Pseudogene HLA-DRB6 Regulates Immune Microenvironment of Cutaneous Melanoma by miR-338/CXCL10 Axis

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Abstract

Background: The relationship between the pseudogene and tumor immune microenvironment in cutaneous melanoma is unclear. In this study, we analyzed the role of the pseudogene HLA-DRB6 and its effect on the tumor immune microenvironment in skin cutaneous melanoma (SKCM) using bioinformatics tools.

Method: The GEPIA database was used to analyze the expression of HLA-DRB6 and CXCL10 mRNA in tumor tissues. The TIMER database was used to analyze the relationship between mRNA levels and the infiltration of immune cells. The enrichment of HLA-DRB6 and CXCL10 in melanoma tissues was analyzed by single cell portal. The binding sites of HLA-DRB6 with its target genes was predicted via starBase database. The gene expression profiling and clinical data from GEO database (GSE94873) was used to verify the potential of CXCL10 as a biomarker.

Result: The expression of HLA-DRB6 in SKCM tumor is higher than in normal tissues, and patients with high HLA-DRB6 expression had a better prognosis (P<0.05). Furthermore, HLA-DRB6 is positively correlated with the infiltration of immune cells such as B cells, $CD4^+$ T, and $CD8^+$ T lymphocytes, and the expression of immune checkpoint molecules such as PD-1, PD-L1, and CTLA-4. Single cell transcriptome sequencing data showed that HLA-DRB6 is mainly enriched in macrophages and had the highest correlation with CXCL10 than other chemokines (cor=0.66, P<0.0001). In addition, we found that CXCL10 can be used as a potential biomarker for predicting responsiveness and survival rate in SKCM patients who treated with Tremelimumab (a human anti-CTLA-4 antibody).

Conclusion: In the microenvironment of SKCM, HLA-DRB6 is mainly enriched in macrophages and regulates the expression of CXCL10 through the ceRNA mechanism. Furthermore, the CXCL10 in peripheral blood can be used as a biomarker to predict the responsiveness and the prognosis for patients treated with tremelimumab.

Background

According to a survey, approximately 55,500 cancer-relateddeaths are caused by Skin cutaneous melanoma (SKCM) each year, accounting for 0.7% of all the cancer-related deaths[1]. Before 2011, metastatic melanoma was life-threatening with overall approximately 9 months survival of patients with advanced-stage melanoma [2]. Therefore, it is necessary to conduct in-depth research on the regulation mechanism of SKCM. The mechanism of tumorigenesis, development and metastasis is very complicated. In the past, research has been focused mainly on the tumor cells themselves, while ignoring the impact of the tumor’s microenvironment on it. As early as 1889, the "seed and soil" hypothesis proposed by Stephen Paget laid the foundation for the concept of tumor microenvironment. Tumor microenvironment is a complex integrated system, which is composed of different kinds of stromal cells and cytokines, including fibroblasts, immune and inflammatory cells, adipocytes, glial cells, smooth muscle cells, vascular cells, etc.[3]. These non-malignant cells in the tumor microenvironment play a key

role in all the stages of carcinogenesis in different ways. Growing studies have shown that immune cells in the tumor microenvironment, such as macrophages, T lymphocytes, B lymphocytes, neutrophils, dendritic cells, NK cells, etc., can promote tumor growth and the metastasic effect[4]. However, the specific regulation mechanism between tumor growth and metastasis, and immune cells in the tumor microenvironment is still unclear.

Pseudogene(s) are defective genomic sequences similar to the homologous coding genes. They exist in almost all life forms. In mammalian genomes, the number of pseudogenes is similar to that of protein-coding genes[5]. Originally pseudogenes were considered to be the product of evolution and did not have any physiological functions[6]. But with the development of biotechnology, it has been found that pseudogenes play a key regulatory role in various human diseases (including cancer) at the level of DNA, RNA or protein[7]. HLA-DRB6 is one of the major histocompatibility complex genes in humans, that lacks exon 1, and is closely related to immune molecules at the genomic position. This exon usually encodes the leader and the first 4 amino acid residues of the mature protein[8]. Currently, almost none data on the role of pseudogene HLA-DRB6 in tumor immunity is available. In this study, we tried to investigate the role of HLA-DRB6 and its downstream target gene in tumor microenvironment and prognosis.

Methods

Analysis of the expression of HLA-DRB6 and CXCL10 in tumor

The gene expression profiling interactive analysis (GEPIA, http://gepia.cancer-pku.cn/index.html) [40] database integrates gene expression data of tumors, paracancerous, and non-tumor samples from The Cancer Genome Atlas (TCGA) and The Genotype-Tissue Expression (GTEx) portals. We used the "General" module in GEPIA to analyze the expression of HLA-DRB6 in different tumors and normal tissues, and the "Expression DIY" module to analyze the differential expression of HLA-DRB6 and CXCL10 in SKCM and normal tissues. Furthermore, , we used the tumor immune estimation resource (TIMER, https://cistrome.shinyapps.io/timer/)[41] database tool to analyze the expression of HLA-DRB6 and CXCL10 in various tumors and the differential expression in SKCM.

Analysis of the relationship between HLA-DRB6, CXCL10 and immune infiltration

Immune cell infiltration determines the prognosis and survival time of SKCM patients to certain extent. The "Gene" module of the TIMER database was used to analyze the relationship between HLA-DRB6 and CXCL10 mRNA levels and the infiltration of B lymphocytes, $CD4^+$ T, $CD8^+$ T lymphocytes, macrophages, neutrophils, and dendritic cells. The correlation between HLA-DRB6 and CXCL10 mRNA levels and PD-1(PDCD1), PD-L1(CD274), CTLA4, LAG3, TIM3(HAVCR2) were analyzed using the "Correlation" module of the TIMER database. Spearman correlation analysis was used to evaluate the statistical significance. Log2 RSEM was used indicate the expression level of the expressed gene. The GEPIA database was used to analyze the correlation between HLA-DRB6 and various chemokines.

Analysis of the distribution of HLA-DRB6 and CXCL10 by single cell sequencing data.

Single Cell Portal (https://singlecell.broadinstitute.org/single_cell) was established by the Broad Institute of MIT and Harvard for the purpose of reducing barriers and accelerating single cell research. We analyzed the enrichment of HLA-DRB6 and CXCL10 in melanoma tissues by single cell portal. To further validate the enrichment of HLA-DRB6 in immune cells, we analyzed the distribution of HLA-DRB6 in hepatocellular carcinoma (HCC) with HCC single cell data (http://cancer-pku.cn: 3838 / HCC /) [11].

Prediction of the intracellular localization of HLA-DRB6

The subcellular localization of HLA-DRB6 in cytoplasm, nucleus, cytosol, ribosomes and exosomes was analyzed according to the sequence information. HLA-DRB6 sequence information was obtained through UCSC [42]. HLA-DRB6 mRNA sequence was entered in the "Upload single lncRNA sequence" frame of the lncLocator (lncRNA subcellular localization predictor, http://www.csbio.sjtu.edu.cn/bioinf/lncLocator) [43] database to predict its subcellular localization.

Candidate miRNA and mRNA of HLA-DRB6

The starBase[44] was used to analyze the binding sites of HLA-DRB6 with its target genes. The candidate target miRNAs of HLA-DRB6 ware predicted by starBase "miRNA-pseudogene" under "miRNA-Target" pane. Then searched the target mRNA of these miRNA by "miRNA-RNA" pane under "RNA-RNA".

Statistics and clinical data analysis

Gene expression profiling and related clinical characteristics data (GSE98743) of cutaneous melanoma patients were downloaded from the GEO (Gene Expression Omnibus) database. Data analysis was performed using SPSS 19.0. Unpaired t-test or paired t-test was used to determine the difference in CXCL10 expression between different groups. Chi-square test and Fisher's exact test were used to determine correlation between CXCL10 expression and clinical characteristics of patients. The receiver operating curve (ROC) was used to analyze the diagnostic value of genes such as CXCL10 in predicting whether patients will respond to immunotherapy and the survival status.

