Role of sphingosine-1-phosphate receptors in the tumor microenvironment: prospects for cancer immunotherapy

Z. WANG[1,3], H.-M. ZHANG[2,3], Y.-R. GUO[2,3], L.-L. LI[2], G.-Z. ZHANG[1]

[1]Department of Dermatology, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, China
[2]Department of Dermatology, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China
[3]Beijing University of Chinese Medicine, Beijing, China

Abstract. – OBJECTIVE: Sphingosine-1-phosphate (S1P) is a sphingolipid protein with anti-apoptotic and pro-survival effects on cancer cells *via* S1P receptors (S1PRs); however, the role of S1PRs in the tumor microenvironment and immune invasion is still unclear. This study investigated the relationship between S1PR expressions and patient survival and clinical manifestations with respect to the tumor microenvironment and immune infiltration.

MATERIALS AND METHODS: The expression levels of five S1PRs were obtained from The Cancer Genome Atlas pan-cancer database and the Kaplan-Meier survival analysis was performed. We predicted the relationship between S1PRs expression levels and patient survival using the univariate Cox proportional hazard regression model. Subsequently, we analyzed correlations between S1PRs expression and infiltrating immune cell subtypes using the Kolmogorov-Smirnov test and the infiltration levels of immune and stromal cells in each tumor using the ESTIMATE algorithm and Spearman's test.

RESULTS: The five S1PRs exhibited significant heterogeneity in their expression levels. The expression levels correlated with overall patient survival; however, anti-apoptotic or pro-apoptotic features varied depending on the cancer type. The variable effects of S1PRs on tumors may be related to TGF-β levels. Our results suggest that S1PRs exert distinct influences on the tumor stem cell index and chemotherapeutic drug sensitivity.

CONCLUSIONS: This research provides comprehensive information on the importance of S1PRs in the immune microenvironment, stemness score, sensitivity of human cancer drugs, and cancer prognosis. Interestingly, our findings indicate variations in the expression levels and functions of different S1PR family members. This study highlights S1PRs as potential new targets for antitumor (adjuvant) therapy.

Introduction

Sphingosine-1-phosphate (S1P) is a lipid mediator with biological activity[1] that acts as an extracellular signaling molecule. S1P is produced *via* the conversion of ceramide into sphingosine by ceramidase, followed by the phosphorylation of sphingosine-by-sphingosine kinase (SK)[2]. Two subtypes of SK have been identified (SK1 and SK2) that can be activated by a variety of cellular signals, including G-protein-coupled receptors (GPCRs), small GTPases, and tyrosine kinase receptor agonists. Outside the cell, S1P can bind to five S1P-specific receptors (*S1PR1-5*)[3], leading to cell signaling in an autocrine, paracrine, or endocrine manner through the action of downstream signaling molecules[4]. S1PRs are a family of GPCRs belonging to the endothelial differentiation gene receptor family. Of note, different S1PRs are differentially coupled with different G proteins, and thus, S1P can stimulate different cell types or signal transduction pathways in the same cell. For example, *S1PR1* is only coupled with the Gi protein[5] and signaling through *S1PR1* results in a decrease in cyclic AMP (cAMP) and the activation of Ras, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and phospholipase C (PLC) pathways[6]. Furthermore, both *S1PR2* and *S1PR3* are coupled with the Gi and G12/13

proteins[7] and can activate the aforementioned pathways[8]. However, the characteristics of *S1PR4* and *S1PR5* remain unclear. S1PRs and their downstream pathways regulate a series of cellular processes, such as cell proliferation, survival, and migration, as well as angiogenesis and lymphangiogenesis[9-12]. Therefore, abnormal expression of S1PR family members and the resulting impact on signal transduction are important factors in the initiation and progression (and, consequently, the prognosis) of different tumors[11-17].

The tumor microenvironment (TME) is closely related to tumor characteristics[18]. Increasing evidence supports the hypothesis that S1P-related signals play key roles in the extracellular environment of tumors[19]. Importantly, in recent years, many studies[20-22] on the expression and function of one or more S1PRs have been conducted in a variety of diseases; however, no systematic study has been performed on the role of S1PR family members in human cancer. Therefore, to address this knowledge gap, in this study, data retrieved from The Cancer Genome Atlas (TCGA) database were used to explore differences in the expression levels of S1PR family members in different tumors (33 primary tumors) and their relationship with the overall survival in patients with cancer. In addition, relationships between the expression levels of different genes and the TME and drug sensitivity were explored. Our findings augment our understanding of the role of the S1PR family in different cancers, especially with regards to immune responses, the TME, and drug resistance, and support the therapeutic potential of targeting S1PRs for cancer treatment.

Materials and Methods

Data Collection

We retrieved data from TCGA database - Xena browser (available at: https://xenabrowser.net/datapages/), including RNA sequencing (RNA SeqV2 RSEM), clinical, and survival data, as well as stemness scores based on mRNA levels (RNA stemness score, RNAss), any available DNA methylation data (DNA stemness score, DNAss), and immune cell infiltration profiles. The analyzed cancer data[23] included 33 cancer types: ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, UCS,

and UVM (see **Supplementary Figure 1** for details). To investigate the relationship between the expression of each S1PR gene and overall patient survival, all tumor samples were subject to survival analysis.

Since the data from TCGA are publicly available and open access, this study do not require approval by Ethics Committees, since it followed TCGA data access policies and publication guidelines.

S1PR Expression Levels and Correlation Analyses

First, the expression levels of S1PR family members in the 33 cancers were analyzed (data are shown as boxplot graphs). Subsequently, based on the $\log_2$ (fold change), we analyzed the differences in the expression of each *S1PR* gene in tumor *vs.* healthy tissues using Wilcoxon tests, and the results are presented in boxplots and heat maps; of note, only tumor types with > 5 related healthy samples available were included in this analysis. Finally, we calculated and visualized the correlation between the expression of the five S1PR family members in 33 cancer types using Spearman's correlation analysis.

Correlation with Patient Survival

In this study, patients with tumors were divided into two groups based on the median expression of S1PR family members (high *vs.* low expression). Then, survival differences between the high and low expression groups were compared using the Kaplan-Meier (K-M) method to produce a K-M curve. In addition, we used a univariate Cox proportional hazard regression model to analyze the relationship between gene expression and the overall survival of patients with cancer to determine the potential prognostic value of the S1PR family members.

Relationship with the Tumor Microenvironment and Immune Infiltration

To better understand the relationship between the expression of S1PR family members and the tumor immune components, we used the Kolmogorov-Smirnov (K-S) test to analyze their correlation in all cancer types. The estimation of stromal and immune cells in malignant tumors was determined using the ESTIMATE algorithm[24]. This algorithm uses gene expression information to infer the ratio of stromal cells and immune cells in tumor samples. We used the ESTIMATE algo-

rithm to score each sample and used Spearman's correlation analysis to analyze the correlation between the expression levels of each gene and the infiltration level of immune cells and stromal cells in each tumor.

