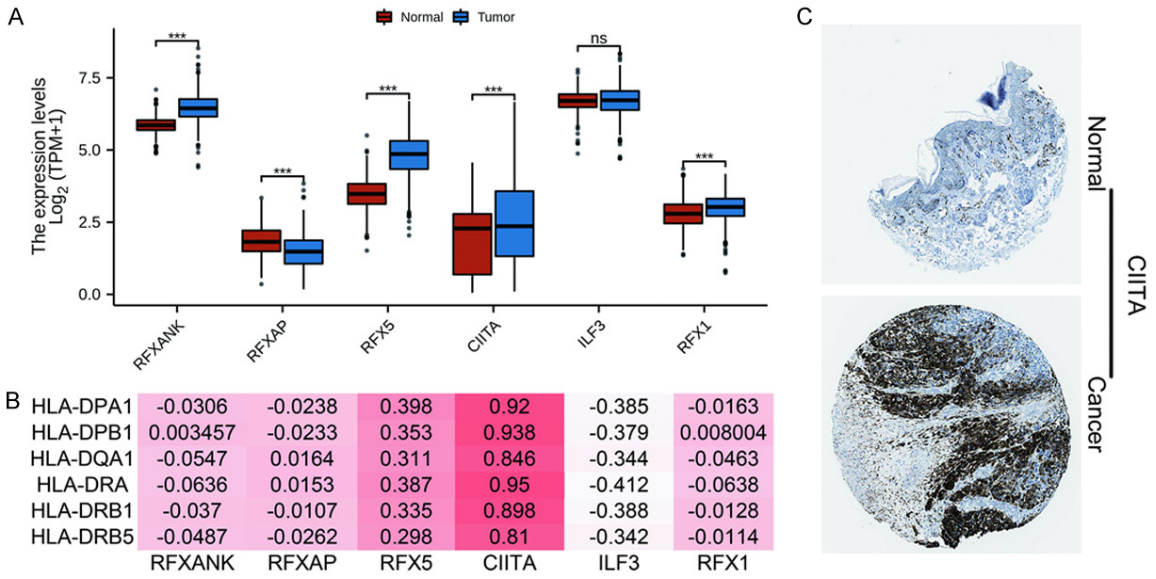

Figure 8. Analysis of the expression of the indicated transcription factors. A. The mRNA expression levels of the indicated transcription factors in SKCM. B. Correlation between transcription factors and hub genes expression (cBioPortal). C. Validation of protein expression levels of the indicated transcription factors in the HPA database.

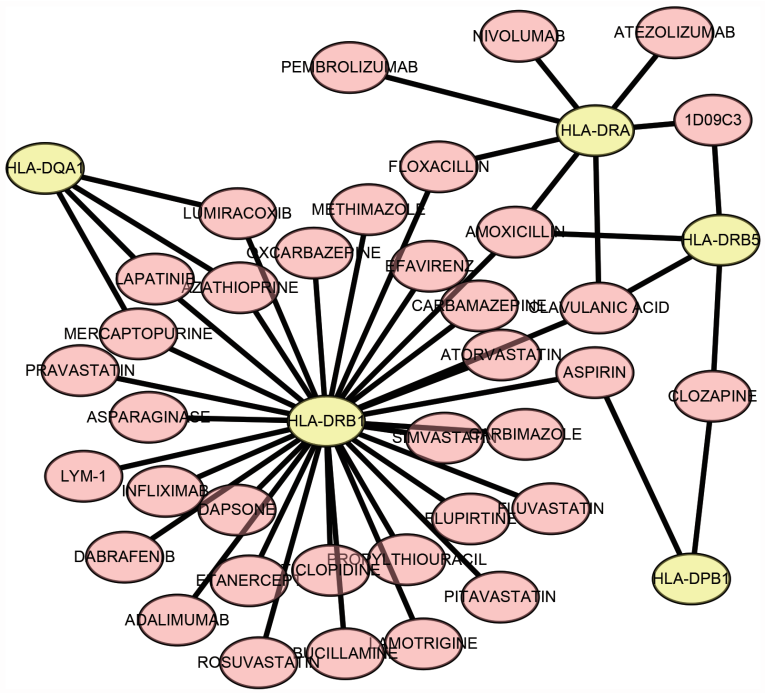

Figure 9. Drug-gene interaction diagram. The yellow circle indicates the related hub gene, and the pink square indicates the drug.