Results

(A) The expression level of HLA-DRB6 in different types of human cancers and the relationship with prognosis of SKCM patients

To compare the expression levels of HLA-DRB6 in various types of cancer, GEPIA was used to analyze the RNA-seq data from the TCGA and GTEx datasets. Compared with normal tissues, the transcription level of HLA-DRB6 were significantly higher in CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), COAD (colon adenocarcinoma), DLBC (lymphoid neoplasm diffuse large B-cell lymphoma), GBM (glioblastoma), KIRC (Kidney renal clear cell carcinoma), LAML (acute myeloid leukemia), LGG (brain lower grade glioma), LIHC (Liver hepatocellular carcinoma), PAAD (pancreatic adenocarcinoma), READ (rectum adenocarcinoma), SKCM, TGCT (testicular germ cell tumors), THCA (Thyroid carcinoma), THYM (thymoma) and UCEC (uterine corpus endometrial carcinoma) (Figure 1A, B).

We also used the TIMER database to analyze the expression of HLA-DRB6. The results show that HLA-DRB6 were highly expressed in BRCA (breast invasive carcinoma), ESCA (esophageal carcinoma), KIRC and STAD (stomach adenocarcinoma), whereas in BLCA (bladder urothelial carcinoma), KICH (kidney Chromophobe), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), the expression was low. Furthermore, the expression of HLA-DRB6 was higher in SKCM with metastasis than in SKCM without metastasis (Figure 1C). We further analysed the relationship between the expression of HLA-DRB6 and the prognosis of SKCM patients.The results showed that high expression of HLA-DRB6 was associated with a better prognosis in SKCM with or without metastasis but not in primary SKCM (Figure 1D).

(B) HLA-DRB6 expression in SKCM is positively correlated with immune cells infiltration

High levels of lymphocyte infiltration are one of the factors for good prognosis in patients with cutaneous melanoma[9, 10]. In order to further understand the relationship between HLA-DRB6 expression and SKCM prognosis, we sought to establish a correlation between HLA-DRB6 and immune cell infiltration into the SKCM tumor microenvironment. Results showed that HLA-DRB6 expression was significantly negatively correlated with the tumor purity (P<0.01), indicating that in tumor microenvironment, HLA-DRB6 might be expressed by the infiltrating immune cells or other interstitial cells. In SKCM and SKCM metastases, HLA-DRB6 RNA levels were positively correlated with infiltrating B cells, $CD4^+$ T, $CD8^+$ T lymphocytes, macrophages, neutrophils and DCs. In SKCM-primary, HLA-DRB6 was positively correlated with $CD4^+$ T, $CD8^+$ T lymphocytes, macrophages, neutrophils, and DCs infiltration (Figure 2A). We also sought to determine a relationship between HLA-DRB6 and some key molecules of immune checkpoints in the SKCM tumor microenvironment and found that HLA-DRB6 is positively associated with PD-1, PD-L1, CTLA-4, LAG3 and TIM3 (Figure 2B).

(C) HLA-DRB6 expression is predominant in macrophages and B cells and is associated with chemokines

Analysis of the single cell sequencing data of Itay tirosh by the single cell portal showed that HLA-DRB6 expression is predominantly concentratedin macrophages and B lymphocytes in melanoma (Figure 3A), which is consistent with the results shown by Livnat jerby-Arnon (Figure 3B)[11]. In order to verify the enrichment of HLA-DRB6 in these cells, we analyzed the single-cell transcriptome data in other types of tumors like Hepatocellular carcinoma (HCC). The result showed that HLA-DRB6 is mainly enriched in monocytic macrophages and B lymphocytes (Figure S1). This illustrates the possibility of the enrichment of HLA-DRB6 in tumors other than SKCM.

Based on the fact that HLA-DRB6 expression is positively correlated with different types of immune cells infiltration and that its expression is mainly concentrated in macrophages, we speculated that HLA-DRB6 might enriches immune cells locally in the tumor by affecting the secretion of chemokines. We sought to establish a correlation between HLA-DRB6 and chemokines using GEPIA. The results showed that HLA-DRB6 was most strongly associated with CXCL10 relative to other chemokines (Figure 3C, Figure S2). Furthermore, the single cell sequencing data of Itay tirosh and Livnat jerby-arnon on/ melanoma showed

that CXCL10 was also mainly distributed in macrophages (Figure S3). Combined with the previous reports that macrophages affect immune cells infiltration by regulating the expression of CXCL10[12, 13], we speculate that the HLA-DRB6-induced increased immune cell infiltration in tumor might occur through regulating the level of CXCL10 in macrophages.

(D) HLA-DRB6 regulates CXCL10 expression through miR-338-3p

Pseudogenes can regulate the expression of target genes through the competitive endogenous RNA (ceRNA) mechanism by combining miRNA, and play a key regulatory role in the development of human cancer[14]. As a special type of lncRNA, the cellular localization of the pseudogenes-derived lncRNA determine the underlying mechanisms to some extent. We used lncLocator to speculate the localization of HLA-DBR6 in subcellular compartments. The results showed that compared with ribosomes, exosomes and cytosol, HLA-DRB6 was highly expressed in the nucleus and cytoplasm (Figure 4A). Then we used starBase dataset to analyze the target gene of HLA-DRB6. We found that miR-338-3p interacts with HLA-DRB6 (Figure 4B). The expression of miR-338-3p and HLA-DRB6 is negatively correlated in SKCM (Figure 4C). In addition, we analyzed the binding site of miR-338-3p and found that miR-338-3p binds with the transcription factors STAT1 and NFKB1 mRNA 3'UTR (Figure 4D). Studies have shown that STAT1[15-17] and NF-κB[18-20] can bind to the CXCL10 promoter and affect the expression of CXCL10. We also found that the expression of HLA-DRB6 and CXCL10 is positively correlated in SKCM (cor > 0.5, Figure 4E). Therefore, we speculate that HLA-DRB6 regulates SKCM tumor development through the miR-338 / CXCL10 axis in macrophages.

(F) CXCL10 is highly expressed in SKCM and associated with good prognosis

CXCL10 is a colony-stimulating factor, mainly secreted by monocytes, endothelial cells, fibroblasts, and cancer cells under the stimulation of cytokines such as IFN-γ. It can suppress tumors by aggregating immune cells and weaken angiogenesis. However, some studies have shown that CXCL10 can promote tumor proliferation and metastasis[21]. In order to study the role of CXCL10 in SKCM, we first analyzed the expression of CXCL10 in SKCM. Like HLA-DRB6, the expression level of CXCL10 was higher in tumor tissues than normal tissues, and higher in SKCM metastases than non-metastatic SKCM (Figure 5A, 5B). Survival analysis resulted that SKCM and SKCM metastases patients with high infiltration levels of B lymphocytes, $CD8^+$ T lymphocytes, neutrophils, DCs and high expression of CXCL10 had better prognosis (Figure 5C).

(G) CXCL10 is closely related to immune cells infiltration in SKCM

As a potential target molecule of HLA-DRB6, the relationship between CXCL10 and immune cells in SKCM was explored here. We found that the expression of CXCL10 was positively correlated with the infiltration of B cells, $CD4^+$ T, $CD8^+$ T cells, macrophages, neutrophils and DCs in general, and particularly positively associated with the immune checkpoint molecule PD-1, PD-L1, CTLA-4, LAG3, TIM3 in SKCM and SKCM-metastases (Figure 6A, 6B). These results are very much in accordance with the correlation between HLA-DRB6 and immune cells infilteration in SKCM.