Relationship with Cancer Stem Cells Drug Sensitivity

First, we evaluated the correlation between stem cell characteristics in each tumor and the expression of different S1PR family members from two viewpoints: RNA transcription and DNA methylation. In addition, we downloaded data of different cancer cell lines from the National Cancer Institute (NCI)-60 database (available at: https://discover.nci.nih.gov/cellminer/) and used Pearson's correlation test to explore the relationship between *S1PR* gene expression and the drug sensitivity of cancer cells. We only considered 263 Food and Drug Administration (FDA)-approved or clinically tested drugs for the correlation analysis and then visualized the 16 combinations with most significant correlations.

Relationship with Melanoma

We explored the correlation between the expression levels of S1PR family members and various parameters (e.g., tumor immune cells, clinical traits, TME, and stem cells) in the cancer type of concern, using skin melanoma (SKCM) as a proof of principle. In addition, to further explore the types of immune cells, we used the CIBERSORT method to calculate the proportions of tumor-infiltrating immune subgroups. Quality filtering was performed using $p < 0.05$ as a standard to analyze the proportion and correlation of immune subgroups. Subsequently, using *S1PR1* as an example, the correlation between immune cell infiltration and gene expression was analyzed. Tumor samples were grouped according to the expression levels (high *vs.* low *S1PR1* expression), and the differences in immune cell infiltration were analyzed. Finally, immune subgroups related to the expression of *S1PR1* family members were obtained.

Statistical Analysis

Statistical analyses were performed using SPSS software v. 22.0 (IBM Corp., Armonk, NY, USA) and R version 3.6.1 (available at: https://www.r-project.org/). A Wilcoxon rank sum test was performed to analyze differential gene expression between tumor and healthy tissues. Univariate and multivariate Cox regression analyses or the Log-ranch test were used to investigate the relationship

between gene expression and patient overall survival. The correlation between gene expression and the stemness, stromal, immune, and estimate scores, as well as drug sensitivity, was determined by calculating Spearman's or Pearson's correlation coefficients. In addition, linear regression was used to investigate the relationship between gene expression and patient clinical characteristics, immune components, and SKCM. The CIBERSORT algorithm was used to analyze the proportion of tumor-infiltrating immune cell subsets. Statistical significance was defined as $p < 0.05$.

Results

Expression of S1PR Genes in Different Types of Cancer

We assessed the expression levels of the different S1PR family members in 33 cancer types using data retrieved from TCGA database. We observed that the expression of *S1PR1-5* in cancer cells decreased sequentially (Figure 1A). For the five S1PR members, there was significant heterogeneity in expression across tumor types. The expression of a given receptor gene may be significantly increased in some tumors, decreased in others, or not affected at all (Figure 1B-F).

Despite the heterogeneity, some interesting trends were observed. For instance, although *S1PR1* expression tended to be downregulated in most tumors, *S1PR4* tended to be upregulated (Figure 1G). Spearman's correlation analysis revealed that the expression levels of *S1PR1* and *S1PR3* were positively correlated (r = 0.49), whereas the expression levels of *S1PR1* and *S1PR2/5* were negatively correlated (Figure 1H).

Relationship Between S1PR Expression and Patient Overall Survival

Next, to understand whether cancer progression or suppression was correlated with one or more S1PRs, we used a univariate Cox proportional hazards regression model to analyze data from the primary tumors of 33 cancer types. Interestingly, the expression levels of a few S1PR family members were correlated with the overall survival rate of patients with some cancers (Figure 2).

S1PR Expression is Associated with Tumor Immune Responses and Tumor Microenvironment

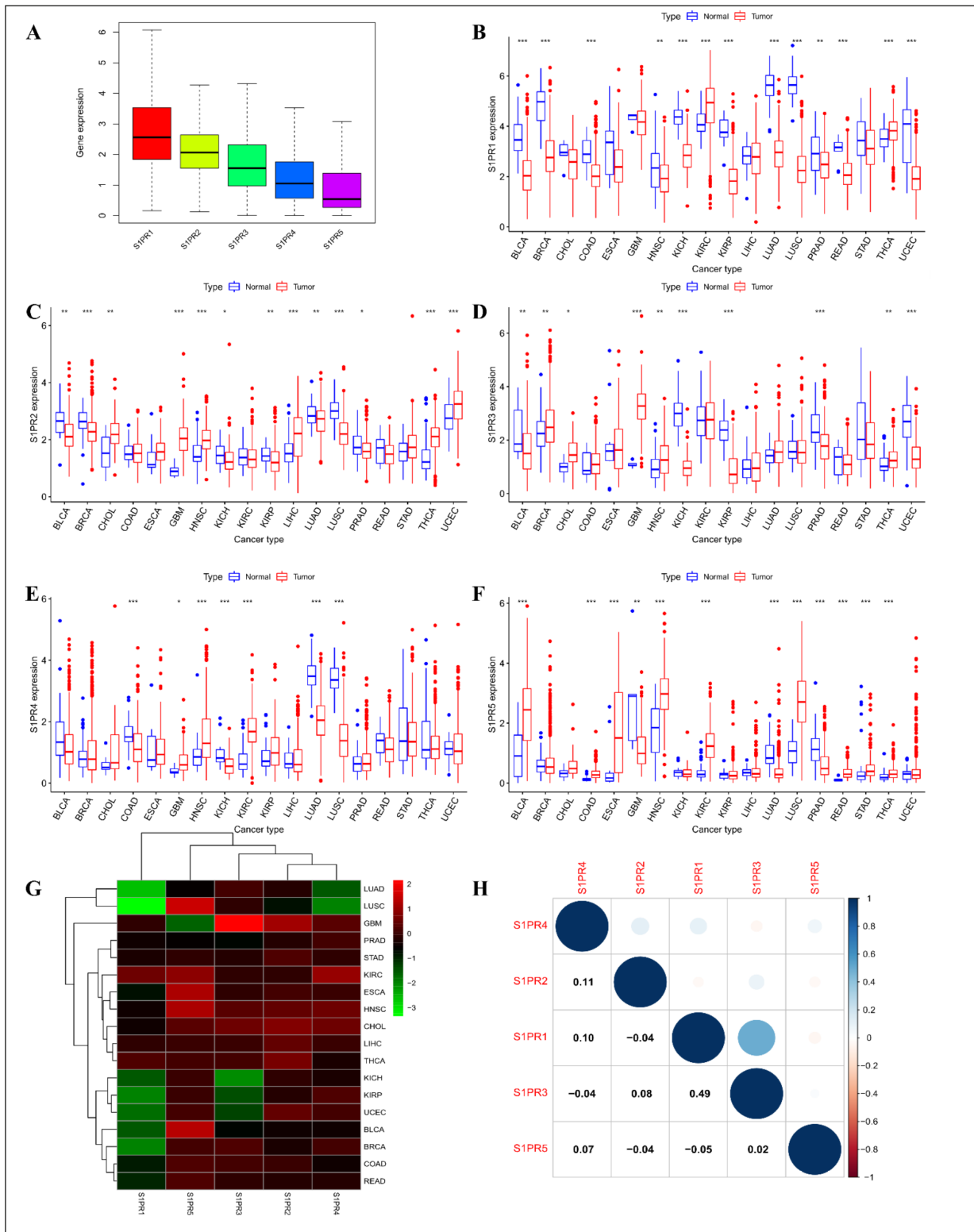To explore the potential relationships between the expression of each S1PR family member and

Figure 1. Expression of S1PR family members in tumor and adjacent healthy tissues. **A**, Expression of the five S1PR family members in 33 tumors. **B-F**, Boxplots of the expression of *S1PR1* (**B**), *S1PR2* (**C**), *S1PR3* (**D**), *S1PR4* (**E**), and *S1PR5* (**F**) in 18 tumor types and associated healthy samples. **G**, Heatmaps of *S1PR1-5* expression in the 18 tumor types that had over five associated healthy samples. **H**, Correlation of the expression levels of different S1PR family members in cancer. Blue indicates a positive correlation, and red indicates a negative correlation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. S1PR, Sphingosine-1-phosphate receptor.
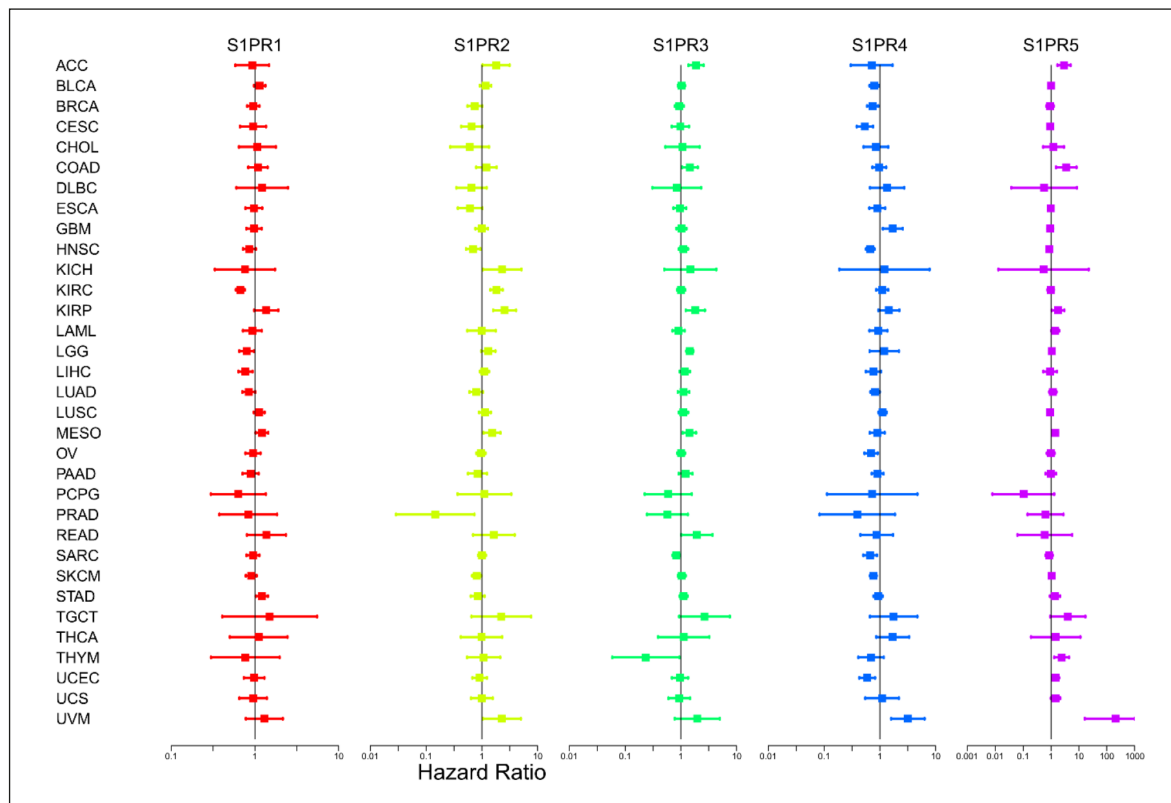
Figure 2. Relationship between *S1PR* gene expression and the overall survival of patients with different types of cancer. S1PR, Sphingosine-1-phosphate receptor.

the immune components involved in cancer, we performed correlation analysis. Six subtypes of immune-infiltrating cells have been defined in human tumors, each with the ability to promote or inhibit tumorigenesis: C1 (wound healing), C2 (IFN-γ dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 [transforming growth factor-β (TGF-β) dominant][25]. Interestingly, we observed that, except for *S1PR5*, genes of the S1PR family were highly expressed in the C6 subtype (Figure 3A); this indicates that most tumors have high TGF-β content. Furthermore, both *S1PR5* and *S1PR1* were significantly upregulated in the C5 subtype, suggesting a relationship between cancer and immune silencing.

We further used the ESTIMATE algorithm to calculate the correlation between the expression levels of S1PR family members and tumor-infiltrating mesenchymal and immune cells. We found that the immune and stromal cell scores varied for different members of the S1PR family, as well as in different cancer types. Surprisingly, across cancer types, *S1PR1* had the highest cor-

relation with the stromal cell score (Figure 3B), whereas *S1PR4* had the highest correlation with the immune cell score (Figure 3C). Notably, we also used the ESTIMATE algorithm to calculate the association between the expression of S1PR family members and tumor purity. Similar to the pattern observed for immune cell score, we found that *S1PR4* expression was highly positively correlated with the ESTIMATE score (Figure 3D); conversely, the tumor purity score was the most negatively correlated with *S1PR4* expression (Figure 3E).

S1PR Expression is Associated with the Characteristics of Cancer Stem Cells

During cancer development, tumor cells gradually lose their differentiated phenotype and acquire progenitor and stem cell-like characteristics. Herein, we used RNAss based on mRNA expression levels and DNAss based on DNA methylation patterns to evaluate the stem cell characteristics of tumors. The results revealed that S1PR expression was associated with RNAss and DNAss to varying degrees in different types
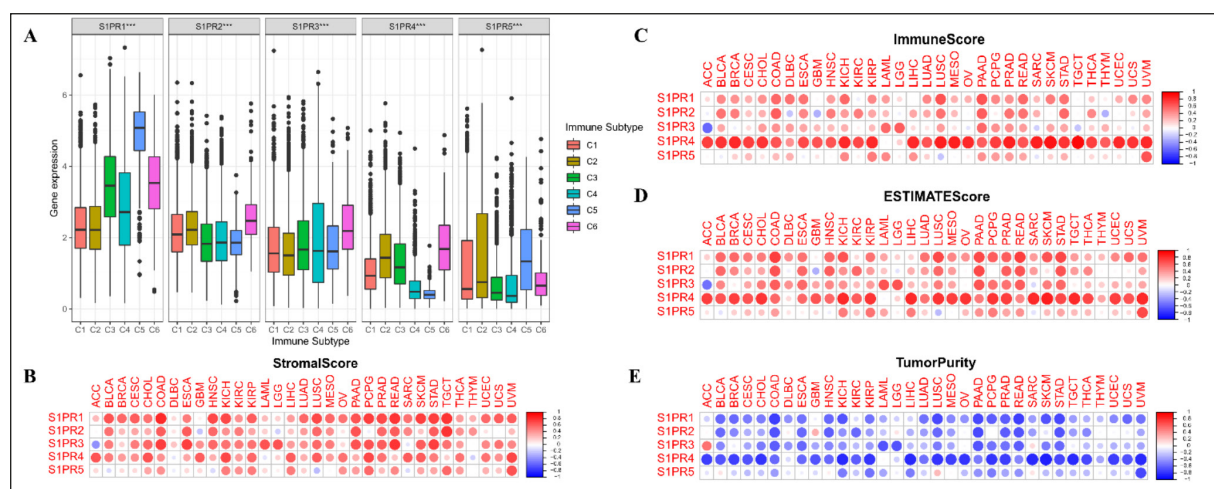
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Figure 3. Relationship between *S1PR* gene expression the tumor microenvironment. **A**, Correlation between the expression of *S1PR* genes and different immune cell subtypes. **B-E**, Correlation between the expression levels of S1PR family members and the stromal score (**B**), immune score (**C**), ESTIMATE score (**D**), and tumor purity (**E**). Red indicates a positive correlation and blue indicates a negative correlation. The size of the dots represents the absolute value of the correlation coefficient; larger size indicates a higher correlation coefficient. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. S1PR, Sphingosine-1-phosphate receptor.

of cancer (Figure 4A-B). Interestingly, the expression levels of most S1PRs were negatively correlated with RNAss; the same was not true for DNAss, which were only weakly correlated, if at all, with S1PR expression. Moreover, we observed that in kidney renal papillary cell carcinoma and thyroid carcinoma, all genes were positively correlated with DNAss and negatively correlated with RNAss. However, in SKCM, all genes were negatively correlated with both DNAss and RNAss. These conflicting results indicate that RNAss and DNAss are associated with cancer cell populations with different characteristics or degrees of stemness.

In addition, we studied the relationship between the expression of *S1PR* genes and sensitivity to chemotherapeutic drugs. The top 16 S1PR-drug combinations with the strongest correlations are presented in Figure 4C. We observed that increasing expression of *S1PR1* was correlated with decreased sensitivity of cells to chemotherapeutic drugs. In contrast, elevated expressions of *S1PR4* and *S1PR3* were associated with higher drug sensitivity.

The Role of S1PR Family Members in Skin Melanoma

Melanoma is the most aggressive skin cancer, and its incidence continues to rise. Several factors have been shown to predict the poor prognosis in these patients, including sex, age, degree of ulceration, mitosis rate, and Clark tumor grade. Sev-

eral previous studies[26-29] have explored the therapeutic effects of targeting S1PR family members in melanoma; however, these studies were mostly based on animal models or cell lines. Therefore, in this study, using human skin melanoma data (retrieved from TCGA database), we explored the relationship between melanoma and the expression of S1PR family members. Regarding the correlation between the expression levels of S1PR family members and different immune cell subtypes in this study, only five of the six immune subtypes were detected in melanoma and *S1PR4* was differentially expressed in distinct immune subtypes (Figure 5A). In particular, *S1PR4* was highly expressed in the C2, C3, and C6 subtypes, which are closely related to inflammation; this is in line with the reported lymphocyte infiltration in melanoma.

Due to the lack of data from healthy control samples from patients with melanoma, we could not assess differences in the expression levels of S1PR family members in melanoma *vs.* the adjacent tissues (Figure 1). However, by studying the relationship between the expressions of S1PR family members in SKCM specimens and different clinicopathological features (Figure 5B-J), we observed that the expression of *S1PR2* was much higher than that of the other family members. The expression of *S1PR4* was also increased to a lesser extent. Interestingly, the opposite trend was observed when considering all other cancer types together. These results suggest that high expres-
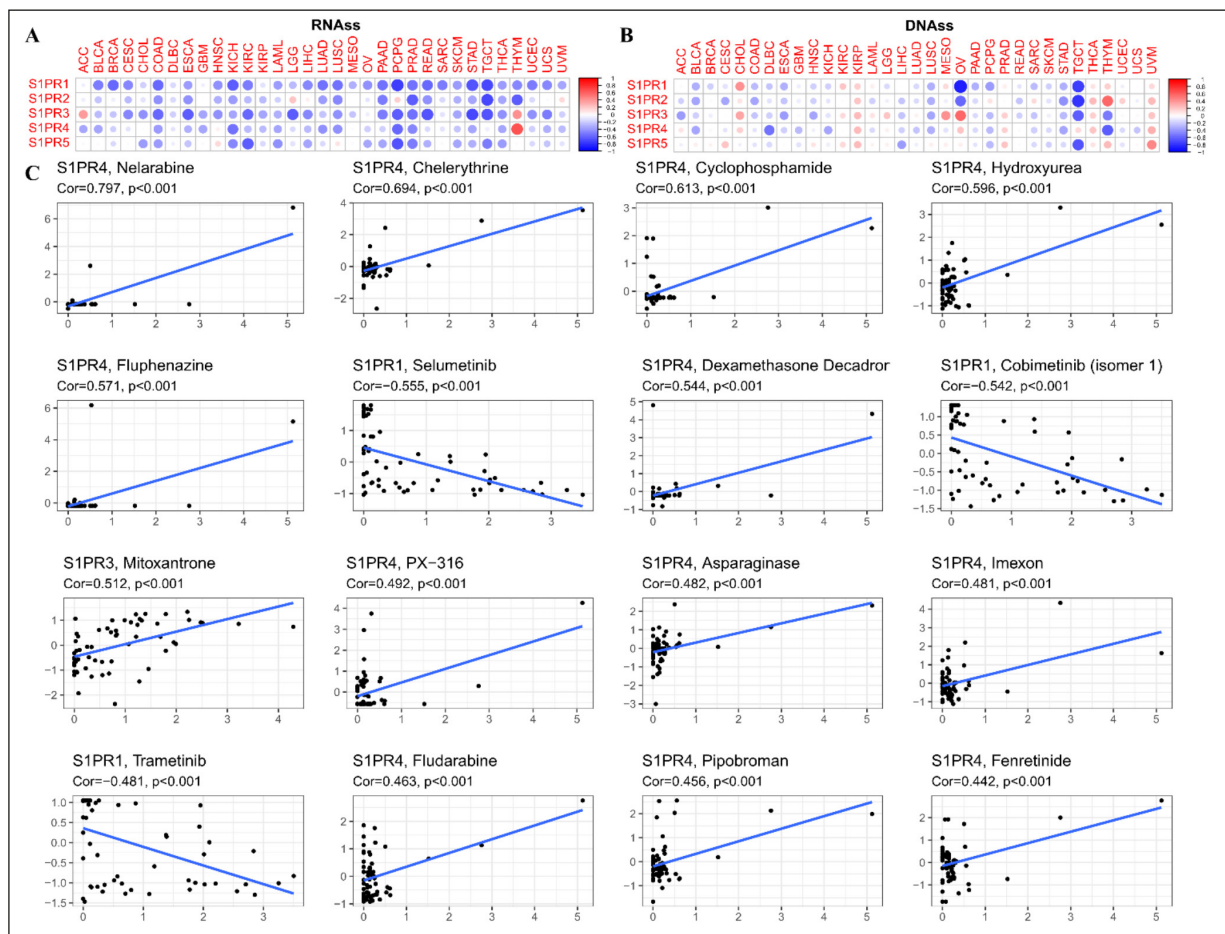
Figure 4. Relationship between *S1PR* gene expression and tumor stemness and drug sensitivity. **A-B**, The relationship between the expression of *S1PR* genes and RNAss (**A**) or DNAss (**B**). **C**, The relationship between the expression of *S1PR* genes and drug sensitivity. S1PR, Sphingosine-1-phosphate receptor.

sion of *S1PR2* and *S1PR4* may be especially relevant in the clinical study of SKCM.

In patients with melanoma, age (Figure 5B) and sex (Figure 5C) were not significantly correlated with the expression levels of S1PRs. Furthermore, different clinical stages were associated with different expression levels of *S1PR1*, *S1PR3*, and *S1PR4* (Figure 5D). In clinical stages III and above (involving lymph nodes or adjacent metastases), the expression of *S1PR4* was significantly reduced. In line with these results, the K-M curve demonstrated that the survival rate of patients with melanoma was significantly higher in patients with high expression of *S1PR4* ($p = 0.010$). Of note, similar trends were observed for the expression levels of *S1PR1* and *S1PR3*; this finding is consistent with the initial positive correlation observed between the expression of *S1PR1* and *S1PR3* (Figure 1H). We also studied the correlation between the expression of S1PRs and the classification of melanoma (Figure 5E) and ulcer formation (Figure 5F). Differences in the expression of *S1PR* genes with tumor thickness and depth of invasion suggest the potential involvement of *S1PR* genes in tumor invasion.

We also explored the correlation between the expression of *S1PR* genes and TME and stem cell index in patients with melanoma. We observed that the expression of S1PR family members was negatively correlated with two stem cell indices and positively correlated with the tumor cell matrix and immune infiltration indices (Figure 6A). Cox analysis revealed that increased expression of *S1PR1/2/4* was associated with a better survival rate in patients with melanoma, whereas increased expression of *S1PR3/5* was associated with poor survival (Figure 2); however, a significant correlation was only detected for *S1PR4* (Figure 6B).
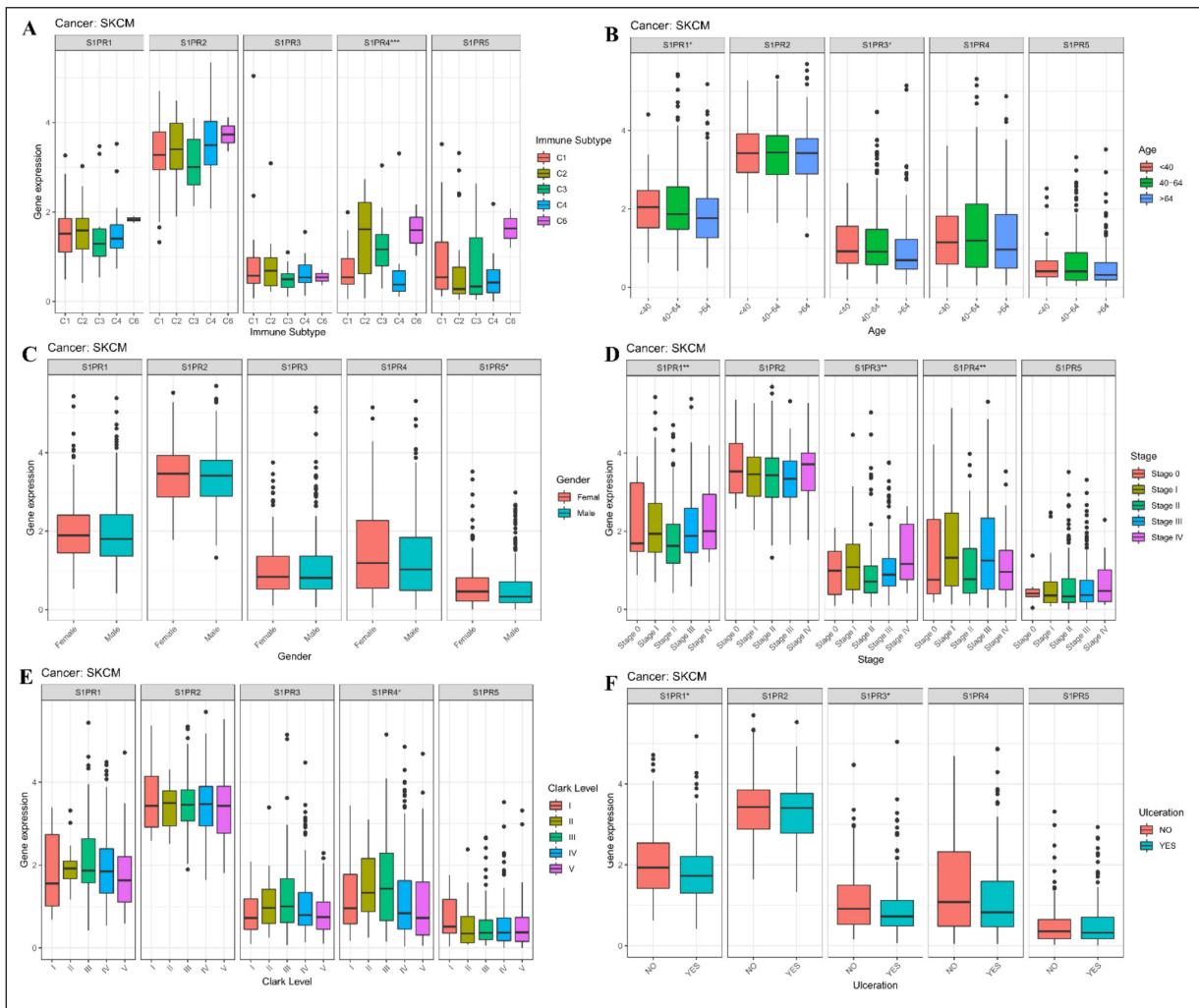
Figure 5. Correlation between *S1PR* gene expression and clinical features and immune infiltration in skin melanoma. **A**, Correlation between the expression of different *S1PR* genes and distinct immune-infiltrating cell subtypes in patients with melanoma. **B-F**, The expression levels of the S1PR family members as a function of age (**B**), sex (**C**), clinical stage (**D**), Clark stage (**E**), and ulcer formation (**F**). S1PR, Sphingosine-1-phosphate receptor; SKCM, skin melanoma.

This suggests that the upregulation of *S1PR3* and *S1PR5* affects the stem cell index, whereas other mechanisms affect the survival of patients.

Finally, we used the CIBERSORT algorithm to analyze immune cell infiltration in melanoma (Figure 6C) and the correlations between cell subsets (Figure 6D). The results revealed that the two immune cell types with the strongest positive correlation were neutrophils and activated mast cells (r = 0.70), whereas the two subtypes with the strongest negative correlation were CD8+ T cells and M0 macrophages (r = -0.62). We further focused on the potential role of *S1PR1* in immune cell infiltration in melanoma (Figure 7A-I). Twenty-two types of immune cells were studied in patients with melanoma with high *vs.* low ex-

pression levels of *S1PR1* (Figure 7J). Interestingly, five types of immune cells were related to the expression of *S1PR1* (Figure 7K). Together, these results indicate that the expression of *S1PR1* affects the TME in melanoma.

Discussion

S1P is a simple membrane-derived phospholipid generated by SKs. It is a widely studied tumor- and inflammation-related factor. Many previous studies[19] have confirmed that SK and S1P are involved in a variety of physiological processes, including the proliferation, migration, and invasion of malignant cells, as well as tumor

neovascularization, lymphatic vessel formation, and cancer patient survival. In fact, SK, S1PR, and S1P-degrading enzymes jointly regulate the graded signal transduction of S1P to control normal physiological functions and the progression of inflammation and cancer[30]. Notably, S1P activates signaling pathways mediated by different members of the S1PR family, and therefore, can mediate overlapping or distinct functions. For example, previous studies[15] have demonstrated the importance of *S1PR1* and *S1PR3* in the promotion of cancer development, whereas *S1PR2* was reported[7] to promote or inhibit cancer development depending on the type of cancer and microenvironment. We reported that the expression levels of different members of the S1PR family are heterogeneous; for example, the expression of *S1PR2* was upregulated in most tumors, whereas conversely, that of *S1PR1* was downregulated in most tumors. Previous studies[31] on S1P and its receptors have often focused on the increase in the content of S1P and the respective producing enzyme (SK1), while ignoring the effect of S1PR

expression on tumors. Interestingly, our Cox survival analysis revealed that the role of *S1PR1* as a proto-oncogene or tumor suppressor gene varies depending on the type of tumor; indeed, the role of *S1PR1* can vary even within the same tumor type depending on other pathological features, as observed in lung adenocarcinoma and lung squamous cell carcinoma. Overall, our findings not only confirmed the adverse effects of *S1PR1* on the prognoses of lung, gastric, and colorectal cancers, which is consistent with previous studies[32-34] for other diseases, but also suggested the importance of S1PR family members in other tumors.

TME is a key influencer of tumor occurrence, progression, and prognosis, and it is considered indispensable in the development of cancer immunotherapies[25]. TME is mainly composed of tumor cells, immune cells, fibroblasts, extracellular matrix, blood vessels, and lymphoid tissues. The interactions between immune cells in the TME vary according to the state of the immune system and are manifested by different infiltration patterns of immune cell subtypes[35]. For example,
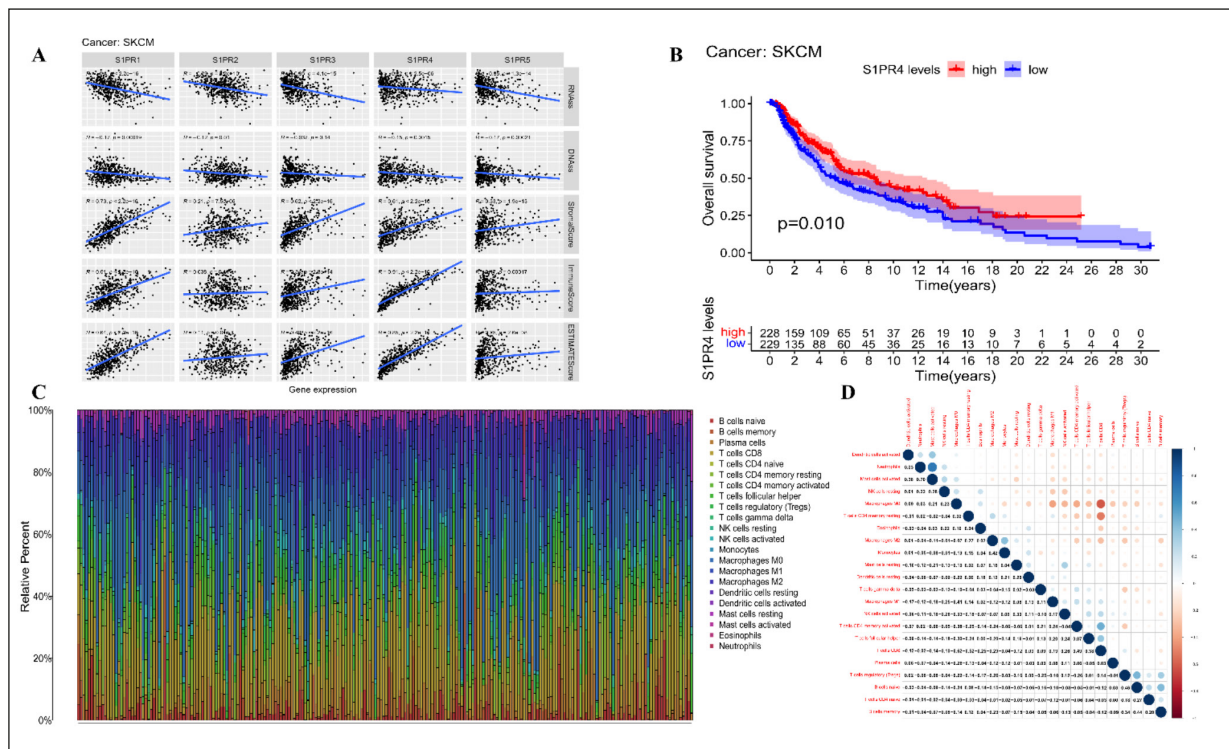

Figure 6. Significance of *S1PR* gene expression in patients with skin melanoma. **A,** The ESTIMATE algorithm was used to calculate the correlation matrix representing the relationships between the expression of *S1PR* genes and the RNAss, DNAss, matrix score, immune score, and ESTIMATE score. **B,** The relationship between the expression of the *S1PR* genes and overall survival of patients with melanoma. Distribution (**C**) and correlation analysis (**D**) of 22 immune cells in melanoma tumor samples. RNAss, RNA stemness score; DNAss, DNA stemness score; S1PR, Sphingosine-1-phosphate receptor; SKCM, skin melanoma.
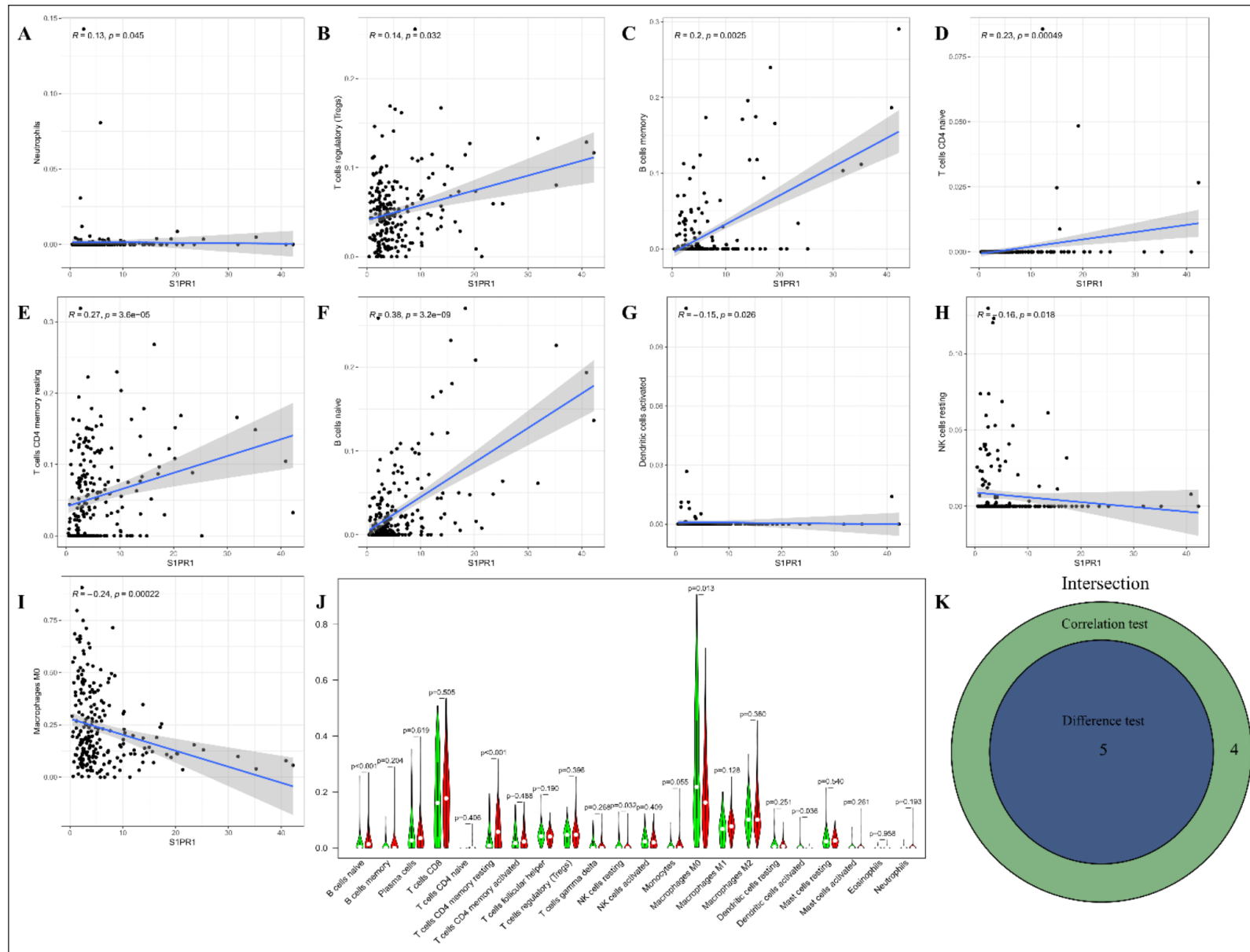
Figure 7. Relationship between *S1PR* gene expression and immune cells in melanoma. **A-I,** Correlation between the expression of *S1PR1* and the number of neutrophils (**A**), regulatory T cells (**B**), memory B cells (**C**), naïve CD4+ T cells (**D**), resting memory CD4+ T cells (**E**), naïve B cells (**F**), activated dendritic cells (**G**), resting NK cells (**H**), and M0 macrophages (**I**) in patients with melanoma. **J,** Difference in immune cell content in patients with high or low *S1PR1* expression. **K,** Venn diagram representing the intersection of immune cells related to *S1PR1* expression. S1PR, Sphingosine-1-phosphate receptor.

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the C2 and C3 subtypes, dominated by type I immune responses, are associated with better prognoses than the C4 and C6 subtypes. The presence of the C1 subtype often indicates higher levels of angiogenesis and hyperproliferation characteristics in tumors. Although the C5 subtype had the lowest expression in pan-carcinoma, its response to macrophages was the highest and was dominated by macrophage M2-type polarization. Of note, cytokines are key molecules in the TME; besides their own humoral regulatory factors, they function as important mediators of antitumor immune responses by controlling the activation of immune cells[36]. The abundance of individual cytokines, especially IFN-γ (C2)[37] or TGF-β (C6)[38], can often be a measure of antitumor immunity. In our study, each member of the S1PR family was distinctly associated with the indices for the TME, with the highest expression in the C6 subtype being the most prominent. In a recent study[35] on the global transcriptome immune classification of solid tumors, the C6 subtype was associated with the levels of TGF-β and lymphocyte infiltration; notably, no obvious differences in T cell distribution were observed in our study. In addition, we detected high expression of *S1PR1* and *S1PR5* in the C5 subtype. Based on these results, we speculate that an immunotherapeutic approach targeting *S1PR1* and *S1PR5* may have a beneficial anticancer effect; however, additional experimental validation is necessary. Furthermore, tumor cells and mesenchymal cells, important constituents of the TME, must be considered not only in metastases but also as providers of factors that promote tumor development and invasion[39]. Nonetheless, our findings provide evidence supporting the use of the S1PR family as an immunotherapy target for patients with cancer.

The results of our correlation analysis indicate that increasing expression of the S1PR family members is correlated with weakened stem cell characteristics of the tumor. Moreover, while an increase in the expression of *S1PR1* was associated with reduced sensitivity to chemotherapeutic drugs, elevated *S1PR4* expression was related to a possible increase in drug sensitivity. Previous work[14] has suggested that, unlike *S1PR1/3*, which promotes tumor growth and invasion, *S1PR2* exhibits tumor specificity. The findings of this study reveal the potential of anti-*S1PR4* agents as possible chemotherapy adjuvant agents.

During the past two decades, the functions of S1P and S1PRs have been extensively studied. Patients with solid tumors, such as breast, gastric, colorectal, liver, and pancreatic tumors, as well as with non-solid tumors, such as large B-cell lymphoma and leukemia, exhibit high S1P levels in the blood. The S1P content in the tumor stroma is also higher than that in healthy tissues adjacent to the cancer site[14]. Interestingly, Lee et al[40] found that increasing the expression of *S1PR1* in mouse bladder tumor MB49 cells promoted tumor cell proliferation and invasion. Chae et al[41] also demonstrated that *S1PR1* promotes tumor angiogenesis using a lung cancer xenograft model. In line with these studies, an *S1PR1* antagonist was demonstrated[42] to inhibit the migration of Hodgkin's lymphoma. Similar studies[43,44] were performed to determine the function of other S1PR family members. For example, the *S1PR3* pathway was demonstrated to promote the proliferation and lung metastasis of breast cancer cells *in vivo*[43]. However, inhibition of *S1PR5* expression promoted proliferation and metastasis of esophageal cancer cells[44]. Interestingly, unlike other members of the S1PR family, *S1PR2* was emphasized as tumor-specific[14]; however, S1PR2 was demonstrated to inhibit tumor cell proliferation in some tumors. For example, *S1PR2* knockout significantly increased tumor growth and angiogenesis in transplanted mouse melanoma cells[45], accelerated the progression of liver cancer in a mouse model[46], and promoted the migration and invasion of multiple myeloma cells[47]. However, some studies[48-50] have found that blocking the function of *S1PR2* inhibited the growth of ovarian cancer cells both *in vivo* and *in vitro*[48], restored the sensitivity of chronic myeloid leukemia cell lines resistant to tyrosine kinase inhibitors to chemotherapeutic drugs and inhibited their proliferation[49], and reversed the 5-fluorouracil resistance of a variety of tumor cells[50]. These contradictory findings were also reflected in our study with respect to melanoma. Although the expression of *S1PR2* was significantly elevated in patients with melanoma, Cox analysis showed that such an upregulation to be beneficial to the survival of patients with SKCM. Thus, future research on the function of *S1PR2* must consider these tumor-specific features.

Recently, research on immunotherapy has intensified. The principle of tumor immunotherapy is to activate the human immune system to achieve a sustained elimination of cancer cells. Cutaneous melanoma accounts for only 1% of all skin cancers; however, an extremely high mortality rate has been reported, making melanoma the most lethal malignant skin tumor[51]. Immunotherapy has clear effects on melanoma; however,

some patients do not benefit from this treatment at present. Therefore, it is important to identify new and effective targets for the diagnosis and treatment of malignant melanoma. In our study, age and sex were nonspecific factors associated with the expression of S1PR family members in patients with melanoma. Conversely, in tumors without the expansion of epidermis, the expression of *S1PR4* increased, whereas in those associated with invasion, a significant downregulation of *S1PR4* was observed. Therefore, we hypothesized that activating *S1PR4* may be important in inhibiting the growth and spread of melanoma and may improve patient survival.

Notably, we also found that higher clinical stages of melanoma were associated with an increase in the expression of *S1PR1*, contradicting the conclusion that *S1PR1* can prolong the survival time of patients with melanoma, which was suggested by the Cox regression analysis. Previous studies[52] have also reported that *S1PR1* is associated with poor prognosis in patients with melanoma. Altogether, these findings suggest that *S1PR1* may be involved in a complicated mechanism that impacts the survival rate of patients with cancer. Upon analyzing the effect of *S1PR1* expression on immune cell infiltration in melanoma, the following five subgroups of immune cells were found to be particularly associated with *S1PR1* expression: naïve B cells, CD4$^+$ resting memory T cells, resting natural killer (NK) cells, M0 macrophages, and dendritic cells. Immune cells, such as T cells and macrophages, are important components of the TME. Studies[53] in a melanoma model have confirmed that tumor reactive CD4$^+$ T cells can activate T cell proliferation and cytotoxic activity in large amounts and induce cancer regression. M0 macrophages have also been associated with poor prognosis in patients with melanoma. Whereas CD8$^+$ T cells were reported[54] to be anergic in melanoma tissues, this loss of function is reversible. Therefore, tumor specific CD8$^+$ T cells can be reprogrammed by immunotherapy to restore cytotoxicity in melanoma.

In conclusion, the expression of S1PR family members in cancer and their potential association with the TME, clinical prognosis, stem cells, and immune characteristics were comprehensively investigated.

Limitations

This study is not without limitations. For instance, the cancer data retrieved from TCGA database were almost entirely derived from Cau-

casian individuals; therefore, our results may be biased. For example, although the incidence of melanoma is higher in Caucasians, people of color are more prone to mucosal melanoma, and this type of cancer was not included in the database. Additionally, although many tumor types were included in this study, not all tumor data were complete or included sufficient paired healthy control samples, and some tumor types were excluded from the analysis. Finally, validation experiments were not conducted to verify the impact of S1PR family members on the clinical features, prognosis, and immune infiltration associated with melanoma. In future studies, with the establishment of cell or animal models based on the deletion or overexpression of S1PR family members and the development of S1PR-specific agonists and antagonists, we expect to clarify in-depth the role of S1PR family members in cancer and contribute to the development of novel therapies.

Conclusions

Using TCGA pan-cancer dataset, we explored and discussed the expression, molecular features, and impact on immunity and the tumor microenvironment of the S1PR gene family in cancer. Our results suggest that different S1PRs play different roles in different cancers, or in different pathological stages of the same cancer. Our findings help elucidate the roles of this gene family in tumorigenesis and progression, especially with regards to the immune response, tumor microenvironment, and drug resistance.

Authors' Contributions
Zi Wang drafted and revised the manuscript. Haomin Zhang, Yuanrui Guo, and Lingling Li contributed equally to the study, including patient recruitment, data collection, and data analysis. All authors reviewed and approved the final version of the manuscript.

ORCID ID

Zi Wang: 0000-0003-3007-7642.
Haomin Zhang: 0000-0001-9994-1262.
Yuanrui Guo: 0000-0002-4193-1325.
Guangzhong Zhang: 0000-0003-2645-672X.
Lingling Li: 0000-0003-4156-7065.

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