MEDICAL SCIENCE MONITOR

DATABASE ANALYSIS

e-ISSN 1643-3750
© Med Sci Monit, 2021; 27: e932052
DOI: 10.12659/MSM.932052

Received: 2021.03.09
Accepted: 2021.06.08
Available online: 2021.07.02
Published: 2021.07.11

Identification of *CXCL13* as an Immune-Related Biomarker Associated with Tumorigenesis and Prognosis in Cutaneous Melanoma Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

DEF Zebing Si
AG Konghe Hu

Department of Orthopedics, The Affiliated Yuebei People's Hospital of Shantou University Medical College, Shaoguan, Guangdong, PR China

Corresponding Author: Konghe Hu, e-mail: hkhgksq@126.com
Source of support: The present study was supported in part by a grant from the Guangdong Provincial Science and Technology Project (2017A020215080)

Background: Melanoma is one of the most lethal tumors and its treatment is still challenging. It is urgent to detect novel therapy targets in melanoma.

Material/Methods: The GEO dataset was used to obtain a list of DEGS (differentially-expressed genes). Integrative bioinformatics analyses, including HPRD database, TCGA data, and TIMER, were performed to determine the role of *CXCL13* in SKCM (skin cutaneous melanoma) progression and the immune environment. Furthermore, Pearson correlation coefficient analysis was used to measure correlations between *CXCL13* and its co-expressed genes. Survival analysis, GO, and KEGG enrichment analysis were performed to investigate the role of *CXCL13* in SKCM.

Results: A total of 41 DEGs were identified in 3 GEO datasets, and 4 out of 41 DEGs are hub genes. Among the 4 hub genes, *CXCL13* is involved in the most KEGG terms. *CXCL13* is co-expressed with well-known immune checkpoint blockade targets, and it was associated with better overall survival. In addition, *CXCL13* levels in infiltrating immune cells (neutrophil and myeloid dendritic cells) affect prognosis and survival in SKCM. Functional enrichment analysis clarified that *CXCL13*-co-expressed top 30 genes were associated with immune signaling pathways. Network analysis identified *CXCL13* as a hub gene that interacts with CXCR5 to participate in immune-related biological process.

Conclusions: This study found that CXCL13 is associated with SKCM tumorigenesis and prognosis and immune infiltrations. Our result suggests that CXCL13 has great potential in development of novel immunotherapy targets in melanoma.

Keywords: CXCL13 Protein, Human • Gene Expression • Melanoma

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/932052

3309 2 6 20

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Melanoma is a malignant tumor that causes approximately three-quarters of skin cancer-related deaths [1]. Melanoma treatment has been revolutionized with the approval of BRAFV600E inhibitors, which can significantly improve response and overall survival for patients with the BRAFV600E mutation [2]. However, patients develop acquired resistance to BRAFV600E inhibitors treatment [3]. Subsequent development of targeted immune checkpoints therapy has prolonged the survival of patients, such as with targeted CTLA-4 (ipilimumab, approved in 2011), and PD-1 (nivolumab, pembrolizumab, approved in 2014) has extended recurrence-free survival in high-risk resected melanoma patients [4,5]. However, melanoma recurrence was approximately 25-30% within 1 year in patients who were targeted by PD1, and PD1 monotherapy was not effective in those patients [5]. To overcome the drug resistance, it is urgent to explore novel therapeutic targets and treatment strategies for melanoma.

In our study, based on 3 datasets from the GEO database, we found that 41 differentially-expressed genes (DEGs) were over-expressed in melanoma compared to normal samples. Through network analysis, 4 (CXCL13, MMP1, SPP1, GZMB) out of the 41 DEGs had a high degree. KEGG enrichment analysis for the 41 DEGs indicated that CXCL1 is involved in 3 pathways. Moreover, Kaplan-Meier survival analysis showed that high *CXCL13* expression is associated with longer survival time for tumor patients. Correction analysis further demonstrated that *CXCL13* is co-expressed with 3 well-known immune checkpoint blockade targets (*PD1(PDCD1), PDL1(CD274),* and *CTLA4*) in SKCM. Functional enrichment analysis showed that *CXCL13* and its highly co-expressed genes are involved in some important biological processes, such as immune pathways. Immune analysis showed that *CXCL13* expression levels in infiltration of immune cells (CD4 T cell, neutrophil, macrophage, and myeloid dendritic cell) were associated with SKCM survival. These immune infiltration cells contribute to the tumor microenvironment, which can directly or indirectly modulate tumor immunity and have anti-tumor effects [6]. Thus, our results suggest *CXCL13* as a new immune target and strategy for SKCM diagnosis and treatment.

Material and Methods

Identification of DEGs

We accessed GEO database to obtain the expression data. GSE15605 (58 melanoma samples and 16 normal samples), GSE46517 (104 melanoma samples and 12 normal samples), and GSE114445 (16 melanoma samples and 18 normal samples) were adopted to perform the differentially expression. The DEGs between melanoma and normal samples were collected using GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r) online service. DEGS were defined as the threshold of |logFC| >1.5 and p value ≤0.01. Next, Venn website (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was applied to detect the overlap DEGs among these 3 datasets.

Network Construction and Functional Gene Sets

The network is an undirected graph G (V, E) where V stands for the genes and edges (i, j) ∈ E are weighted by PCC. Protein interaction networks were sourced from HPRD (http://www.hprd.org) database [7], the version is release 9. Protein self-interactions were removed, resulting in 39240 interactions among 9616 proteins. Cytoscape can create network interaction maps based on input genes and gene interaction [8]. The HPRD was used to extract the protein interaction of DEGs. 41DEGs were mapped to the HPRD, and the interaction was plotted by Cytoscape software (version 3.8.2, https://cytoscape.org/). The degree of each DEG was defined as the number of each DEG connected directly to its neighbor in HPRD. Only the nodes with degree greater than 5 were regarded as high-degree nodes.

Survival Analysis

The gene expression RNAseq and the survival time of TCGA-SKCM were downloaded from UCSC Xena (https://xenabrowser.net/datapages/). Gene expression data were log2(x+1) transformed, and include 20 531 identifiers and 474 samples (473 melanoma samples and 1 normal samples). The survival data include 479 samples. There were 457 common samples overlapped with 473 melanoma samples and 479 samples. The samples with gene expression greater than the 50% quantile of the gene expression were regarded as high expression, and otherwise were regarded as low expression. For CXCL13 gene survival analysis, we first classified samples into CXCL13 high-expression and CXCL13 low-expression groups. Then, we used the overall survival and event information from survival data to evaluate the survival difference between these 2 groups. Overall survival (OS) was defined as the time from the date of SKCM diagnosis to the date of patient death (from any cause) or last contact (whichever occurred first). Survival and survminer packages were utilized to perform survival analysis in R (version 3.6, https://www.r-project.org/).