(H) CXC10 can be used as a potential biomarker in peripheral blood to predict the efficacy and prognosis of immunotherapy

We used the GEO database (GSE94873) to analyze the gene expression and clinical data of cutaneous melanoma patients who had been treated with CTLA-4. Comparing the difference in gene expression in peripheral blood of patients before treatment with the Tremelimumab, a human monoclonal antibody against CTLA-4, we found that the expression of CXCL10, IL10, NME4, NEDD4L, and BLVRB was higher in patients who responded to the treatment than those who did not respond to Tremelimumab, but the expression of ZBTB10, PDE3B, NAB2, ITGA4 were decreased in respondent patients(Figure 7A, Table S1). And in the patients who responded to tremelimumab therapy, CXCL10 was decreased after treatment (Figure 7B,Table S2). By analyzing the clinical data, we found that there was no correlation between CXCL10 and age, sex and tumor stage, but was related to the treatment response and the 12-month survival status (Table 1). The ROC curve was used to evaluate the ability of CXCL10 in peripheral blood before immunotherapy to predict the cutaneous melanoma patients' responses to the tremelimumab treatment. Result showed that the area under the curve (AUC) of CXCL10 is 0.618 (Figure 7C). In order to improve the predictive ability, we combined the nine differentially expressed genes CXCL10, IL10, ITGA4, NME4, BLVRB, ZBTB10, NEDD4L, PDE3B, and NAB2 to predict the immunotherapy response, and the AUC raised to 0.741 (Figure 7D). We then used these indicators to evaluate the one-year survival rate. The results showed that the AUCs of CXCL10 and 9-index combination were 0.58 and 0.6996 respectively (Fig. 7E, 7F). The sensitivity and specificity of these markers are shown in Table 2. Combining these results, we speculate the gene expression of CXCL10 in peripheral blood can be used to predict the therapeutic effect and prognosis.

Discussion

Increasing evidences show that the tumor immune microenvironment of melanoma patients determines the development and prognosis of the tumor to some extent[22]. Primary melanoma with active TIL (Tumor infiltration lymphocyte) infiltration has been reported to have a lower sentinel lymph node positive rate compared to melanoma without infiltrated TIL[23]. And the expression of immune-related genes in metastatic lymphoma has been shown to be beneficial for patient survival[24]. Moreover, the genomic classification of cutaneous melanoma found that only the transcriptome subgroups characterized by the enrichment of immune gene expression are associated with an improvement in the quality of life of the patients[25]. As we all know, immune checkpoint blocking therapy has revolutionized the treatment of cancer. Antibodies that block immune checkpoint proteins, including CTLA-4, PD-1 and PD-L1, have been approved by the FDA for the treatment of melanoma[26]. Although anti-CTLA-4 and anti-PD-1/PD-L1 treatments have achieved some success in clinical practice, the response rate of these drugs can only reach 40%-50% at most[27]. Therefore, in-depth understanding of the immune regulation mechanism in cutaneous melanoma and the discovery of biomarkers that could predict the therapeutic effect of the immune checkpoints are still a dilemma that needs to be resolved urgently.

There are only a few reports about pseudogenes and the immune regulation[28, 29]. As a special type of lncRNA, pseudogenes may play an important role in tumor immunity. In this study, we analyzed the immunoregulatory role of the pseudogene HLA-DRB6 in SKCM usingbioinformatics tools. It has been reported that DNA methylation of HLA-DRB6 is related to immunity in rheumatoid arthritis[30], but the relationship between HLA-DRB6 transcript and immunity has not been reported. We analyzed the relationship between the pseudogene HLA-DRB6 and immunity in SKCM, and found that the expression of HLA-DRB6 was positively correlated with various immune cells and immune molecules, indicating that HLA-DRB6 may be involved in the immune regulation of SKCM. Single cell transcriptome sequencing analysis showed that HLA-DRB6 and CXCL10 are enriched mainly in macrophages, and there was a strong correlation between them in melanoma (cor=0.66, P<0.0001). We speculated that HLA-DRB6 may affect immune cell infiltration in tumor by regulating CXCL10 based on the fact that CXCL10 is closely associated with immune cell enrichment.

Similar to the mechanism of lncRNA, pseudogenes can also play a regulatory role through a competitive endogenous RNA (ceRNA) mechanism[31]. Some scholars believe that the prerequisite for the ceRNA mechanism is the presence of lncRNA in the cytoplasm[32, 33]. Therefore, we predicted the location of HLA-DRB6 in subcellular compartments, and the results showed that part of HLA-DRB6 is expressed in the cytoplasm. Bioinformatics analysis found that HLA-DRB6 may play a role through the miR-338/CXCL10 axis. Interestingly, the expression trend of CXCL10, the target gene of HLA-DRB6, and its correlation with prognosis are highly consistent with HLA-DRB6 in SKCM, which may further indicate that HLA-DRB6 may affect the development of SKCM by regulating target gene CXCL10. It has been reported that CXCL10 plays an immunomodulatory role in melanoma[34, 35], but its relationship with immune cells and immune molecules is not very clear. Our analysis of the relationship between CXCL10 and immunity was similar to the relationship between HLA-DRB6 and immunity in SKCM. These results show that HLA-DRB6 can affect the SKCM immune microenvironment by regulating the expression of CXCL10.

Although some patients with cutaneous melanoma can recover after receiving immune checkpoint blockade treatment, the low response rate and high drug prices make some patients to give up[36]. If there are some biological markers that can predict the therapeutic effect before treatment, it will provide a great help to clinicians. At present, the most widely used detection method for judging the therapeutic outcome of immune checkpoints is the immunohistochemistry to detect the expression of PD-L1 in tumor tissue[37]. However, there exist some problems in judging the prognosis based on the PD-L1 level of tumor tissue. For example, a certain proportion of patients with low-expression PD-L1 can respond to treatment, local tumor tissue sampling may cause deviations in the results, and invasive examination may bring the risk of tumor cells spreading[38]. Compared to tissue specimens, blood specimens have some advantage, including its low invasiveness and risk, low acquisition cost and continuous acquisition, and low requirements for collection personnel. Since CXCL10 is one of the essential components for an effective anti-tumor immune response after receiving immune checkpoint blockade therapy[39], we speculate that CXCL10 can be used as a predictive marker. Based on the above purpose, we analyzed the data of peripheral blood of patients receiving tremelimumab treatment (GSE94873). Analyzing this data,

we found that the mRNA level of CXCL10 in peripheral blood before treatment can be used to judge the treatment reactivity and one-year survival rate in advance.

In summary, in this study we found that the pseudogene HLA-DRB6 is highly expressed in SKCM, and was related to the prognosis and tumor immune microenvironment status. Mechanism analysis predicted that HLA-DRB6 may regulate the SKCM immune microenvironment through the miR-338/CXCL10 axis. Based on the present study, we propose the following hypothesis: Increased HLA-DRB6 levels in the SKCM is associated with elevated HLA-DRB6 in macrophage, and HLA-DRB6 competitively bind to miR-338 through a ceRNA mechanism, inhibit the binding of miR-338 to STAT1 and NFKB1, and thus upregulate CXCL10 in macrophages. Moreover, high levels of CXCL10 further aggregated immune cells to the tumor, thus leading to a better prognosis for patients (Figure 8). This provides a new approach for the treatment of SKCM. Furthermore, the CXCL10 in peripheral blood can be used as a biomarker to predict the responsiveness and the prognosis for patients treated with tremelimumab.