DQA1, HLA-DRA, HLA-DRB1, HLA-DRB5) are important biomarkers associated with the occurrence and progression of SKCM tumors. Their expression levels are closely related to prognosis and immune infiltration and can help us to better understand the tumorigenesis of SKCM. However, it is imperative that further functions be studied in order to verify these findings.

Acknowledgements

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Disclosure of conflict of interest

None.

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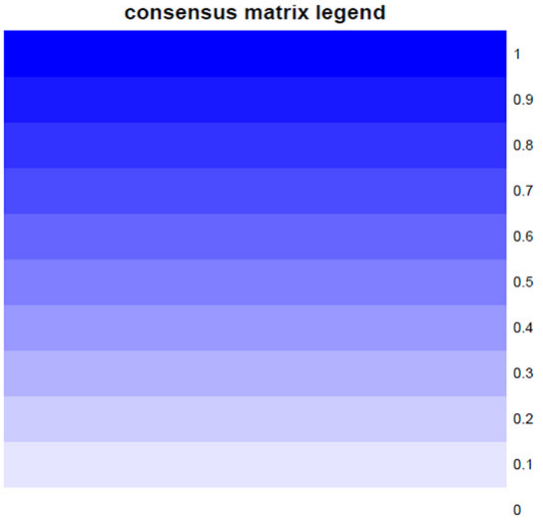
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0.7
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0.2