Survival curves were estimated using the Kaplan-Meier method and compared across groups with the log-rank test. The t test was used to evaluate the statical significance. A p value <0.05 was statistically significant.

Co-Expression Analysis

Gene and gene co-expression were analyzed statistically using Pearson's correlation coefficient (PCC). The sample size of the

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

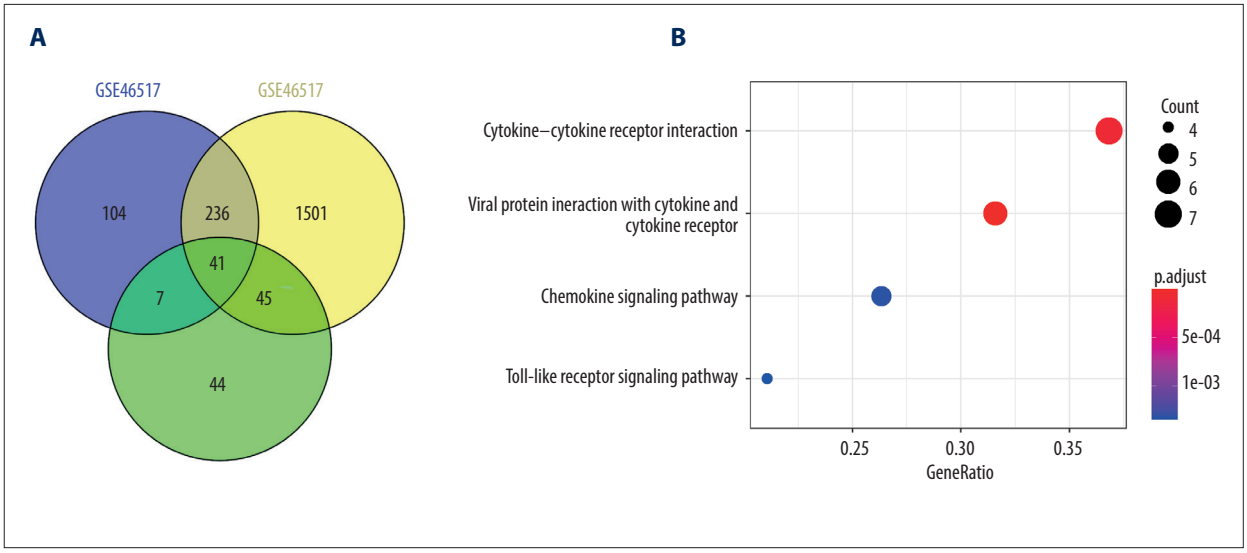

Figure 1. Venn diagram and the most significant KEGG pathways of 41DEGs. (**A**) The Venn diagram displays the number of DEGs in 3 datasets from the GEO database. (**B**) Four significant KEGG pathways of 41 DEGs. Adjusted p value <0.01. Figure 1A was produced using the Venny website (https://bioinfogp.cnb.csic.es/tools/venny/index.html, version 2.1). Figure 1B was produced using clusterProfiler packages in R (version 3.6, https://www.r-project.org/).

gene expression was 473 melanoma samples. The scatter plots of 3 known immune genes (PD1, PDL1 and CTLA4) correlated with CXCL13 were plotted using ggplot2, ggpubr, and ggpmisc packages in R (version 3.6). The heat map of the top 30 highly co-expressed genes with CXCL13 was plotted using the pheatmap package in R (version 3.6, https://www.r-project.org/).

Enrichment Analysis

We then performed GO and KEGG enrichment analyses of the top 30 high-correction-associated genes using the "clusterProfiler" package in R (version 3.6, https://www.r-project.org/).

TIMER

TIMER (http://timer.cistrome.org) is a comprehensive resource for systematic analysis (immune association, cancer exploration, and immune estimation) of immune infiltrates across diverse cancer types [9]. The association between immune infiltrates (CD4 T cells, neutrophils, macrophages, and myeloid dendritic cells) and clinical outcome for *CXCL13* was explored using the TIMER online service.

Results

Identification of Differential Genes

To find the potential and reliable target genes, we first extracted genes which were differentially expressed in melanoma and normal samples. Through differential expression analysis, each of these datasets (GSE15065, GSE46517, and GSE114445) produced the corresponding list of DEGs. The 41 overlapped genes (**Supplementary Table 1**), as shown in the Venn diagram, are common DEGs in the 3 datasets (**Figure 1A**)

KEGG Enrichment Analysis of the DEGs

KEGG enrichment of 41 DEGs was performed using the clusterProfiler package in R software, then displayed in bubble charts ($P<0.05$, **Figure 1B**). The size of the bubble represents the number of genes in the KEGG terms, and the color of bubbles represents the adjusted *P* value in the KEGG terms. KEGG enrichment analysis indicated that changes in biologic pathways were significantly enriched in cytokine-cytokine receptor interaction, chemokine signaling pathway, and Toll-like receptor signaling pathway. These results revealed that immune response and inflammatory response play important roles in melanoma tumorigenesis.

PPI Network Construction and Hub Genes Identification

We mapped 41 DEGs to the HPRD database and extracted their directly connected proteins, including 113 nodes (gene) and 92 edges (gene and gene interaction). Then, we input the information of 92 edges into Cytoscape. In **Figure 2A**, we display the high-degree genes-associated network. **Figure 2A** shows 4 (MMP1, SPP1, CXCL13, and GZMB) hub genes (the node with yellow color) with a degree greater than 5. Combined with the KEGG enrichment information of 41 DEGs, and among the 4 hub genes, CXC13 was found to be involved in 3 KEGG pathway terms (**Supplementary Table 2**).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

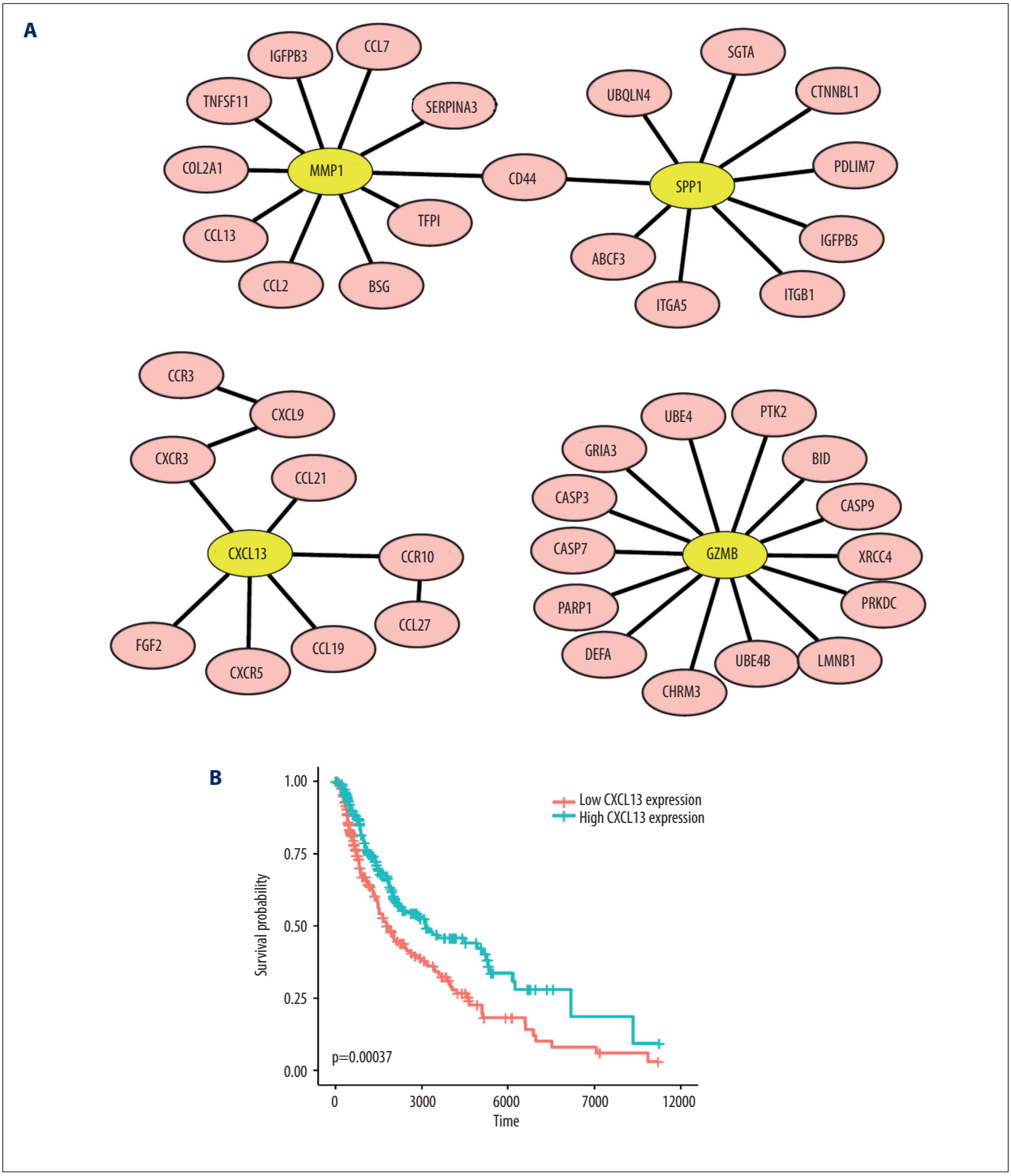

Figure 2. The network and survival plot. (**A**) The PPI (Protein-Protein Interaction) network of 4 high-degree DEGs (differentially-expressed genes). (**B**) Kaplan-Meier survival curves show that the expression of CXCL13 is consistently associated with better overall survival (OS) in SKCM. Figure 2A was produced using Cytoscape (version 3.8.2, https://cytoscape.org/). Figure 2B was produced using survival and survminer packages in R (version 3.6, https://www.r-project.org/).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Figure 2A shows that CXCL13 is connected with CXCR5. To investigate whether CXCL13 is co-expressed with CXCR5, we calculated the Pearson correlation coefficient. There was a positive correlation between CXCL13 expression and CXCR5 expression (PCC=0.74). High CXCL13 and CXCR5 expression were significantly related to longer survival time in SKCM patients (P=0.001). *CXCL13* can interact with its ligand *CXCR5* and play vital roles in apoptosis, proliferation, and differentiation of immune cells. It has been revealed that *CXCL13* makes pivotal contributions to the biological process of multiple cancers via *CXCL13/CXCR5* signaling [10]. For SKCM, in a previous study, Huang et al found that *CXCL13* acts as an immune-related biomarker [11], which is in agreement with our results. The prognostic value and biological function of *CXCL13* in SKCM are unclear and need to be better characterized. Here, we screened out available datasets associated with SKCM from public databases to systematically investigate the mechanism of CXCL13 on the progression, prognosis, and microenvironment of SKCM.

CXCL13 Level in Patients with SKCM

Early detection and treatment of SKCM is still a challenge that perplexes clinicians. Therefore, new therapy targets are needed to improve treatment efficacy. We first detected and compared the mRNA level of *CXCL13* between SKCM samples and normal samples. Expression information was based on TCGA database, showing that the mRNA expression of CXCL13 was significantly lower in SKCM (the mean of CXCL13=-1.99) than in normal samples (the mean of CXCL13=6.78). We suggest that low CXCL13 expression occurs in SKCM and deserves further clinical validation as a potential therapy marker.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

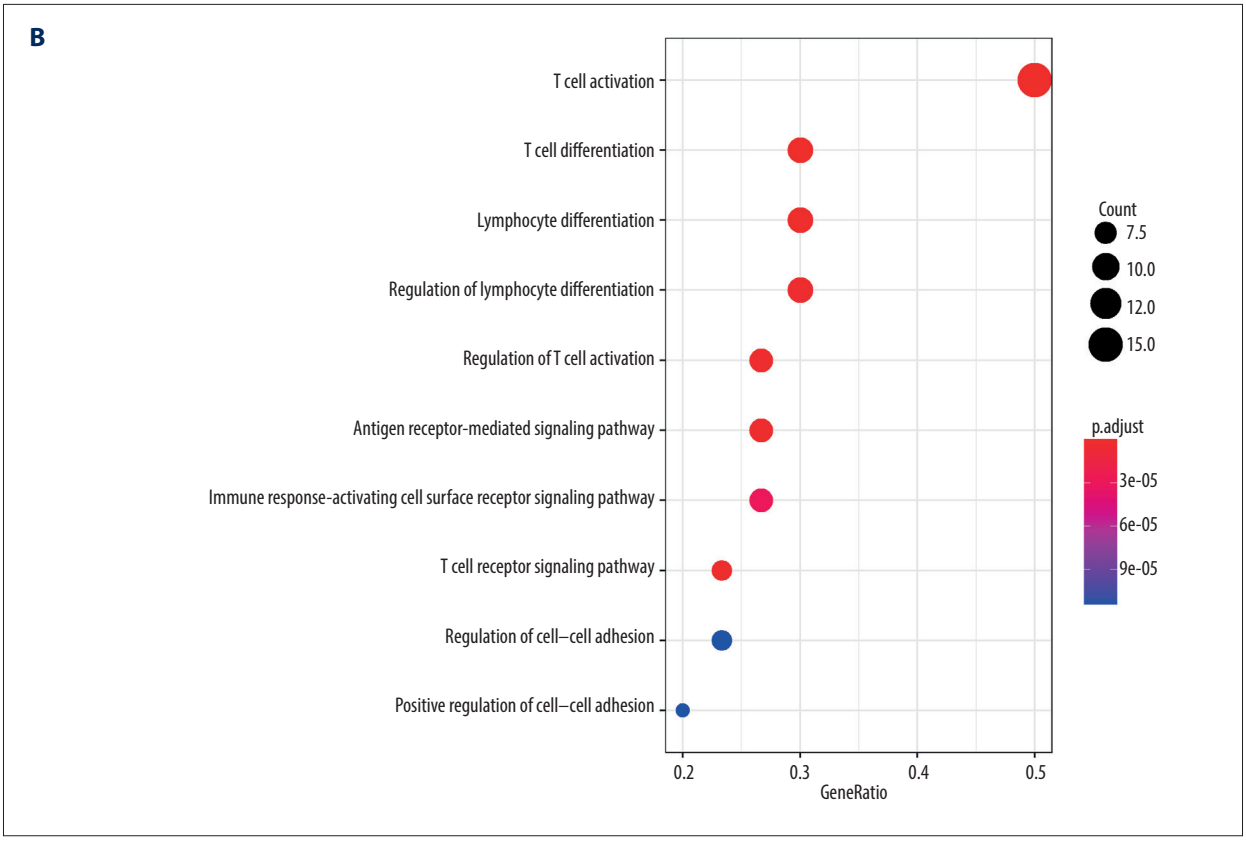

Figure 3. *CXCL13* associated top 30 co-expressed genes and functional enrichment analysis in SKCM. (**A**) PCC was used to calculate correlations between *CXCL13* and expressed genes in SKCM. Heat map showing top 30 genes positively correlated with *CXCL13* in SKCM. (**B**) The significantly enriched GO annotations of the top 30 high *CXCL13* co-expression genes in SKCM were analyzed. Figure 3A was produced using pheatmap packages in R (version 3.6, https://www.r-project.org/) and Figure 3B was produced using clusterProfiler packages in R (version 3.6, https://www.r-project.org/).

The Relationship Between CXCL13 Expression and Clinical Outcomes of SKCM Patients

We also evaluated the prognostic value of *CXCL13* in SKCM from TCGA. Kaplan-Meier survival curves of OS were produced utilizing *CXCL13* expression data and survival information of SKCM. The sample with CXCL13 expression greater than the 50th percentile of CXCL13 expression was treated as high CXCL13 expression, and otherwise was treated as low CXCL13 expression. The survival analysis showed that low expression of *CXCL13* (P =0.00037) predicted poor OS (**Figure 2B**). In the above analysis, CXCL13 had lower expression in SKCM than in normal samples. This supports that CXCL13 low expression is related to poor OS. Therefore, it is conceivable that high *CXCL13* expression is an independent risk factor and leads to a better prognosis in melanoma patients.

CXCL13 Co-Expression Genes Are Involved in Immune Pathways

We then focused on the function of the top 30 highly co-expressed genes with CXCL*13* using GO enrichment analysis, showing that co-expression genes were correlated with *CXCL13* in SKCM. PCC was utilized to analyze *CXCL13* co-expressed genes from TCGA data. As shown in **Figure 3A**, the heat map displays the genes that showed significant positive correlations with *CXCL13*. The X axis represents SKCM samples, and the Y axis represents the rank of genes which are positive correlations with *CXCL13*. To explore the function of the top 30 highly co-expression genes, GO enrichment analyses were performed. LCK is a kinase that is among the top 30 highly co-expressed genes. Kinases play a crucial role in cancer formation and progress by modulating cancer cell migration, progression, and apoptosis [12]. The results of our study indicated that LCK is the vital regulator of CXCL13 in SKCM.

Figure 3B displays the top 10 most statistically significant GO terms. Biological processes (BP) were significantly enriched

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

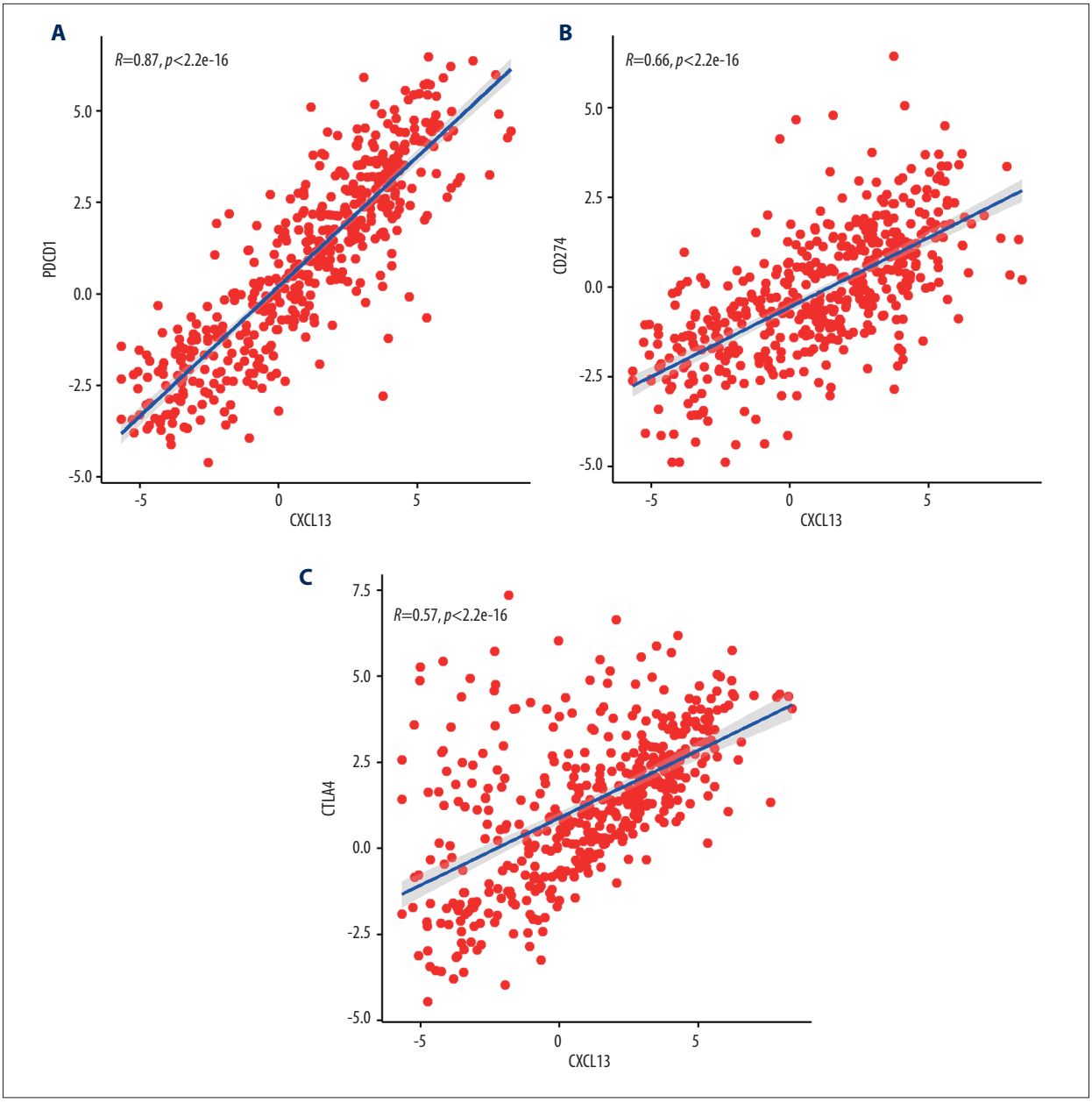

Figure 4. Gene expression correlation analysis. The scatter plot shows Pearson correlation of CXCL13 expression with expression of PDCD1 (**A**), CD274 (**B**), and CTLA4 (**C**). These figures were produced using ggplot2, ggpubr, and ggpmisc packages in R (version 3.6, https://www.r-project.org/).

in T cell activation, lymphocyte differentiation, regulation of lymphocyte activation, regulation of T cell action, and regulation of cell-cell adhesion, which suggests *CXCL13* has an effect on the transcriptome. Therefore, we found that the biological functions of these co-expressed genes are primarily related to immune-related pathways. Previous studies have demonstrated that the immune microenvironment plays key roles in immune evasion and metastasis of various cancers [13]. *CXCL13* interacts with other genes to regulate the tumor immune environment, and *CXCL13* co-expression genes make a crucial contribution to immune-related pathways. In summary, *CXCL13* makes an important contribution to immune biological process.

CXCL13 is Co-Expressed with 3 Well-Known Immune Checkpoint Blockade Targets

To assess the potential of *CXCL13* as an immune target, the correction of *CXCL13* with 3 well-known checkpoint genes was measured. The most well-known checkpoint blockade

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

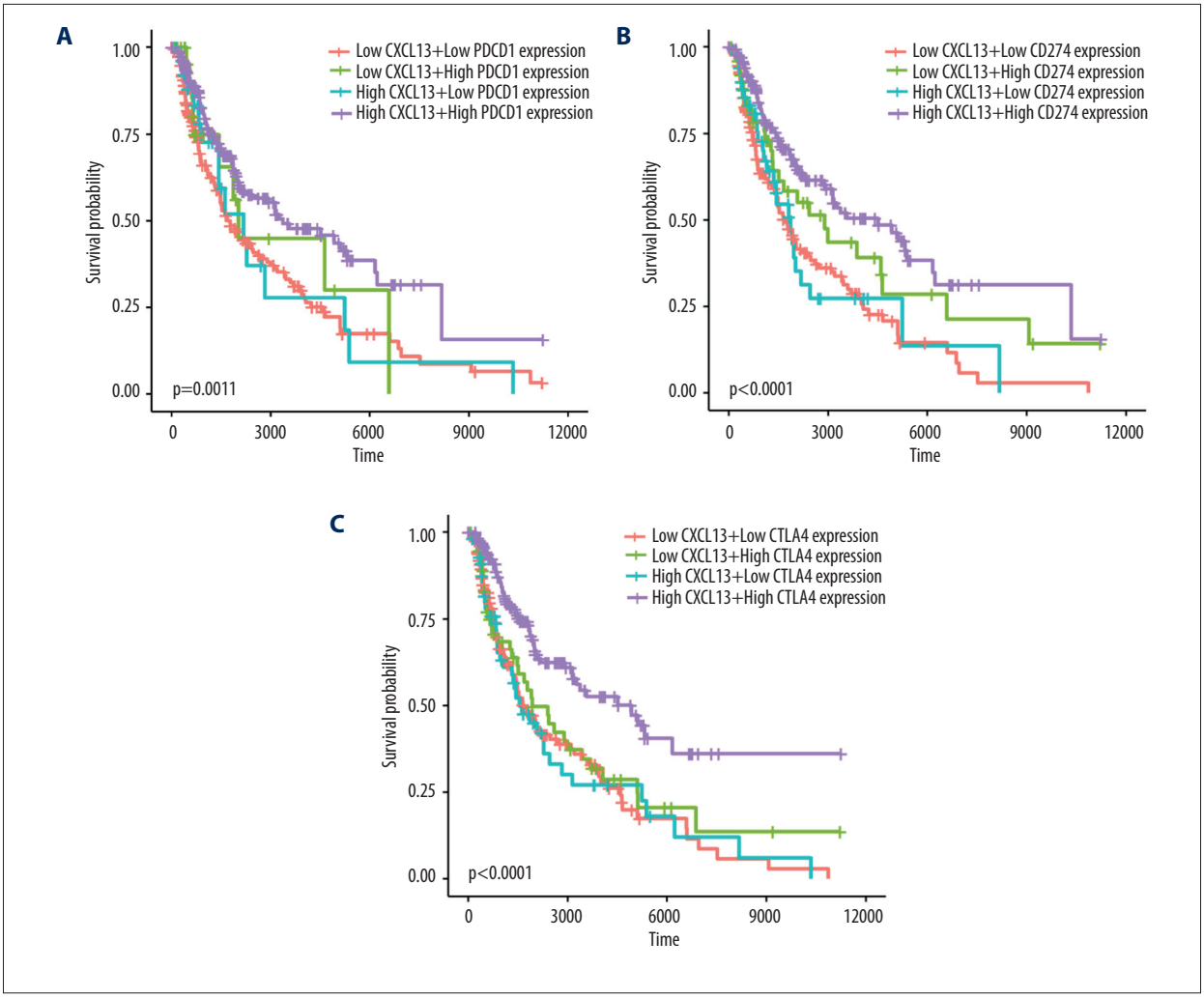

Figure 5. Kaplan-Meier survival curves show that CXCL13 expression and PDCD1/CD274/CTLA4 expression are significantly associated with OS in TCGA-SKCM cohorts (**A**) *PDCD1*, (**B**) *CD274*, (**C**) *CTLA4*. These figures were produced using survival and survminer packages in R (version 3.6, https://www.r-project.org/).

used as cancer immunotherapies are anti-CTLA4 and -PD-1 and -PD-L1 antibodies.

To explore the biological effects of *CXCL13* in SKCM, we examined *CXCL13* co-expression in SKCM. *CXCL13* is co-expressed with *CTLA4*, *PD-1* (*PDCD1*), and *PD-L1* (*CD274*) in SKCM and the relationship is statically significant (**Figure 4**). Immune checkpoint therapies, including *PD-1* or *PD-L1,* and *CTLA4*, have extended patient survival times in multiple cancer types. In **Figure 4A**, X represents the CXCL13 expression data across 473 SKCM samples and the Y represents the PDCD1 expression data across 473 SKCM samples. We found that *CXCL13* is co-expressed with these immune checkpoint blockade genes. *CXCL13* expression showed a strong positive association with expression of PDCD*1* (PCC=0.87, *P*<0.01), *PDL1* (PCC=0.66, *P*<0.01), and *CTLA4* (PCC=0.57, *P*<0.01). This suggests *CXCL13* interacts with immune checkpoint blockade genes and plays a vital in immune function. The positive correlation between the expression of these proteins and CXCL13 expression indicates that CXCL13 could be a predictor for active response to anti-PD-1/PD-L1 immunotherapy in SKCM since PD-L1 expression is a biomarker for predicting the anti-PD-1/PD-L1 immunotherapy response in cancer. The cooperative anticancer effects of anti-CD137 with anti-CTLA4 or/and -PD-1 antibodies in several cancer types exceed that of a single antibody, which shows the advantage of antibody combinations in the immune checkpoint therapies in cancer treatment. PD-L1 expression can act as a indictor for the anti-PD-1/PD-L1 immunotherapy response in cancer [14]. The positive correlation between the expression of PD-1/PD-L1/CTLA4 and *CXCL13* expression indicates that *CXCL13* upregulation could be a biomarker for the response to anti-PD-1/PD-L1/*CTLA4* immunotherapy in SKCM.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

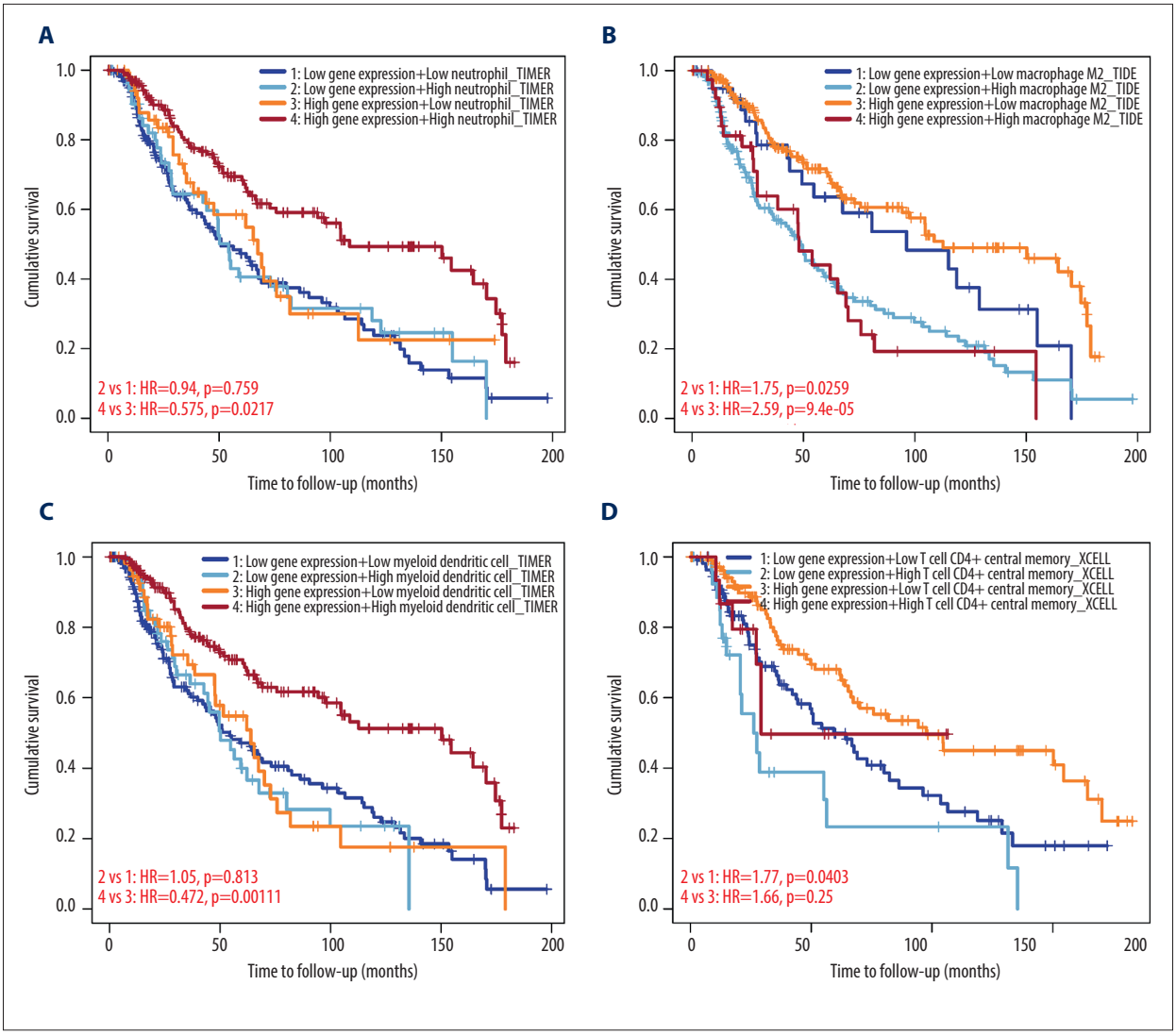

Figure 6. Correlation of *CXCL13* expression with immune infiltration level in the TIMER database. (**A**) Neutrophil cells, (**B**) macrophages, (**C**) myeloid dendritic cells and (**D**) CD4+ T cells. These figures were produced using the TIMMER website (http://timer.cistrome.org, version 2.0).

To investigate whether the CXCL13 and the immune checkpoint gene have an effect on survival time, we performed survival analysis. We used the 50th quantile as a threshold to create high-expression and low-expression groups. **Figure 5A** shows the correlation between *CXCL13* expression in SKCM expression data, *CTLA4* expression in SKCM expression data, and survival time. Patients with high *CXCL13* gene expression and high PD-1 gene expression had a longer survival time in SKCM (p=0.0011). In **Figure 5B**, patients with high *CXCL13* gene expression and high PD-L1 gene expression had a longer survival time in SKCM (*P*<0.0001). In **Figure 5C**, patients with high *CXCL13* gene expression and high CTLA4 gene expression had a longer survival time in SKCM (*P*<0.0001). This suggests that the efficacy of the combination of CXCL13 and PD-1/PD-L1/CTLA4 antibodies to treat SKCM. Thus, more investigations are urgently needed on the combination of *CXCL13* and other antibodies to advance treatment effects and determine the underlying mechanisms.

Modules Exploring the Association Between Immune Infiltrates and CXCL13 Expression or Clinical Uutcome in TCGA

CXCL13 mediates the migration and localization of immune cells. Increasing information shows that immune cell infiltration can accelerate tumor progression and recurrence and affect immunotherapy and clinical outcome. Correlation between *CXCL13* in SKCM expression, abundance of immune infiltrates (neutrophil cells, macrophages, myeloid dendritic cells and CD4+ T cells), and survival time are shown in **Figure 6**. Patients

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

with high *CXCL13* gene expression and high neutrophil infiltrates had a longer survival time than patients with high gene expression and low neutrophil infiltrates ($P<0.01$). Patients with high *CXCL13* gene expression and low macrophage infiltrates tended to have a longer survival time than those with high *CXCL13* gene expression and high macrophage infiltrates ($P<0.01$). Patients with high *CXCL13* gene expression and high myeloid dendritic cell infiltrates tended to a have longer survival times ($P=0.003$). Patients with low *CXCL13* gene expression and low macrophage infiltrates tended to have a longer survival time than those with low *CXCL13* gene expression and high macrophage infiltrates ($P=0.02$). $CD4^+$ T cells can inhibit cancer growth via activating M1 macrophages. Patients with low *CXCL13* gene expression and low T cell CD4+ infiltrates had a longer survival time than patients with low gene expression and high T cell CD4+ infiltrates ($P=0.04$). Thus, cumulative curve analysis showed that immune infiltrates were significantly associated with *CXCL13* in SKCM, indicating that immune infiltrate cells significantly affect the prognosis. In this study, we found a significant correlation between the survival time and the expression of *CXCL13* in the infiltration of the 4 immune cell types – neutrophils, $CD4^+$ T cells, macrophages, and myeloid dendritic cells – indicating that *CXCL13* expression in combination with immune cell status can predict prognosis. Further research is needed to explore the potential immune therapy using *CXCL13*.

Discussion

Melanoma is a malignant tumor. Although PD1 monotherapy and BRAF inhibitors were widely used in patients with melanoma, these therapies were not active in those patients with drug resistance. Therefore, it is urgent to detect more therapeutic targets and prognostic biomarkers.

In our study, 41 common DEGs were found in 3 datasets. KEGG enrichment analysis indicated that these DEGs play a crucial role in immune response and inflammatory response. After inputting 41 DEGs and their PPI into Cytoscape software, we obtained 4 (MMP1, SPP1, CXCL13, and GZMB) hub genes with a high degree. KEGG enrichment revealed that CXCL13 was enriched in the most KEGG pathway terms. Recent studies have reported that chemokine receptors expressed on melanoma and immune cells are closely associated with the prognosis and the efficacy of melanoma immunotherapy [15,16]. *CXCL13* is a chemokine with a pivotal role in the immune system [17]. Through literature searching, we know that *CXCL13* is differentially expressed in the skin cutaneous melanoma (SKCM) [18]. Kazanietz et al suggest that how *CXCL*13 regulates the relationship between tumor cell and cancer microenvironment is vital to detect novel therapeutic targets for cancer treatment [16]. Jiao et al revealed the associations of CXCL13 and immune cell infiltration signature in renal cancer and the potential of CXCL13 as an immune response biomarker in patients with renal cancer [19]. In melanoma, no studies have systematically investigated the interaction between CXCL13 and the microenvironment. Herein, we performed comprehensive analyses to investigate the mechanisms of *CXCL13* in the SKCM microenvironment and immunotherapy efficacy.

Our analysis suggests that *CXCL13* is differentially expressed in TCGA-SKCM samples. Through network analysis, CXCL13 was shown to have a high degree and can interact with CXCR5. *CXCL13/CXCR5* signaling has been reported in many cancers. High CXCL13 expression is an independent risk factor and leads to a better prognosis in TCGA-SKCM samples. We then focused on the function of the top 30 highly co-expressed genes with CXCL13 using GO enrichment analysis. Our results suggested that CXCL13 interacts with other genes to regulate the tumor immune environment, which makes a crucial contribution to immune-related pathways. Further, the positive correlation between the expression of PD-1/PD-L1/CTLA4 and CXCL13 expression indicates that CXCL13 could be a biomarker for the response to anti-PD-1/PD-L1/CTLA4 immunotherapy in SKCM.

In addition, the expressions of CXCL13 were positively correlated with infiltration of 4 immune cell (neutrophils, CD4+ T cells, macrophages, and myeloid dendritic cells). Previous research reported that the degree of immune cell infiltration was related to favorable outcomes [20]. This is similar to our results that increasing levels of neutrophils and myeloid dendritic cells have a relationship with longer survival time in TCGA-SKCM patients.

Our results provide insights for future research on immunotherapy of SKCM. *CXCL13* and associated pathways involved in SKCM immunity could be a target to design more effective therapeutic strategies. We anticipate our study results can help design new immunotherapeutic drugs, and to guide clinicians in using appropriate drugs for patients.

It also could be highly useful in the identification of comorbidity patterns between diseases. Bioinformatics tools are novel alternatives for conventional approaches to get these data and integrate them in a biologically meaningful manner. Exploiting the bioinformatics tools, we have introduced *CXCL13*-related genes and pathways obtained from our network-based analysis. These genes and pathways could be subjected to further empirical investigations to attain additional insights about the biology and immunology of *CXCL13* as well as its relationship to other diseases. Our study has some limitations. Analysis on the transcriptional level can reflect some aspects of immune status, but not global changes. Moreover, other independent cohort and in vitro or in vivo studies should be performed to validate our results.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Conclusions

In summary, we systematically analyzed CXCL13 expression, prognosis, biological function, immune infiltration, and its relationship with 3 well-known immune checkpoint blockade targets. CXCL13 expression was closely related to survival rates of SKCM patients. CXCL13 can interact with other genes to regulate the immune pathways. It also has a correction with the expression of PD-1/PD-L1/CTLA4. In addition, CXCL13 is associated with infiltration of multiple immune cells. Research shows that CXCL13 may serve as potential therapeutic target in cutaneous melanoma, and it could be a biomarker for the response to anti-PD-1/PD-L1/CTLA4 immunotherapy in SKCM.

Data Availability Statement

The data pertaining to the present study are included in tables and/or figures in the manuscript.

Conflict of interest

None.

Declaration of Figures Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Supplementary Material

Supplementary Table 1. The 41 common DEGs in GSE15605, GSE46517, and GSE114445.

41 differential expressed genes
LAMB4, CHP2, EXPH5,CDHR1, SCGB1D2, CCL27, GATA3, PRAME, CYP3A5, RORA, POU2F3, SCEL, PDZD2, DSC3, SPP1, KLF5, WIF1, GZMB, PHACTR1, TFAP2B, GDF15, RGS1, IGF2BP3, SCGB2A2, CCL3L3, C1QB, MAGEA6, CYP39A1, IL37, MAGEA6, CITED1, MAGEA12, FCGR2A, SLAMF7, MMP1, CXCL13, MAGEA2B, CXCL9, CCL5, UBD, LCE2B

Supplementary Table 2. The KEGG enrichment of 41 DEGs.

ID	Description	p value	p. adjust	Gene ID	Count
hsa04061	Viral protein interaction with cytokine and cytokine receptor	7.23E-08	4.55E-06	10850/414062/27178/10563/4283/6352	6
hsa04060	Cytokine-cytokine receptor interaction	2.73E-06	8.58E-05	10850/9518/414062/27178/10563/4283/6352	7
hsa04062	Chemokine signaling pathway	6.28E-05	0.00132	10850/414062/10563/4283/6352	5
hsa04620	Toll-like receptor signaling pathway	8.55E-05	0.001347	6696/414062/4283/6352	4

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Olszanski AJ. Current and future roles of targeted therapy and immunotherapy in advanced melanoma. J Manag Care Spec Pharm. 2014;20:346-56
2. Leonardi GC, Falzone L, Salemi R, et al. Cutaneous melanoma: From pathogenesis to therapy (review). Int J Oncol. 2018;52:1071-80
3. Czarnecka AM, Bartnik E, Fiedorowicz M, Rutkowski P. Targeted therapy in melanoma and mechanisms of resistance. Int J Mol Sci. 2020;21:4576
4. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369:122-33
5. Owen CN, Shoushtari AN, Chauhan D, et al. Management of early melanoma recurrence despite adjuvant anti-PD-1 antibody therapy. Ann Oncol. 2020;31:1075-82
6. Chow MT, Luster AD. Chemokines in cancer. Cancer Immunol Res. 2014;2:1125-31
7. Keshava Prasad TS, Goel R, Kandasamy K, et al. Human protein reference database – 2009 update. Nucleic Acids Res. 2009;37 D767-72
8. Shannon P, Markiel A, Ozier O, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-504
9. Li T, Fan J, Wang B, et al. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108-10
10. Hussain M, Adah D, Tariq M, et al. CXCL13/CXCR5 signaling axis in cancer. Life Sci. 2019;227:175-86
11. Huang B, Han W, Sheng ZF, Shen GL. Identification of immune-related biomarkers associated with tumorigenesis and prognosis in cutaneous melanoma patients. Cancer Cell Int. 2020;20:195
12. Zhou X, Peng M, He Y, et al. CXC Chemokines as therapeutic targets and prognostic biomarkers in skin cutaneous melanoma microenvironment. Front Oncol. 2021;11:619003
13. Tauriello DVF, Palomo-Ponce S, Stork D, et al. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature. 2018;554:538-43
14. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther. 2015;14:847-56
15. Jacquelot N, Duong CPM, Belz GT, Zitvogel L. Targeting chemokines and chemokine receptors in melanoma and other cancers. Front Immunol. 2018;9:2480
16. Kazanietz MG, Durando M, Cooke M. CXCL13 and its receptor CXCR5 in cancer: Inflammation, immune response, and beyond. Front Endocrinol (Lausanne). 2019;10:471
17. Razis E, Kalogeras KT, Kotsantis I, et al. The role of CXCL13 and CXCL9 in early breast cancer. Clinical Breast Cancer. 2020;20:e36-53
18. Huang B, Han W, Sheng Z-F, Shen G-L. Identification of immune-related biomarkers associated with tumorigenesis and prognosis in cutaneous melanoma patients. Cancer Cell Int. 2020;20:1-15
19. Jiao F, Sun H, Yang Q, et al. Association of CXCL13 and immune cell infiltration signature in clear cell renal cell carcinoma. Int J Med Sci. 2020;17:1610-24
20. Rivadeneira DB, DePeaux K, Wang Y, et al. Oncolytic viruses engineered to enforce leptin expression reprogram tumor-infiltrating T cell metabolism and promote tumor clearance. Immunity. 2019;51:548-60.e4

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]