Conclusions

Bioinformatics analysis shown that HLA-DRB6 is highly expressed in SKCM and is associated with a good prognosis. The expression of HLA-DRB6 is positively correlated with the infiltration of immune cells and the expression of immune checkpoint molecules in the tumor microenvironment. Single-cell sequencing data indicated that HLA-DRB6 was mainly enriched in macrophages and B cells in the SCKM tumor microenvironment. Further analysis showed that HLA-DRB6 can regulate the expression of CXCL10 through miR-338/CXCL10 axis. At the same time, the CXCL10 in peripheral blood can be used as a biomarker to predict the responsiveness and the prognosis for SKCM patients treated with tremelimumab.

Abbreviations

HLA-DRB6:Major Histocompatibility Complex, Class II, DR Beta 6; SKCM:Skin cutaneous melanoma GEPIA:Gene Expression Profiling Interactive Analysis; CXCL 10:C-X-C motif chemokine ligand 10; GEO: Gene Expression Omnibus; CD: cluster of differentiation; PD-1: programmed cell death protein 1; PD-L1: Programmed cell death 1 ligand 1; cytotoxic T-lymphocyte-associated protein 4; TCGA: The Cancer Genome Atlas; GTEx: The Genotype-Tissue Expression; CESC : cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD : colon adenocarcinoma; DLBC: lymphoid neoplasm diffuse large B-cell lymphoma; GBM: glioblastoma; KIRC: Kidney renal clear cell carcinoma; LAML: acute myeloid leukemia; LGG: brain lower grade glioma; LIHC: Liver hepatocellular carcinoma; PAAD: pancreatic adenocarcinoma; READ: rectum adenocarcinoma; TGCT: testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: thymoma; UCEC: uterine corpus endometrial carcinoma; TIMER:the tumor immune estimation resource; BRCA: breast invasive carcinoma; ESCA: esophageal carcinoma; STAD: stomach adenocarcinoma; BLCA: bladder urothelial carcinoma; KICH: kidney Chromophobe; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; DCs: Dendritic cells; LAG3: Lymphocyte-activation gene 3; TIM3:T cell immunoglobulin and mucin domain-containing protein 3; HCC: hepatocellular carcinoma; STAT1: Signal Transducer And Activator Of Transcription 1; NFKB1:nuclear

factor kappa B subunit 1; IL10:Interleukin 10; NME4:Nucleoside Diphosphate Kinase 4; NEDD4L:neural precursor cell expressed, developmentally down-regulated 4-like; BLVRB: Biliverdin Reductase B; ZBTB10: Zinc Finger And BTB Domain Containing 10; PDE3B: Phosphodiesterase 3B; NAB2: NGFI-A binding protein 2; ITGA4: Integrin Subunit Alpha 4; AUC: area under the curve; TIL: Tumor infiltration lymphocyte; UCSC: University of California–Santa Cruz; lncRNA: Long Noncoding RNAs; ROC: The receiver operating curve.

Declarations

Conflict of Interest

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

Author contributions

Conceptualization, Ren Jianmin and Yu Jinglu; Formal analysis, Yu Jinglu and Shi Yang; Data Curation, Ren Jianmin and Inam Ullah Khan;writing—original draft preparation, Ren Jianmin and Yu Jinglu; writing—review and editing, Inam Ullah Khan and Huang Jiansheng; supervision,Huang Jiansheng. All authors have read and agreed to the published version of the manuscript.

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Consent for publication

Written consent for publication was obtained from all study participants.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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Tables

Table1. Correlations between CXCL10 and clinical parameters.

Parameters	n	CXCL10		χ^2	*P*
		High	Low		
Age (years)					
≤54	183	96	87	0.9	0.343
>54	177	84	93		
Gender					
Female	140	68	72	0.187	0.665
Male	220	112	108		
Stage of disease					
IIIC	14	5	9	1.73	0.188
IV M1A	41	20	21		
IV M1B	81	37	44		
IV M1C	224	118	106		
Immunotherapy response					
Responder	46	30	16	4.885	0.027
Nonresponder	314	150	164		
Survival status 12 months					
Alive	161	90	71	4.056	0.044
Dead	199	90	109		

Table 2. Parameters of CXCL10 alone or combination with other genes used to evaluate the potential of CXCL10 as a biomarker for Tremelimumab treatment.

	Response		Survival	
	CXCL10	9 parameters	CXCL10	9 parameters
Sensitivity	63.00%	54.40%	52.80%	50.10%
Specificity	60.80%	84.40%	62.80%	85.1
AUC	0.6182	0.7406	0.58	0.6996
P	0.009616	< 0.0001	0.009037	< 0.0001

Figures

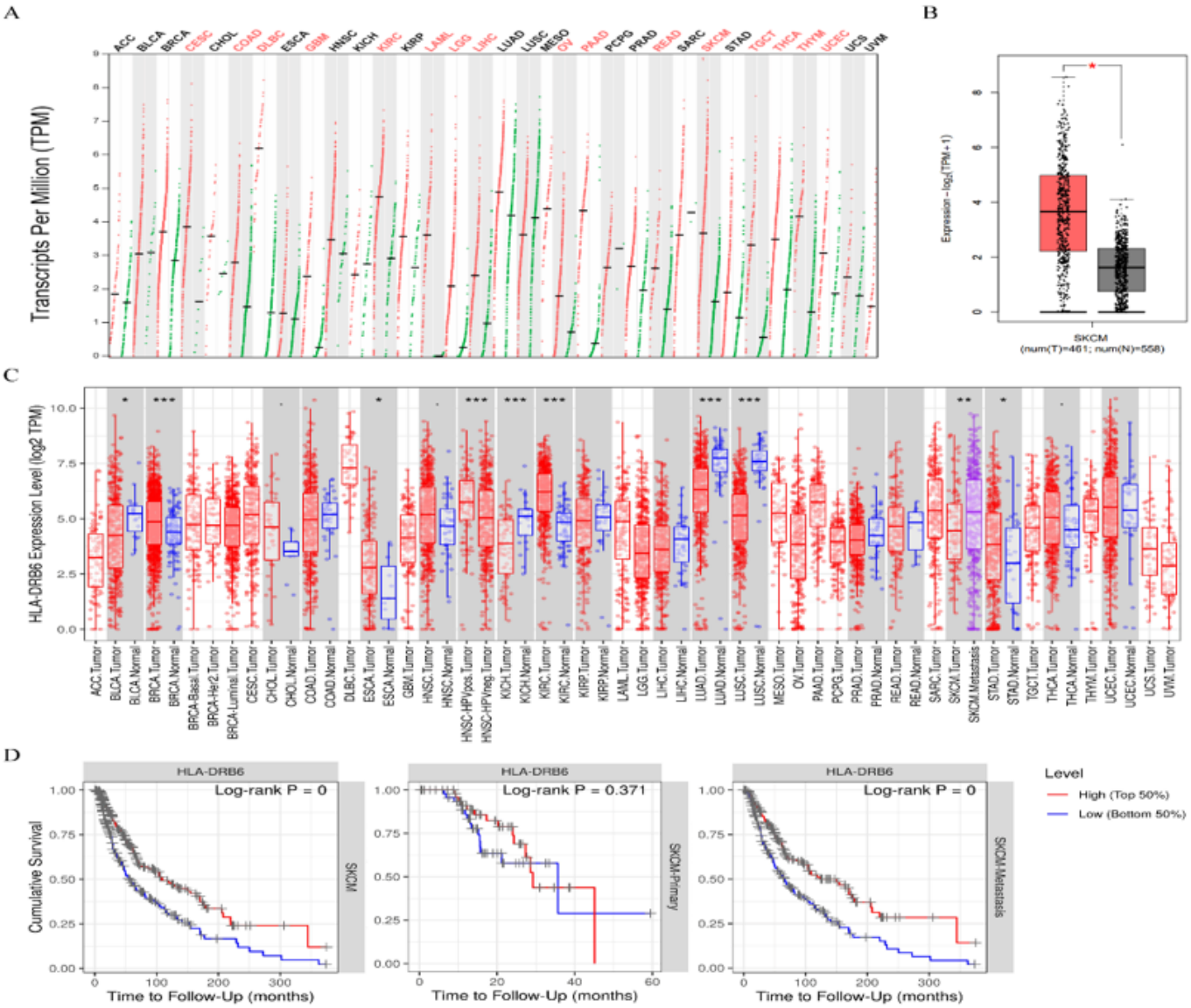

Figure 1

HLA-DRB6 expression levels in human cancers and its relationship with prognosis in the SKCM patients.