0.1
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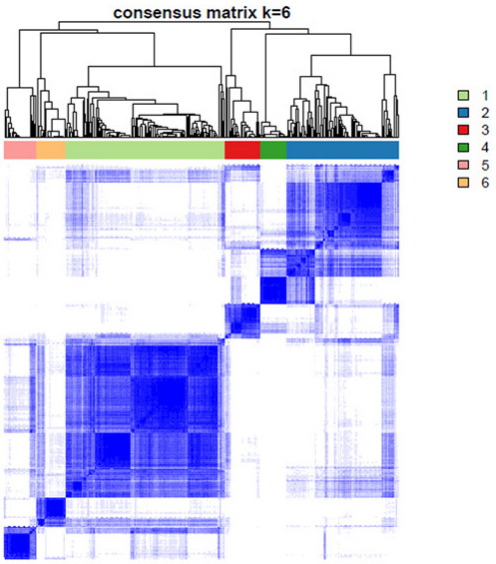
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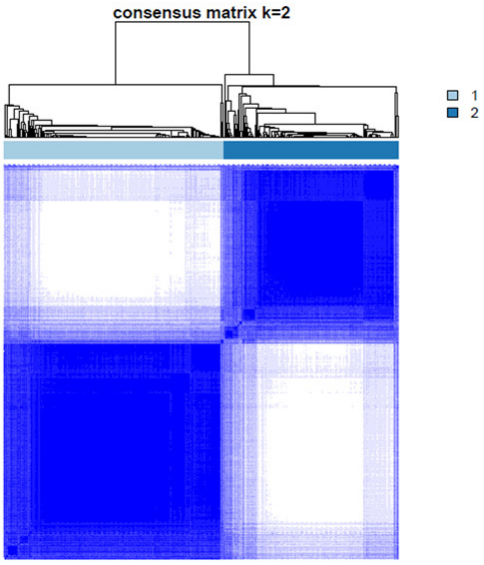
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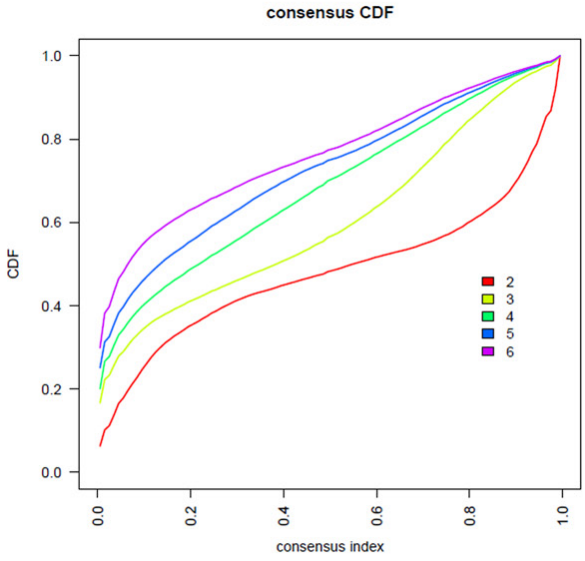
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0.6
0.4
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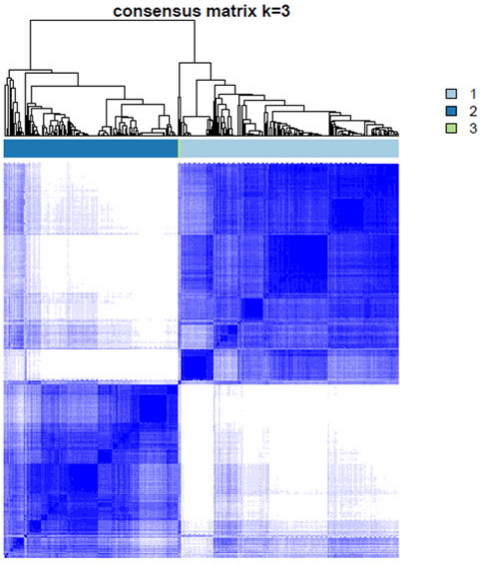
0.8
1.0
consensus index
2
3
4
5
6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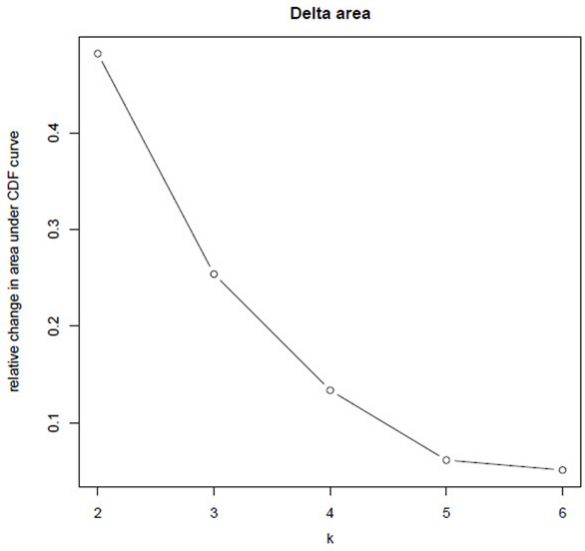
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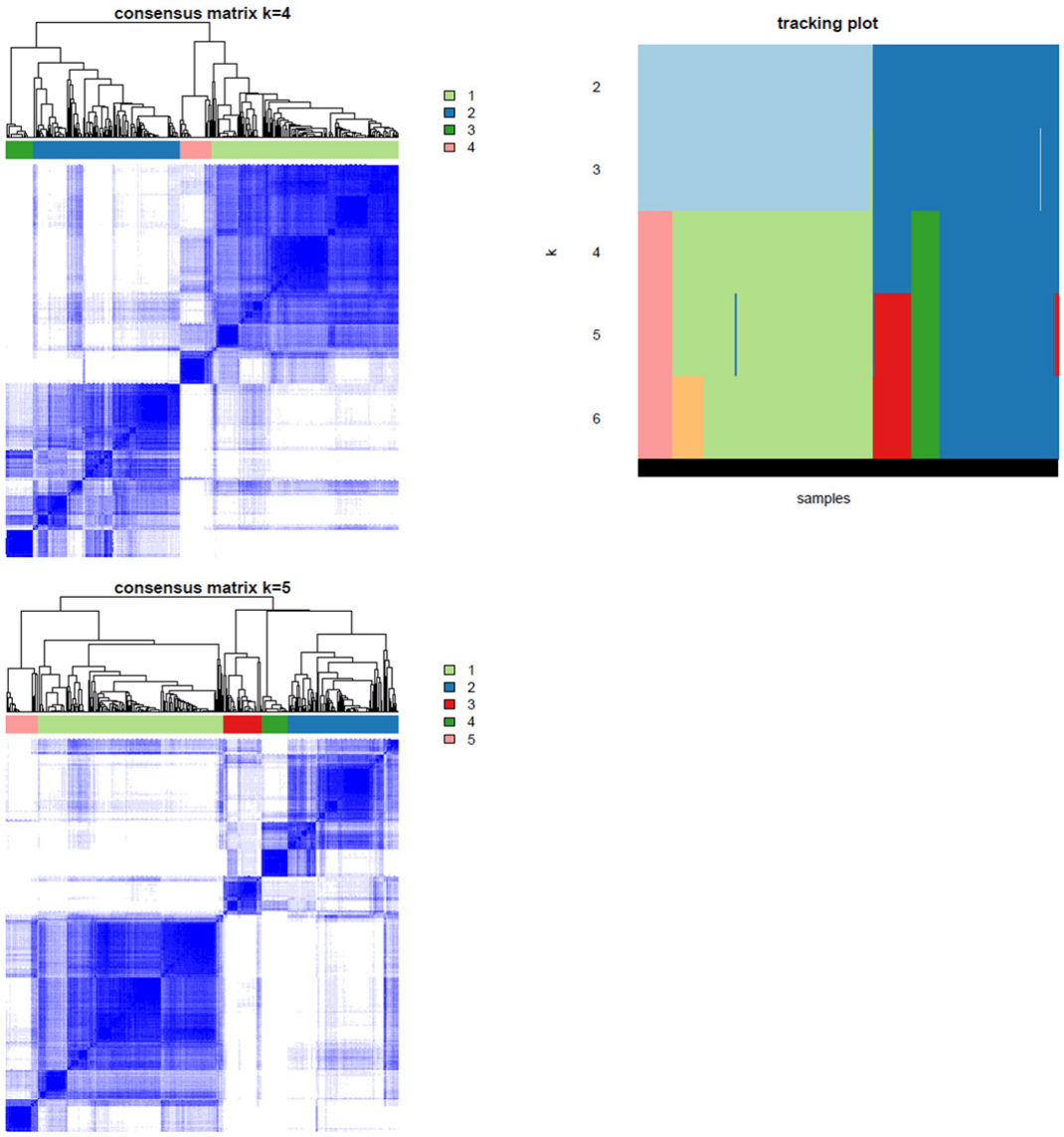
0.2
0.1
2
3
4
5
6
k



Supplementary Figure 1. The results of CDF curve and CDF Delta area curve.

Supplementary Table 2. Univariate and multivariate Cox regression analysis of OS in TCGA cohort

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
T stage (T3&T4 vs. T1&T2)	361	2.085 (1.501-2.895)	<0.001	1.929 (1.352-2.753)	<0.001
N stage (N1&N2&N3 vs. N0)	402	1.752 (1.304-2.354)	<0.001	1.946 (1.395-2.715)	<0.001
M stage (M1 vs. M0)	430	1.897 (1.029-3.496)	0.040	2.632 (1.134-6.107)	0.024
HLA-DPA1 (High vs. Low)	456	0.454 (0.345-0.598)	<0.001	0.469 (0.229-0.958)	0.038
HLA-DPB1 (High vs. Low)	456	0.503 (0.382-0.661)	<0.001	0.840 (0.267-2.644)	0.766
HLA-DQA1 (High vs. Low)	456	0.481 (0.365-0.632)	<0.001	1.071 (0.542-2.119)	0.843
HLA-DRA (High vs. Low)	456	0.493 (0.374-0.649)	<0.001	0.942 (0.311-2.851)	0.916
HLA-DRB1 (High vs. Low)	456	0.514 (0.391-0.676)	<0.001	1.115 (0.455-2.730)	0.812
HLA-DRB5 (High vs. Low)	456	0.542 (0.413-0.712)	<0.001	1.026 (0.617-1.706)	0.922

Supplementary Table 3. Key transcriptional factors (TFs) of six HLA-genes in SKCM (TRRUST database)

Key TF	Description	*P* value	Q value	Regulated genes
RFXANK	regulatory factor X-associated ankyrin-containing protein	7.97E-20	2.39E-19	HLA-DRA, HLA-DQA1, HLA-DRB5, HLA-DPB1, HLA-DPA1, HLA-DRB1
RFXAP	regulatory factor X-associated protein	7.97E-20	2.39E-19	HLA-DPB1, HLA-DRB1, HLA-DRB5, HLA-DRA, HLA-DPA1, HLA-DQA1
RFX5	regulatory factor X, 5 (influences HLA class II expression)	2.96E-19	5.91E-19	HLA-DRB5, HLA-DRA, HLA-DRB1, HLA-DPA1, HLA-DPB1, HLA-DQA1
CIITA	class II, major histocompatibility complex, transactivator	8.91E-11	1.34E-10	HLA-DPB1, HLA-DRB1, HLA-DRB5, HLA-DRA
ILF3	interleukin enhancer binding factor 3, 90 kDa	6.25E-10	7.50E-10	HLA-DRB1, HLA-DRA, HLA-DQA1
RFX1	regulatory factor X, 1 (influences HLA class II expression)	8.83E-06	8.83E-06	HLA-DRA, HLA-DPB1

Supplementary Table 4. Drug-gene interaction pairs

search_term	match_term	match_type	gene	drug	sources	pmids
HLA-DPB1	HLA-DPB1	Definite	HLA-DPB1	CLOZAPINE	PharmGKB	11266078
HLA-DPB1	HLA-DPB1	Definite	HLA-DPB1	ASPIRIN	PharmGKB	15007363|15784113|19392989|9179433
HLA-DQA1	HLA-DQA1	Definite	HLA-DQA1	LUMIRACOXIB	PharmGKB	20639878
HLA-DQA1	HLA-DQA1	Definite	HLA-DQA1	AZATHIOPRINE	PharmGKB	25217962
HLA-DQA1	HLA-DQA1	Definite	HLA-DQA1	LAPATINIB	PharmGKB|FDA	24687830|21245432
HLA-DQA1	HLA-DQA1	Definite	HLA-DQA1	MERCAPTOPURINE	PharmGKB	25217962
HLA-DRA	HLA-DRA	Definite	HLA-DRA	PEMBROLIZUMAB	CIViC	26822383
HLA-DRA	HLA-DRA	Definite	HLA-DRA	AMOXICILLIN	PharmGKB	30664875
HLA-DRA	HLA-DRA	Definite	HLA-DRA	ATEZOLIZUMAB	CIViC	26822383
HLA-DRA	HLA-DRA	Definite	HLA-DRA	FLOXACILLIN	PharmGKB	30664875
HLA-DRA	HLA-DRA	Definite	HLA-DRA	NIVOLUMAB	CIViC	26822383
HLA-DRA	HLA-DRA	Definite	HLA-DRA	1D09C3	DrugBank	16452241
HLA-DRA	HLA-DRA	Definite	HLA-DRA	CLAVULANIC ACID	PharmGKB	30664875
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	FLUVASTATIN	PharmGKB	27839692
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	AZATHIOPRINE	PharmGKB	25217962
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	EFAVIRENZ	PharmGKB	18301070
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	ASPARAGINASE	PharmGKB	24970932|25987655
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	TICLOPIDINE	PharmGKB	17339877
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	ETANERCEPT	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	OXCARBAZEPINE	PharmGKB	27666425
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	ADALIMUMAB	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	INFLIXIMAB	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	LYM-1	ChemblInteractions|TTD	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	BUCILLAMINE	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	CARBIMAZOLE	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	ROSUVASTATIN	PharmGKB	27839692
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	CARBAMAZEPINE	PharmGKB	24399721|23830818
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	MERCAPTOPURINE	PharmGKB	25217962
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	ASPIRIN	PharmGKB	15784113|19392989
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	METHIMAZOLE	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	ATORVASTATIN	PharmGKB	27839692
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	PRAVASTATIN	PharmGKB	27839692
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	FLUPIRTINE	PharmGKB	26959717
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	DAPSONE	PharmGKB	29233746
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	DABRAFENIB	PharmGKB	27023328
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	PROPYLTHIOURACIL	PharmGKB	
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	SIMVASTATIN	PharmGKB	27839692
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	PITAVASTATIN	PharmGKB	27839692
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	AMOXICILLIN	PharmGKB	20800921|10535882|30664875
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	LUMIRACOXIB	PharmGKB	20639878
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	LAPATINIB	PharmGKB|FDA	24687830|28786423
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	CLAVULANIC ACID	PharmGKB	20800921|10535882|30664875
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	LAMOTRIGINE	PharmGKB	19668019
HLA-DRB1	HLA-DRB1	Definite	HLA-DRB1	FLOXACILLIN	PharmGKB	30664875
HLA-DRB5	HLA-DRB5	Definite	HLA-DRB5	CLAVULANIC ACID	PharmGKB	10535882
HLA-DRB5	HLA-DRB5	Definite	HLA-DRB5	AMOXICILLIN	PharmGKB	10535882
HLA-DRB5	HLA-DRB5	Definite	HLA-DRB5	1D09C3	TdgClinicalTrial	
HLA-DRB5	HLA-DRB5	Definite	HLA-DRB5	CLOZAPINE	PharmGKB	11146763