(A, B) Expression of HLA-DRB6 in SKCM and different other types of cancer through GEPIA. (C) (the expression of HLA-DRB6 in SKCM with and without metastasis). (D) Kaplan-Meier survival curves comparing the high and low expression of HLA-DRB6 in SKCM. *P < 0.05, **P <0.01, ***P < 0.001.

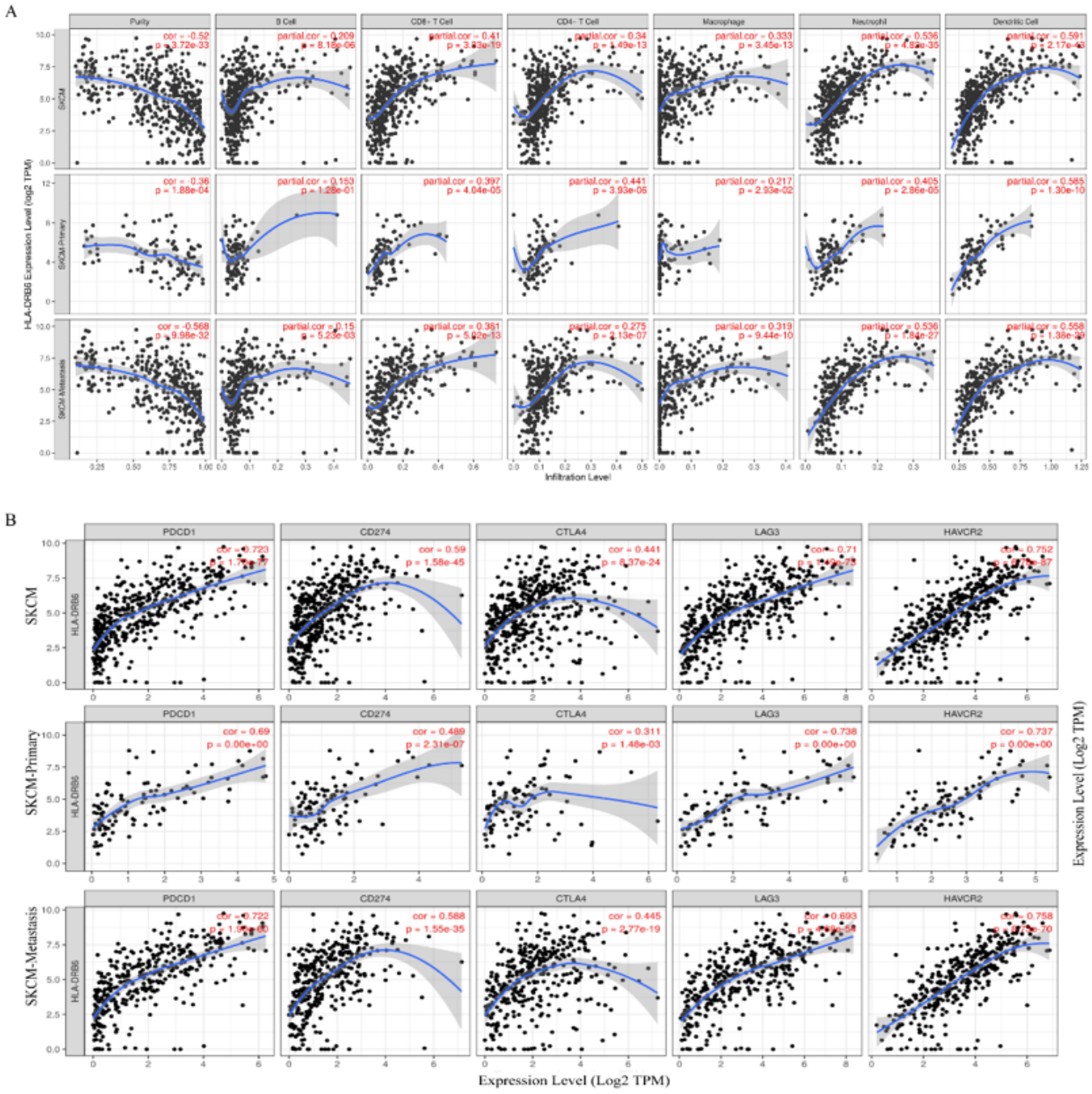

Figure 2

Correlation of HLA-DRB6 expression with immune infiltration in SKCM. (better to mention briefly the how and with the help software you determine the correlation)

(A) Expression of HLA-DRB6 is significantly negatively correlated with tumor purity and positively correlated with infiltrating levels of CD4 $^{+}$T cells, CD8 $^{+}$T cells, macrophages, neutrophils, and dendritic cells in SKCM, SKCM-Primary and SKCM-Metastasis, positively correlated with B cell in SKCM and SKCM-Metastasis but not in SKCM-Primary. (B) Expression of HLA-DRB6 is significant positively correlated with

the mRNA levels of immune checkpoints molecules PD-1, PD-L1, CTLA-4, LAG3, TIM3 in SKCM, SKCM-Primary and SKCM-Metastasis.

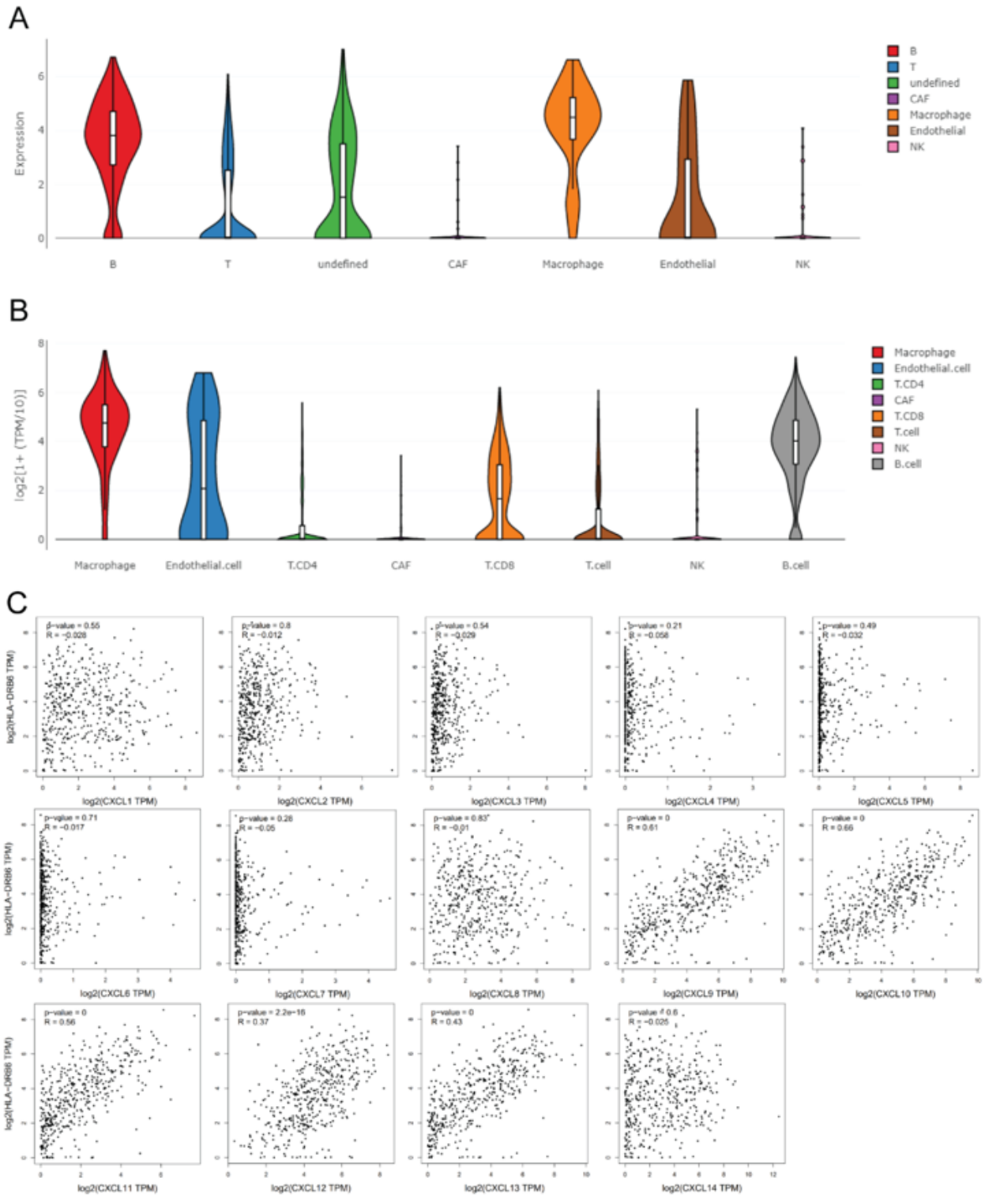

Figure 3

The enrichment of HLA-DRB6 in cells and the correlation of HLA-DRB6 with chemokines

(A) Analysis the single cell transcriptome data of Itay tirosh for understanding the distribution of HLA-DRB6 in cells. (B) Distribution of HLA-DRB6 in cells shown by the data of Livnat jerby-Arnon. (C) Analysis of the relationship between the expression of HLA-DRB6 and multiple chemokines in SKCM by GEPIA

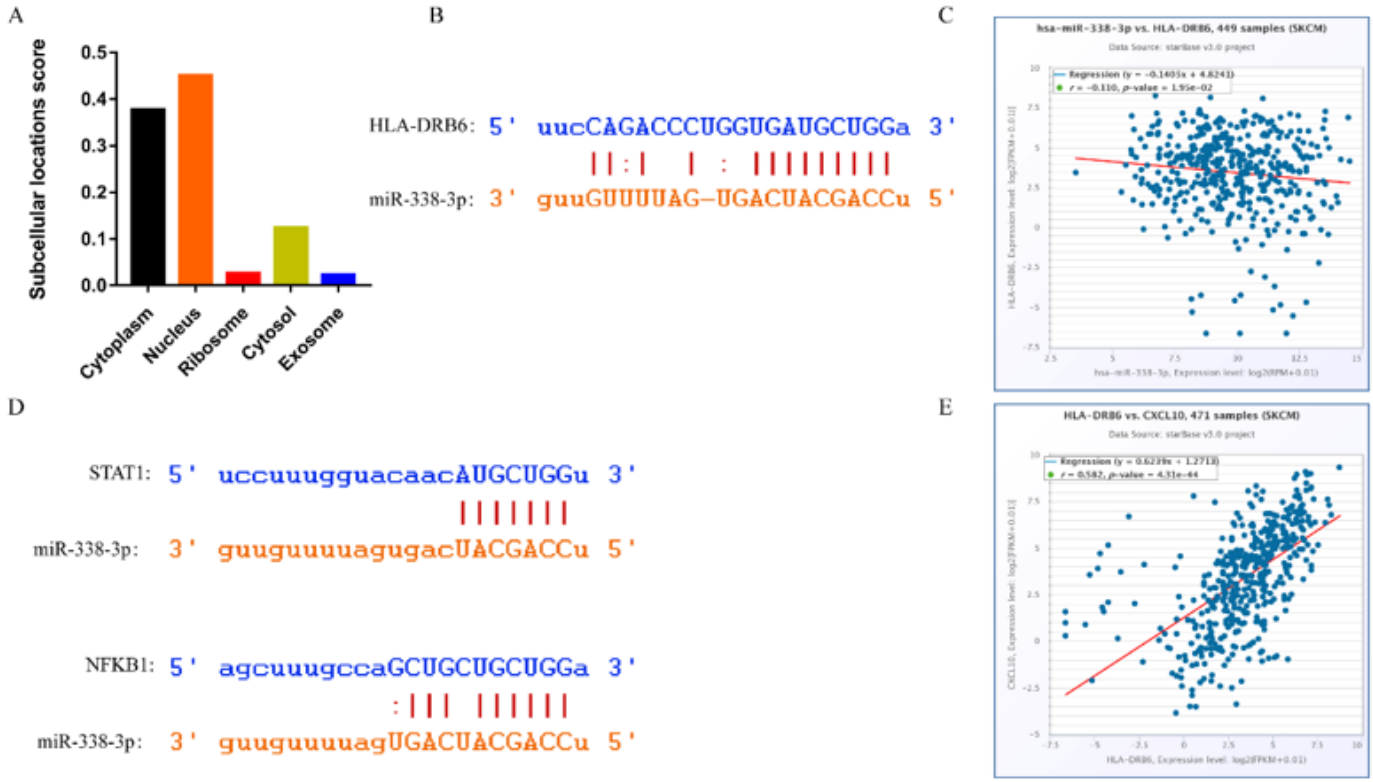

Figure 4

HLA-DRB6 may regulate SKCM tumor immune microenvironment by miR-338/CXCL10 axis.

(A) Prediction of cellular localization for HLA-DRB6 using lncLocator. (B) The binding sites between HLA-DRB6 and miR-338 predicted by starBase v2.0. (C) Correlation of HLA-DRB6 and miR-338-3p expression in SKCM analyzed by starBase. (D) Base pairing between miR-338-3p and STAT1, miR-338-3p and NFKB1. (E) Co-expression relationship betweenHLA-DRB6 and CXCL10 expression in SKCM.

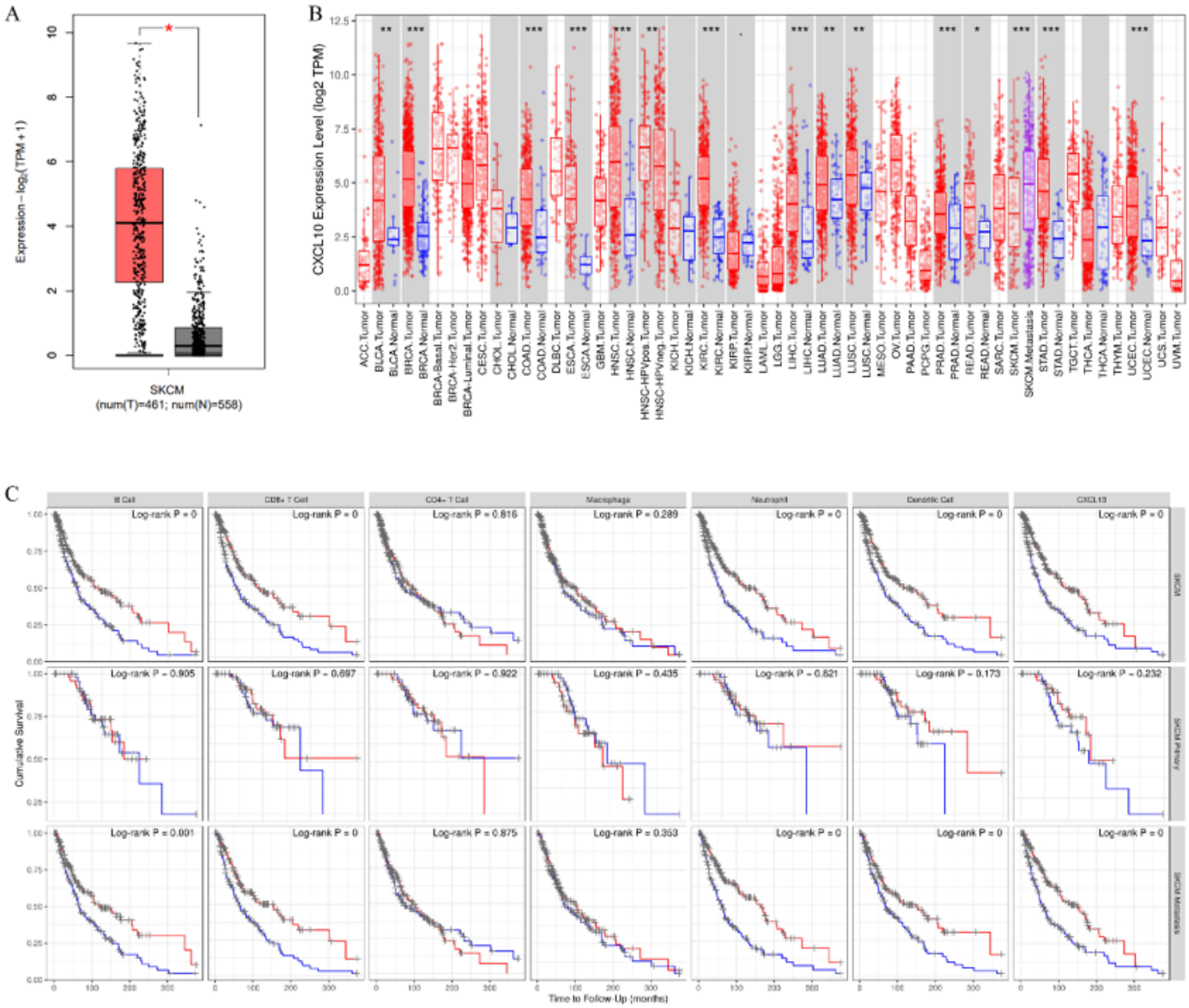

Figure 5

The expression level of CXCL10 and its relationship with prognosis in SKCM.

(A) The expression of CXCL10 in tumor is higher than normal tissues analyzed through GEPIA. (B) The mRNA level of CXCL10 was higher in SKCM-metastatic analyzed by TIMER. (C) High level of the infiltration of B cell, CD8 $^{+}$T cell, neutrophil, dendritic cells, and CXCL10 expression associated with good prognosis.

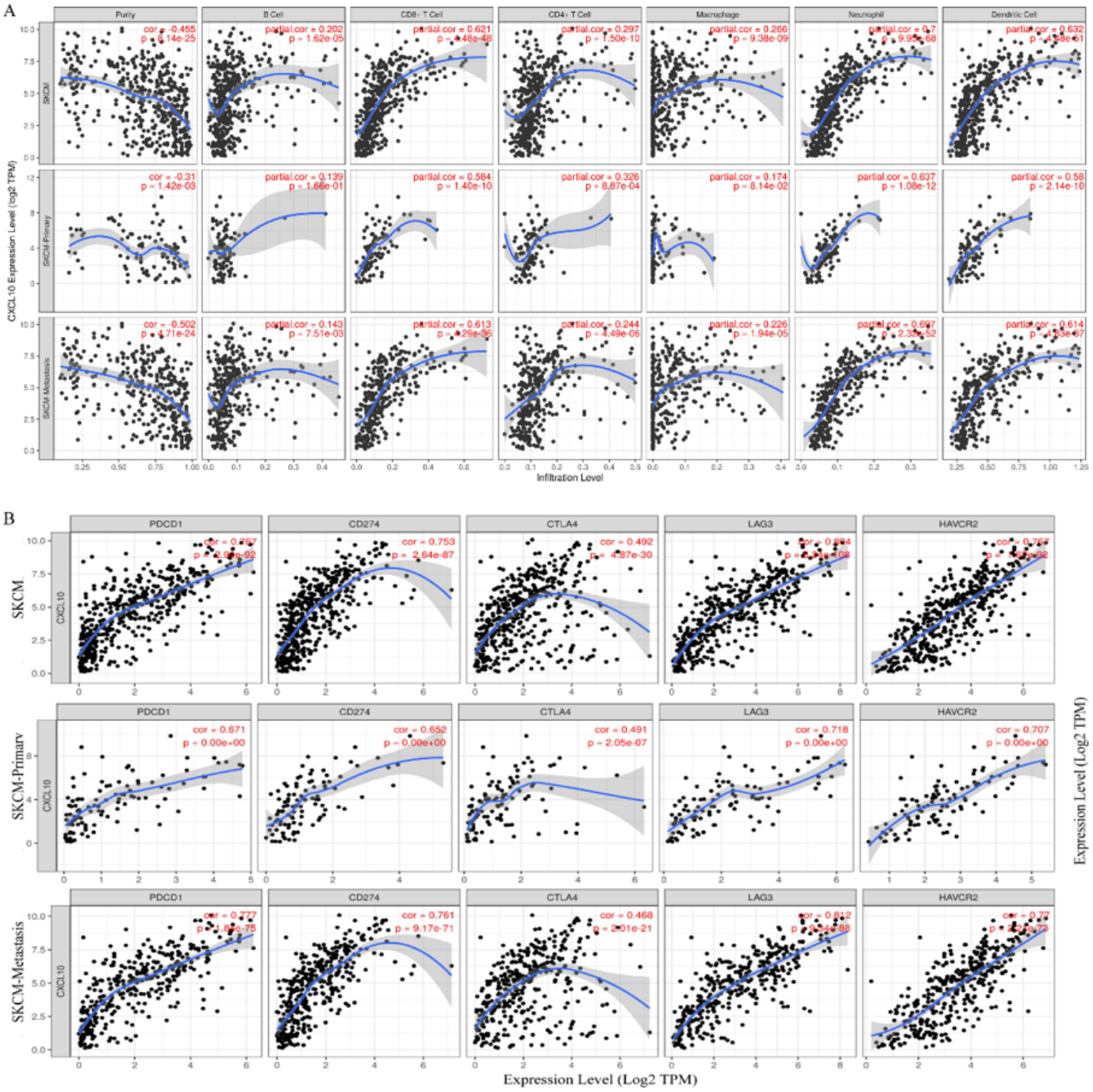

Figure 6

The relationship between the expression of CXCL10 and immune infiltration in SKCM.

(A) The expression of CXCL10 is negatively correlated with tumor purity and positively correlated with infiltrating levels of $CD8^+$ T cells, $CD4^+$ T cells, neutrophils, and dendritic cells in SKCM, SKCM-Primary and SKCM-Metastasis, and positively correlated with B cells, macrophages in SKCM and SKCM-Metastasis. (B) The transcriptional level of CXCL10 is positively correlated with the expression of PD-1, PD-L1, CTLA-4, LAG3 and TIM3.

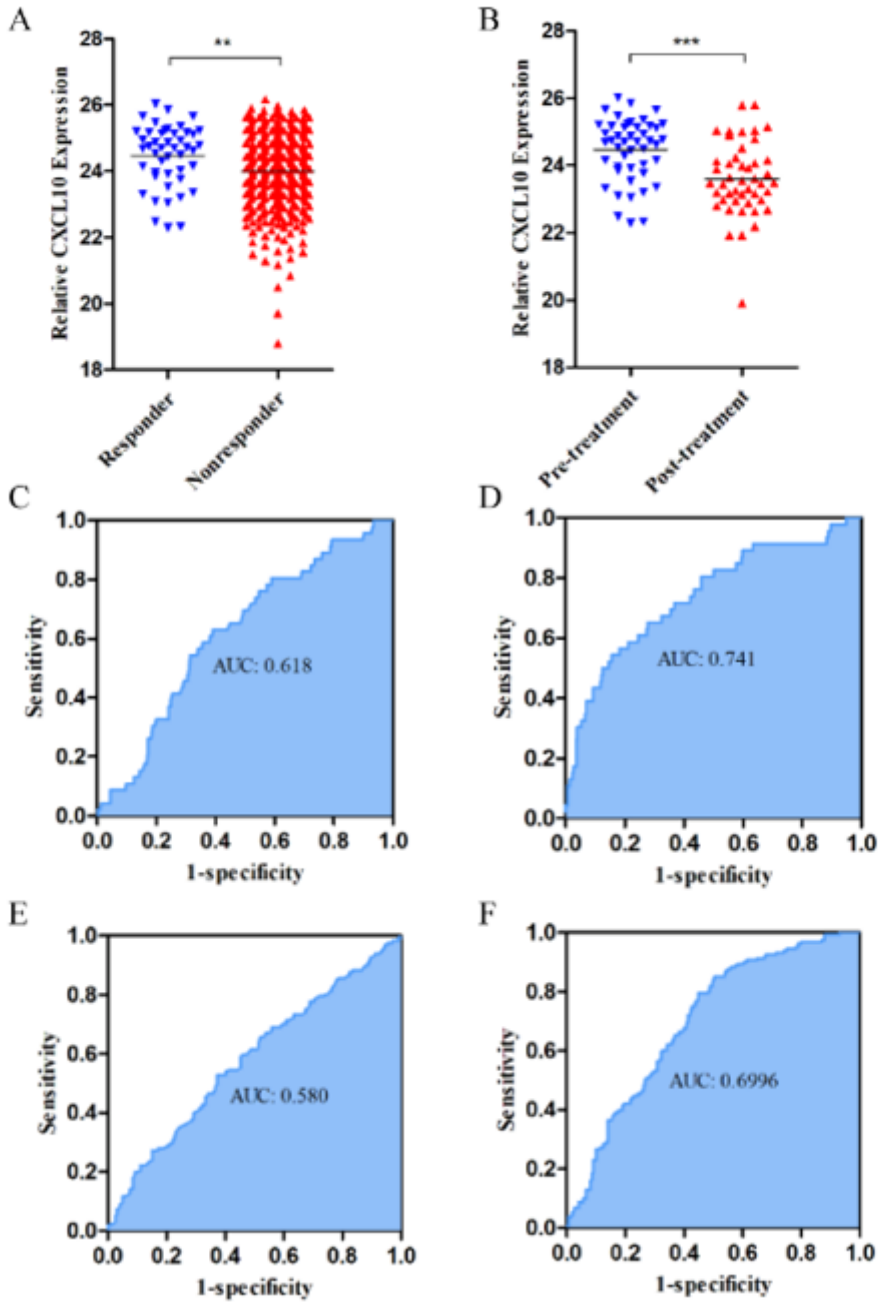

Figure 7

CXCL10 has the potential to be a biomarker to prediction immunotherapy responsiveness and prognosis in patients treated with tremelimumab.

(A) The expression level of CXCL10 in peripheral blood of patients before tremelimumab treatment. (B) Comparing the expression of CXCL10 before and after accept tremelimumab in peripheral blood for patients who had responded to the treatment. (C) Overlapping genes of differentially expressed that is analyzed with A (red) and B (green). (D, E, F) ROC curve was used to evaluate the ability of CXCL10, 3 genes and 9 genes combination as a biomarker to assessment the responsiveness of tremelimumab

therapy. (G, H, I) CXCL10, 3 genes and 9 genes combination used to evaluate 1 year survive status after tremelimumab treatment.

*P < 0.05, **P <0.01, ***P < 0.001.

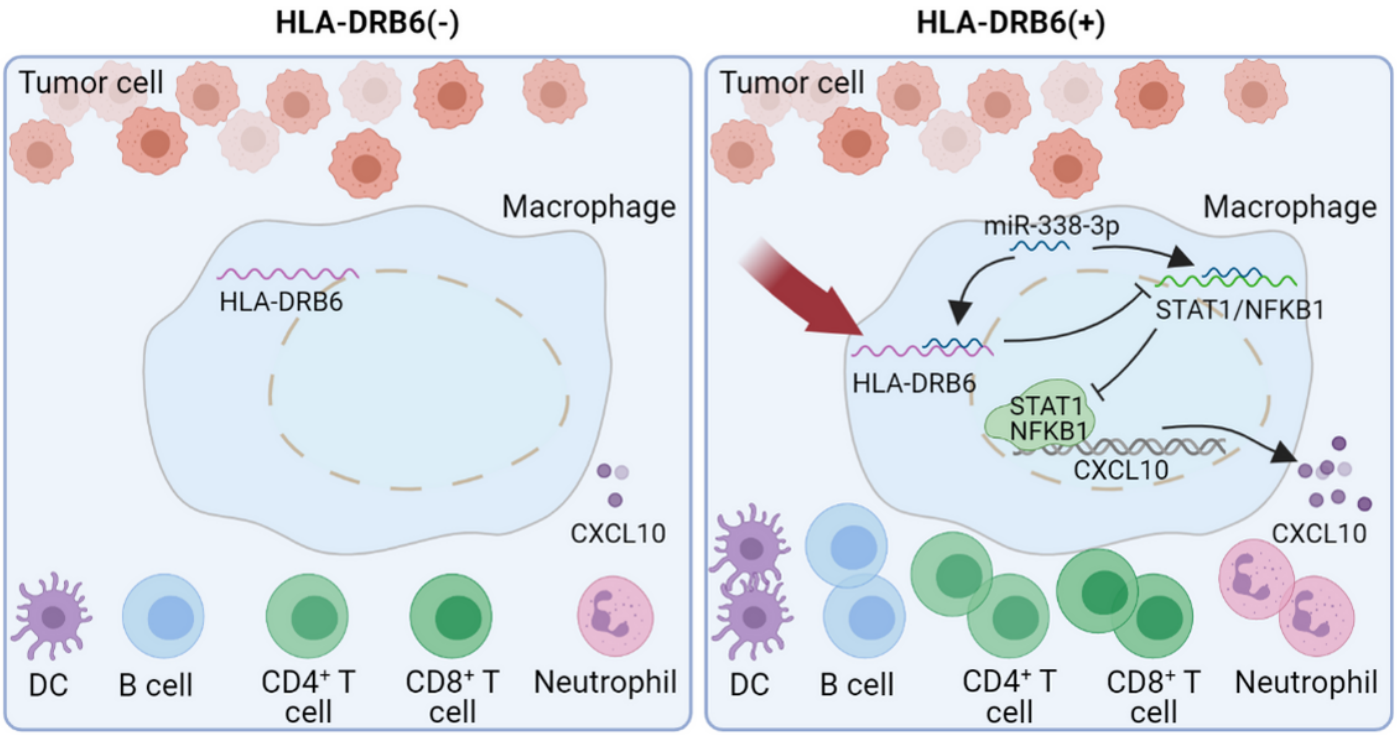

Figure 8

A hypothesis diagram of immune cell infiltration induced by HLA-DRB6 in SKCM

Tumor microenvironment with fewer HLA-DRB6, the level of CXCL10 and the infiltration of immune cells is lower. Reciprocally, HLA-DRB6 levels in macrophages were elevated concomitantly with higher HLA-DRB6 in the tumor microenvironment. HLA-DRB6 inhibited miR-338-3p binding to STAT1 and NFKB1 by competitively adsorbing miR-338-3p, upregulating CXCL10 and promoting immune cell infiltration.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx