Identification of therapeutic targets and prognostic biomarkers in the CCL family in the SKCM microenvironment

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Abstract

Background: Skin cutaneous melanoma (SKCM) is one of the most common malignancies and is one of the most aggressive and dangerous types of skin cancer. CC chemokines in the tumor microenvironment can promote intercellular communication and modulate the transport of immune cells, thus exerting anti-tumor immune effects. The expression of CC chemokines in SKCM, their prognostic value and their relationship with immune cell infiltration are unclear.

Methods: Studies were performed using GEPIA, UALCAN, cBioPortal, STRING, GeneMANIA, Omicshare, David 6.8, Metascape, TRRUST, LinkedOmics and Timer.

Results: In SKCM we found that CCL3, CCL4, CCL5, CCL8, CCL18 were highly expressed in tumor tissue, while a proportion of CC chemokines CCL14, CCL21, CCL22, CCL23, CCL27 were found to be expressed at low levels. The expression of CCL4, CCL5, CCL8, CCL23, CCL27 was closely related to the pathological stage of SKCM. High expression of CCL3, CCL4, CCL5, CCL8, CCL15, CCL21, CCL22, CCL23 tended to correlate with a good prognosis for patients with SKCM. The functions of the differentially expressed CC chemokines are mainly Chemokine signaling pathway, Cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, Cytosolic DNA-sensing pathway. RELA and NFKB1 are key transcription factors for CC chemokines. We identified a number of kinases associated with differentially expressed CCL. Among them CASP7, SERPINE1, LCK and PRKAA1 are strongly correlated. We also found that expression of CC chemokines was significantly associated with infiltration of multiple immune cells.

Conclusion: Our findings may provide new insights into the selection of therapeutic targets and prognostic biomarkers for patients with SKCM.

1. Introduction

Melanoma is a cancer of the skin that occurs when pigment-producing melanocytes become malignant[1, 2]. Depending on the anatomical location where melanoma occurs, we refer to the cells located in the basal layer of the skin's epidermis as skin cutaneous melanoma[2]. The main causative risk factor for melanoma is due to exposure to ultraviolet light, and its frequency is closely related to the color and geographical area of the skin composition[3-5]. Skin cutaneous melanoma is the most aggressive and dangerous type of malignancy among skin cancers, causing the highest number of skin cancer-related deaths[3, 6-8]. The incidence of skin cutaneous melanoma has been on the rise globally for nearly five decades, leading to serious public health concerns[1, 9].

Skin cutaneous melanoma can be divided into early-stage tumors in situ and late-stage metastatic tumors. Early-stage treatment is based on local surgery and has a good prognosis[10]. In the advanced stage, treatment of metastatic tumors is limited, challenging and ineffective, with a five-year survival rate of only 25%. Early detection of skin cutaneous melanoma would significantly reduce its morbidity and mortality[1, 8, 9, 11]. In recent years, the development of targeted therapeutics coupled with the use of immunotherapy has led to significant improvements in clinical outcomes for patients with skin cutaneous

melanoma. Targeted therapy with BRAF and MEK inhibitors is one of the key modalities in the treatment of metastatic melanoma. Immunotherapy is mainly based on the blockade of one or two immune checkpoints (cytotoxic T-lymphocyte-associated protein-4, programmed cell death-1 and programmed cell death ligand-1) [8, 12-15]. The advent of targeted therapy and immunotherapy has led to a significant change in the treatment of SKCM, with positive treatment outcomes and prolonged progression-free and overall survival of patients[9, 16, 17]. At the same time we have also identified gaps in tumor resistance to treatment and in targeted therapy and immunotherapy: the depth and durability of response to targeted therapy are not guaranteed, and the predictability of response to immunotherapy is low[11, 13]. SKCM is now an extremely serious tumor, and finding the right therapeutic target remains a meaningful and urgent matter.

Chemokines are a heterogeneous group of short chain proteins (7-12 kDa), which can be divided into two subgroups, ligands and receptors, based on their structure[18, 19]. The human chemokine system consists of 50 chemokine ligands and 20 chemokine receptors[20]. Chemokine genes are found on two chromosomes, forming two gene clusters, one for CXC chemokines and the other for CC chemokines[21]. The CC chemokine family is a family of 28 chemotactic cytokines with an N-terminal CC structural domain, a family of proteins that share structural, biochemical and physiological features. There are actually a total 27 chemokines, as CCL9 and CCL10 represent the same chemokine[22]. Chemokines are secreted by tumor cells and other cell types (including immune cells and mesenchymal cells in the tumor microenvironment). Acting between cells, they are essential bridges for intercellular communication[23]. Chemokine receptors and ligands carry out different signaling in a specific manner. Early on, chemokines were only found to recruit leucocytes in inflammation, and in later studies they were found to play a role in the immune system[21]. In tumors, it has an effect on tumor cell proliferation, migration, invasion, organ-specific metastasis, drug resistance, and on angiogenesis and lymph angiogenesis. Recruit tumor-associated immune cells such as eosinophils, myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), tumor-associated neutrophils (TAN), regulatory T cells (Treg), tumor-infiltrating lymphocytes (TIL) and dendritic cell subsets to get the right cells to the right place at the right time. They act as important mediators in immune surveillance and cancer progression, performing anti-cancer and cancer-promoting functions[22, 24-26].

For CC Chemokines, we found that they have been studied in a variety of cancers. CCL27 was found to be overexpressed in skin squamous cell carcinoma[27]. In primary human choroidal melanocytes, a range of CCL and CXCL cytokines are involved in driving mechanisms of choroidal homeostasis and inflammation. In colorectal cancer, CCL-20 promotes cancer progression through chemotactic recruitment of CC-chemokine-receptor-6 (CCR-6) expressing B cells and γδ T cells[28]. CCL8 and CCL21 are prominently expressed in breast cancer in terms of prognosis, metastasis and chemoresistance[29]. In pancreatic ductal adenocarcinoma, the anti-tumor effect of CCL-5 blockade in a mouse model with high C- FOXP3 levels[30]. CCL21 and CCL11 are differentially expressed in prostate cancer[31]. CCL-5 regulates tumor cell viability and metastasis[32]. High level CCL7 and CCL21 are expressed in gastric cancer[33].

For melanoma, the investigators verified the dysregulation of CCL-5 gene transcription in melanoma[34]. CCL-2 and IL-8 were found to be produced in adherent melanoma cells and melanocytes[35]. In vivo studies in melanoma mice revealed high CCL-2 production detected in the hypoxic zone of melanoma tumors[36]. CTL migration in melanoma patient studies was mediated by CTL-expressed CCR-4 and CCL2 secreted by tumor cells[37]. The expression and function of CC chemokines in melanoma have been investigated in a number of studies, but no study has yet comprehensively and systematically analyzed the expression and function of CC chemokines in skin cutaneous melanoma, and the description of the CC chemokine family in skin cutaneous melanoma is still unclear. The search for suitable CC chemokines as therapeutic targets and prognostic biomarkers in skin cutaneous melanoma remains a pressing concern.

In our study, we conducted a series of bioinformatic analyses of CC chemokine expression in skin cutaneous melanoma, using data from various databases and online analysis methods to investigate the expression of CC chemokines in skin cutaneous melanoma and their role in tumorigenesis, progression and prognosis, and to assess their potential as prognostic biomarkers and therapeutic targets to find appropriate therapeutic approaches to improve prognosis and clinical outcomes of patients in skin cutaneous melanoma, providing a referenceable basis for the better prognosis and efficacy of skin cutaneous melanoma.

2. Materials And Methods

2.1 Gene Expression Profiling Interactive Analysis (GEPIA)

GEPIA (http://gepia2.cancer-pku.cn/) is a convenient website for biomarker analysis developed by a team from Peking University. The analysis of 9,736 tumor and 8,587 normal tissue RNA sequencing expression data in the The Cancer Genome Atlas (TCGA) and the type-Tissue Expression (GTEx) yields differential expression analysis of tumor and normal tissue, analysis by cancer type or pathological stage, patient survival analysis, similar gene detection, correlation analysis and downscaling analysis.[38] In the single gene analysis module, we analyzed 32 cancer types in TCGA by retrieving 28 different genes in the CCL family to find cancer types with meaningful differential transcript levels in tumor and normal tissues, and obtained their boxplots. |Log2FC| cutoff values we took 1 and p-value Cutoff 0.01. We used log2(TPM+1) for log-scale. we further analyzed the differential expression of genes in CCL family in the single gene module for different pathological stages in skin cutaneous melanoma. Resulting plots of the correlation between gene expression and cancer stage were obtained. We used the multi-gene expression module to obtain the expression of multiple CCL target genes in skin cutaneous melanoma. Survival analysis is also a part of our research focus and GEPIA provides rapid survival analysis. We input CCL family genes, selected the skin cutaneous melanoma to be analyzed and obtain survival graphs such as overall survival (OS) and disease-free survival (DFS), and automatically generated Log rank and HR values.

2.2 UALCAN

UALCAN (http://ualcan.path.uab.edu/) is a web resource for conducting online analysis and mining of cancer data, providing easy access to publicly available cancer OMICS data, allowing users to identify biomarkers or perform computer validation of potential genes of interest, providing charts and graphs depicting protein-coding expression profiles and patient survival information[39]. We investigated the prognostic relevance of CCL expression to patients with skin cutaneous melanoma by entering the CCL genes and using RNA sequencing data in TCGA to obtain patient survival curves. We chose a p-value of 0.05 as the threshold.

2.3 PrognoScan

PrognoScan (http://www.abren.net/PrognoScan/) is a large collection of publicly available cancer microarray datasets with clinical annotations, a tool for assessing the biological relationship between gene expression and prognosis. The relationship between gene expression and patient progression, such as overall survival (OS) and disease-free survival (DFS), was searched in a large collection of publicly available cancer microarray datasets[40]. We obtained survival prognosis results associated with CCL expression in skin cutaneous melanoma by searching for genes in CCL families and plotting their survival curves. *p=0.05.*

2.4 cBioPortal

The cBioPortal (www.cbioportal.org) is a convenient web resource for visualizing and analyzing multidimensional cancer genomics data. Based on the TCGA database, results for gene alterations, co-expression and neighboring genes were obtained from cBioPortal[41]. We obtained the gene mutations associated with CCL in skin cutaneous melanoma and the expression of neighboring genes by entering multiple CCL genes found to be differentially expressed in skin cutaneous melanoma in GEPIA.

2.5 STRING

STRING (https://string-db.org/) is an analytical tool that yields networks of various known and predicted protein interactions. STRING database now contains 24584628 proteins in 5090 tissues[42]. We performed PPI network analysis on differentially expressed CCLs to explore their interactions.

2.6 GeneMANIA

GeneMANIA (http://genemania.org/) uses a very large set of functional association data to find other genes associated with a set of input genes. The association data includes information on protein and gene interactions, pathways, co-expression, etc[43]. We entered differentially expressed CCL genes and obtained a relationship network map of differentially expressed CCL and associated gene interactions.

2.7 Omicshare

The omicshare (https://omicshare.com/) cloud platform was developed independently by Guangzhou Kidio Bio and launched in April 2016, aiming to provide a comprehensive platform for data processing

and analysis, bioinformatics learning, and scientific knowledge sharing for researchers. We obtained the results of Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis by inputting a list of CCL genes that are differentially expressed in skin cutaneous melanoma. processes (BP), cellular components (CC), and molecular function (MF) were included in the GO enrichment analysis.

2.8 DAVID6.8

DAVID (https://david.ncifcrf.gov/home.jsp) now provides researchers with a comprehensive set of functional annotation tools to understand the biological significance behind a large list of genes[44]. We obtained the Functional Annotation Table by entering a list of differentially expressed CCL genes, selected melanomas, using GO enrichment analysis and KEGG pathway enrichment analysis, which was further visualized through the Microbiology website.

2.9 Metascape

Metascape (https://metascape.org/gp//) integrates over forty bioinformatics databases and provides researchers with easy access to comprehensive data analysis through a simple interface for rapid analysis. Not only does it include biological pathway enrichment analysis, protein interaction network structural analysis and rich gene annotation capabilities, but it also presents the results in a high quality graphical language[45]. We have posted the list of differentially expressed CCL genes in the submission to obtain the results of the CCL gene biopathway enrichment analysis.

2.10 TRRUST

TRRUST (https://www.grnpedia.org/trrust/Network_search_form.php) contains 8444 and 6552 transcription factor (TF) target regulatory relationships for 800 human TFs and 828 mouse TFs. They are derived from 11237 PubMed articles and provide information on the mode of regulation (activation or repression). There were 8972 regulatory relationships with known modalities of regulation[46]. We searched for differentially expressed CCLs and found their associated transcription factors.

2.11 LinkedOmics

LinkedOmics (http://www.linkedomics.org/)is a publicly available portal that includes multi-omics data from all 32 TCGA cancer types and 10 Clinical Proteomics Tumor Analysis Consortium (CPTAC) cancer cohorts[47]. We obtained meaningful kinase targets enriched for CCL by analysis of the LinkInterpreter module. Our p-values were chosen as 0.05.

2.12 TIMER

TIMER (http://timer.comp-genomics.org/) is a comprehensive resource for the systematic analysis of immune infiltration in different types of cancer. It provides estimates of the abundance of immune infiltrates by multiple immune deconvolution methods, including TIMER, CIBERSORT, quanTIseq, XCELL,

MCP-counter and several other algorithms[48]. The results of RNA-seq for all TCGA cancers based on different algorithms are computed to obtain results for differentially expressed CCL genes and various types of immune cell infiltrates. The results of the correlation analysis of 20 immune-related cells, including T cells CD4+, B cells, Tregs, Neutrophil, etc., were obtained. We used a purity-adjusted p-value of less than 0.05 as a meaningful screening criterion.

2.13 Ethical statement

The data were acquired from open resource databases, no ethical approval was needed.

3. Results

3.1 Expression of CC chemokine in patients with skin cutaneous melanoma

Firstly, to investigate the role of the 28 CCL chemokines in tumorigenesis, progression and prognosis, we examined the RNA sequencing data of all 32 cancer types in TCGA for CCL transcript levels in GEPIA. We found that in Breast invasive carcinoma (BRCA), the highly expressed chemokines were CCL5 and CCL11 and the low expressed chemokines were CCL7, CCL14, CCL21, CCL23 and CCL28. In colon adenocarcinoma (COAD), CCL3, CCL4, CCL5, CCL15, CCL18, CCL20 and CCL24 were highly expressed and CCL2, CCL11, CCL13, CCL14, CCL21 and CCL23 were lowly expressed. In Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), the highly expressed chemokines were CCL3, CCL4, CCL17, CCL18, CCL20 and CCL22; the low expressed chemokines were CCL14, CCL21, CCL23 and CCL24.In Head and Neck squamous cell carcinoma (HNSC), the highly expressed chemokines were CCL3, CCL5, CCL11, CCL18 and CCL20; the low expressed chemokines were CCL2 and CCL14. In lung squamous cell carcinoma (LUSC), the highly expressed chemokines were CCL20 and CCL26; the low expressed chemokines were CCL2, CCL13, CCL14, CCL15, CCL21 and CCL23. In Liver hepatocellular carcinoma (LIHC), the highly expressed chemokines were CCL15, CCL18, CCL20 and CCL21; the low expressed chemokines were CCL14, CCL19 and CCL23. In Ovarian serous cystadenocarcinoma (OV), the highly expressed chemokines were CCL2, CCL3, CCL4, CCL5, CCL8, CCL11, CCL18, CCL20 and CCL28; the low expressed chemokines were CCL14, CCL21 and CCL26.In Pancreatic adenocarcinoma (PAAD), the highly expressed chemokines were CCL2, CCL3, CCL4, CCL5, CCL11, CCL13, CCL15, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL22, CCL24, CCL26 and CCL28. Specific results can be found in exhibits Supplement Figure 1-9. In skin cutaneous melanoma we found that CCL3, CCL4, CCL5, CCL8 and CCL18 were highly expressed in tumor tissue, while some CC chemokines CCL14, CCL21, CCL22, CCL23 and CCL27 were found to be expressed at low levels in tumor tissue. The results are presented in Figure 1. Our results suggest that CCL family is differentially expressed in a variety of cancer tissues, including skin cutaneous melanoma. We also compared the relative expression levels of individual CC chemokines in SKCM and found that CCL5 had the highest relative expression of all the CC chemokines we evaluated, as shown in Figure 2.

To identify CC chemokines associated with staging of SKCM, we investigated the correlation between CCL expression levels and staging of differentially expressed CCL. The results revealed that the

expression of CCL4 (*p=0.00168*), CCL5 (*p=0.00137*), CCL8 (*p=0.000343*), CCL23 (*p=0.0137*) and CCL27 (*p=0.0156*) were significantly correlated with pathological staging (Figure 3).

The expression of CCL4, CCL5, CCL8, CCL23 and CCL27 increased with tumor progression. These data suggest that these CC chemokines play an important role in the development and progression of SKCM.

3.2 Effect of CC chemokine on the prognosis of patients with skin cutaneous melanoma

We used three methods, UALCAN, GEPIA and Prognoscan, to assess the phototropism of CC chemokine expression and clinical prognosis of SKCM and obtained relatively consistent results. We found that CCL expression significantly affected the prognosis of SKCM. By UALCAN analysis, we found that high expression of CCL3 (*p=0.0018*), CCL4 (*p<0.0001*), CCL5 (*p<0.0001*), CCL8 (*p<0.0001*), CCL23 (*p=0.021*) was associated with a better prognosis for patients with SKCM, and the results of the survival curves can be seen in Figure 4. Our study in GEPIA found that SKCM OS had a better prognosis associated with CCL3 (*p=0.0018*, r=0.65), CCL4 (*p=8.2e-08*, r=0.48), CCL5 (*p=3.2e-06*, r=0.53), CCL8 (*p=e-06*, r=0.52), CCL22 (*p=0.0063*, r=0.69), CCL23 (*p=0.0031*, r=0.67) were associated with high expression, and better DFS prognosis was associated with high expression of CCL4 (*p=0.0026*, r=0.76), CCL5 (*p=0.0027*, r=0.76). The results are presented in Figure 5. In our analysis using Prognoscan, we found that a better prognosis for OS in SKCM was associated with high expression of CCL15, CCL19, CCL21 and CCL23. In dataset GSE19234 using probe HG-U133_Plus_2, High expression of CCL15 had a good prognostic outcome in SKCM (OS *p=0.044292*, HR=0.42, 95%CI=0.18-0.98). High expression of CCL19 had a good prognostic outcome (OS *p=0.001221*, HR=0.62, 95%CI=0.46-0.83). High expression of CCL21 had a good prognostic outcome in SKCM (OS *p=0.046*, HR=0.74, 95%CI=0.55-1.00). High expression of CCL23 had a good prognostic outcome in SKCM (OS *p=0.034096*, HR=0.58, 95% CI=0.35-0.96). (figure 6).

3.3 Analysis of genetic alterations, co-expression, neighborhood gene networks and interactions in CCL families of skin cutaneous melanoma patients

To investigate the molecular characterization of differentially expressed CCL, we used cBioPortal on the TCGA dataset to analyze genetic alterations in differentially expressed CCL. As shown in Figure 7a and 7b, genetic alterations were dominated by mutations, profound deletions and multiple alterations. CCL3, CCL4, CCL5, CCL8, CCL18, CCL14, CCL21, CCL22, CCL23 and CCL27 were found in 0.5, 0.5, 1.4, 0.9, 0.9, 0.9, 0.9, 1.4, 0.5 and 0.9% of SKCM samples were altered. We also obtained genes related to CCL family genes in SKCM patients include PKP4, OSGIN1, LYG1, LMAN2L, CPNE2, LINC01242, LOC100506422, TLK1, ZSCAN25 and NCK2. The results are shown in Table 1.

We further analyzed the relationship of differentially expressed genes in skin cutaneous melanoma, their co-expression genes and interactions using the STRING database and GeneMANIA to explore how our CCL family and its associated genes function in concert. In STRING, we obtained 10 nodes and 22 edges in the PPI network. We found that the functions of these differentially expressed CCLs were associated with chemokine signaling pathways, viral protein-cytokine, cytokine receptor interactions, and inflammatory responses, as shown in Figure 8a. GeneMANIA results also showed that the functions of

the differentially expressed CCLs and related genes were mainly associated with cytokine activity, chemokine receptor binding, cellular responses to chemokines, targeting of chemokines, lymphocyte migration, neutrophil migration, G protein-coupled receptor binding, granulocyte chemotaxis, monocyte migration and cytokine receptor binding. The specific results can be seen in Figure 8b and Table 2.

3.4 Functional enrichment analysis of CCL family genes in skin cutaneous melanoma patients

We performed a functional enrichment analysis of differentially expressed CCL genes in SKCM using three sites to explore the biological processes involved and the functions acted. The richest features of CC in omicshare were extracellular region, cytosol cytoplasmic part, cytoplasm, intracellular part, intracellular, cell, cell part and cellular component. The richest features in MF in omicshare were CCR chemokine receptor binding, chemokine activity, chemokine receptor binding, cytokine activity, cytokine receptor binding, G protein-coupled receptor binding, receptor ligand activity, receptor regulator activity, CCR1 chemokine receptor binding and signaling receptor binding. In category BP, lymphocyte chemotaxis, lymphocyte migration, monocyte chemotaxis, chemokine-mediated signaling pathway, mononuclear cell, migration, response to chemokine, cellular response to chemokine, neutrophil chemotaxis, leukocyte chemotaxis, neutrophil migration and other functions were enriched. The KEGG pathway analysis revealed that the functions of Signaling molecules and interaction, Immune system, Infectious diseases Signal transduction and Neurodegenerative diseases, etc. are closely related to SKCM. These are shown in Figure 10.

We obtained these results in DAVID 6.8: the most abundant functions in the BP category were monocyte chemotaxis, cellular response to interferon-gamma, chemokine-mediated signaling pathway, cellular response to interleukin-1 response to interleukin-1, cellular response to tumor necrosis factor, neutrophil chemotaxis, lymphocyte chemotaxis, positive regulation of ERK1 and ERK2 cascade, immune response and cell-cell signaling. The most abundant functions in the CC category were extracellular space, extracellular region, cell and intracellular. The richest features in MF were chemokine activity, CCR chemokine receptor binding, CCR1 chemokine receptor binding, phospholipase activator activity, CCR5 chemokine receptor binding, chemokine receptor binding, chemoattractant activity and protein kinase activity. The first 8 KEGG pathways were Chemokine signaling pathways, Cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway and Cytosolic DNA-sensing pathway. The results are shown in Figure 10. The results of Metascape were similar to the first two analyses. The CCL family plays an important role in the development and progression of SKCM. The results are shown in Figure 11 and Table 3.

3.5 CCL family gene transcription factor targets, kinase targets in skin cutaneous melanoma patients

TRRUST analysis of differentially expressed genes in SKCM identified two transcription factors associated with SKCM, v-rel reticuloendotheliosis viral oncogene homolog A (avian) (RELA) and nuclear factor of kappa light polypeptide gene enhancer in B-cell 1 (NFKB1). RELA is the target of transcription factors for CCL4, CCL22, CCL3 and CCL5 and NFKB1 is the transcription factor for CCL5, CCL3, CCL4 and CCL22. The results are shown in Table 4.

In LinkedOmics, we identified the top three kinase targets associated with CC chemokine. SYK, BCL2, and CASP7 are the three most important targets in the CCL3 kinase-target network. The composition of the CCL4 kinase target network is mainly associated with CASP7, LCK and SYK. CASP7, LCK and ANXA7 are considered to be the CCL5. CASP7, SYK, and CDH1 are mainly associated with CCL8. BCL2L11, SETD2 and MAP2K1, SERPINE1, 53BP1 and MAPK14 are the three most important targets in the CCL14 and CCL18 kinase-target network, respectively. The composition of the CCL 21and CCL22 kinase-target network is mainly associated with SRC, RPS6KB1 and MYH11 as well as MAPK14, BAP1 and YWHAZ. The kinase target network of CCL23 was mainly composed of NDRG1, MAP2K1 and YBX1. CCL27 was associated with PRKAA1, TSC2 and RBM15. The results can be found in Table 5.

3.6 Immune infiltration of the CCL family gene in skin cutaneous melanoma patients

Using the TIMER database, we performed an analysis of the correlation between differentially expressed genes of SKCM and the abundance of immune infiltrates in the CCL family. Tumor infiltrating lymphocytes were an independent predictor of tumor sentinel lymph node status and survival[49]. The results showed that CCL3, CCL4, CCL5, CCL8, CCL11, CCL14, CCL18, CCL21 and CCL23 expression was negatively correlated with tumor purity.

CCL3 expression and B Cell, CD8+ T Cell, CD4+ T Cell, Macrophage, Neutrophil, Dendritic Cell infiltration level were positively correlated. CCL4 expression and B Cell, CD8+ T Cell, CD4+ T Cell, Macrophage, Neutrophil, Dendritic Cell infiltration level were positively correlated. CCL5 expression was positively correlated with B Cell, CD8+ T Cell, CD4+ T Cell, Macrophage, Neutrophil and Dendritic Cell infiltration level. CCL8 expression was positively correlated with B Cell, CD8+ T Cell, CD4+ T Cell, Macrophage, Neutrophil and Dendritic Cell infiltration level. CCL14 expression was positively correlated with CD4+ T Cell and Macrophage infiltration. CCL18 expression was negatively correlated with CD8+ T Cell and Dendritic Cell infiltration level. CCL21 expression was positively correlated with B Cell, CD8+ T Cell, CD4+ T Cell, Macrophage, Neutrophil, Dendritic Cell infiltration level were positively correlated. The results of CCL22 and CCL23 were consistent with those of CCL21. Specific results can be found in Table 6 and Figures 12.

Discussion

SKCM continues to be one of the most serious malignancies in the world. Finding the right treatment options for metastatic skin cutaneous melanoma remains an urgent problem for researchers and doctors. The treatment of melanoma has evolved into a new phase of targeted therapy, immunotherapy, cancer vaccines and so on[11]. We are gradually shifting the target of treatment from tumor cells to the tumor microenvironment, exploring the interactions between tumor and mesenchymal cells. As for CCL family, it plays an important role in the tumor microenvironment. In addition to its earliest findings of transport and recruitment of leukocytes, more and more studies have found that it has an immunomodulatory function in tumor and is closely associated with a variety of tumor-infiltrating immune cells[22, 23]. The aim of our study is to investigate the prognostic impact of CCL expression in SKCM, to identify genetic alterations in

CCL genes, related genes and their biological functions, to seek for suitable transcription factor targets and kinase targets in CCL family species for SKCM, and to determine the abundance of immune molecules expressed in the tumor microenvironment, thus providing new ideas and basis for the treatment of SKCM.

We first explored the expression of the CCL family in the 32 cancers of the TCGA dataset, including SKCM. We found differential expression of CCL genes in SKCM. CCL3, CCL4, CCL5, CCL8, CCL18 were found to be upregulated in tumor tissue compared to normal tissue, and CCL14, CCL21, CCL22, CCL23, CCL27 were downregulated in tumor tissue. Moreover, we found a significant correlation between CCL expression and the staging of SKCM, indicating the impact of CCL expression on tumor progression. We further analyzed the prognostic impact of CCL in SKCM and we found that high expression of CCL3, CCL4, CCL5, CCL8, CCL15, CCL19, CCL21, CCL22 and CCL23 tended to correlate with a good prognosis for SKCM patients. We found that CCL19 was not a differentially expressed gene in SKCM, so it was not very useful for our subsequent study. The rest of our data suggest that differentially expressed CCL genes play a great role in the occurrence, development and prognosis in SKCM and that differentially expressed CCL genes can be used as markers of prognosis in SKCM patients.

We further investigated the molecular characterization of CCL differentially expressed in SKCM. We found that genetic alterations in SKCM are mainly dominated by mutations, profound deletions and multiple alterations. Genetic alterations play an important role in the occurrence and development of SKCM. We also found that the genes differentially expressed in SKCM of the CCL family are interlinked and traffic with each other, acting synergistically in skin cutaneous melanoma. Through the study of genes associated with differentially expressed CCL, we have identified that our differentially expressed CCLs play a role in relation to cytokine activity, chemokine receptor binding, cellular responses to chemokines, targeting of chemokines, lymphocyte migration, neutrophil migration, G protein-coupled receptor binding, granulocyte chemotaxis, monocyte migration and cytokine receptor binding.

We followed up with a functional enrichment analysis of differential CCL genes in SKCM, where we used GO enrichment analysis and KEGG pathway enrichment analysis to focus on the function of differentially expressed CCL. We obtained consistent results and we found that the functions of these genes are mainly related to Chemokine signaling pathway, Cytokine-cytokine receptor interaction, Toll-like receptor signaling pathway, Cytosolic DNA-sensing pathway. Previous studies have found that chemokine signaling pathways play a role in promoting cancer development, metastasis, and modulating immunity[50-53]. Cytokine-cytokine receptor interactions are essential in inflammatory infiltration and tumor immunity[54, 55]. The Toll-like receptor signaling pathway has been extensively studied and has been identified in many studies. There are many studies reporting the role of Toll-like receptor signaling pathway in immunity system[56-58]. Cytosolic DNA-sensing pathway has been associated with the production of interferons, pro-inflammatory factors and chemokines[59-61].

We have also identified transcription factor targets of SKCM differentially expressed genes: RERA and NFKB1. RELA/NFKB1 dimers are common forms of NF-kB in mammals[62]. RERA phosphorylation is

interlinked with disease, for example, inflammation and cancer, through regulation of NF-kB signaling[63-66]. Similarly, NFKB1 is also involved in the development and progression of disease. It acts as a suppressor of inflammation and cancer and plays an inhibitory role in the development and progression of many cancers[67-69]. We identified a number of kinases associated with differentially expressed CCLs. Among them, CASP7, SERPINE1, LCK and PRKAA1 have strong correlations. We found that CASP7 is associated with aspartate-type endopeptidase activity, cysteine-type endopeptidase activity, protein binding, peptidase activity and cysteine-type peptidase activity[70-73]. The main functions of SERPINE1 are protease binding, serine-type endopeptidase inhibitor activity, signal receptor binding, and protein binding[74-76]. LCK binds mainly to phosphotyrosine residues[77]. The main functions of PRKAA1 are activated protein kinase activity, AMP-activated protein kinase activity and protein serine/threonine kinase activity[78-80]. Our study provides kinase targets for differentially expressed CCLs in SKCM, providing a reference for further mapping the mechanisms of SKCM development and progression, and advancing cancer research by investigating the function of related kinase targets. We can promote or inhibit cancer development and progression by modulating these transcription factor targets and kinase targets.

Moreover, we have analyzed the association of differentially expressed CCL genes expression with immune-associated cells in SKCM. Our results suggest that multiple CCLs are associated with the immune infiltration status of the tumor and that CCL family can reflect the immune status to some extent. It is undeniable that the differentially expressed CCL in SKCM cannot be associated with every type of immune cell. This is due to the fact that each of our CCLs has its own structure and function, in addition to its synergistic role in the biological whole. Our research on immune status is still at the cellular level, and further experimental exploration and validation is needed for the study of immune status in terms of the organism as a whole.

Conclusion

Overall, we have identified differentially expressed CC chemokine within the CC chemokine family in SKCM, and we further understand the impact of the expression of these differentially expressed genes on the prognosis of SKCM patients. We found that the higher the expression level of differentially expressed genes, the better the prognosis of patients, indicating that these CC chemokine ligands can be used as prognostic biomarker. We have correlated these differentially expressed genes with genetic variation and functional enrichment to help us understand the important role of CCL in SKCM. We followed up by studying transcription factor targets and kinase targets to provide new ideas for the development of targeting studies. The analysis of immune infiltration suggests that our differentially expressed chemokine ligands influence the immune status of the tumor microenvironment in SKCM. We hope that our results will provide new insights into the design of new targeted, immunotherapeutic agents, help patients with SKCM to select appropriate therapeutic drugs and prognostic biomarkers, and make our own contribution to the prolongation of life and remission of disease in patients with SKCM.

Abbreviations

SKCM: Skin cutaneous melanoma; MDSC: myeloid-derived suppressor cells; TAM: tumor-associated macrophages; TAN: tumor-associated neutrophils; Treg; regulatory T cells; CCR: CC-chemokine-receptor; GEPIA: Gene Expression Profiling Interactive Analysis; TCGA: The Cancer Genome Atlas; GTEx: the type-Tissue Expression; OS: overall survival; DFS: disease-free survival; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological process; CC: Molecular function; MF: Molecular functions; TF: transcription factor; CPTAC: Clinical Proteomics Tumor Analysis Consortium; BRCA: Breast invasive carcinoma; COAD: colon adenocarcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; HNSC: Head and Neck squamous cell carcinoma; LUSC: lung squamous cell carcinoma; LIHC: Liver hepatocellular carcinoma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; RELA: v-rel reticuloendotheliosis viral oncogene homolog A (avian); NFKB1: nuclear factor of kappa light polypeptide gene enhancer in B-cell 1.

Declarations

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Author Contributions

Jinlu Ma and Jie Chen conceived and designed the study; Xinyue Zhang, Jin Wang, Chao Ji, Jue Pan and Mengjiao Cai collected the data; Jinlu Ma and Jie Chen analyzed the data; Mengjiao Cai, Jin Wang and Xinyue Zhang contributed to drawing/analysis tools; Jie Chen wrote the manuscript. Ma Jinlu revised the manuscript.

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Data availability and materials

The data were acquired from open resource databases. GEPIA: http://gepia2.cancer-pku.cn/; UALCAN: http://ualcan.path.uab.edu/; PrognoScan: http://www.abren.net/PrognoScan/; cBioPortal: www.cbioportal.org; STRING: https://string-db.org/; GeneMANIA: http://genemania.org/; omicshare : https://omicshare.com/; DAVID: https://david.ncifcrf.gov/home.jsp; Metascape: https://metascape.org/gp//; TRRUST: https://www.grnpedia.org/trrust/Network_search_form.php; LinkedOmics: http://www.linkedomics.org/; TIMER: http://timer.comp-genomics.org/.

Ethics approval and consent to participate

Not acceptable.

Consent for publication

Not acceptable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Neighboring related genes of differentially expressed CC chemokines in SKCM patients through cBioPortal.

Gene	Cytoband	Log ratio	*p*	*q*
PKP4	2q24.1	4.26	0.000	0.017
OSGIN1	16q23.3	6.11	0.000	0.017
LYG1	2q11.2	5	0.000	0.017
LMAN2L	2q11.2	5.53	0.000	0.017
CPNE2	16q13	>10	0.000	0.017
LINC01242	9p21.1	>10	0.000	0.017
LOC100506422	9p21.2	>10	0.000	0.017
TLK1	2q31.1	5.07	0.000	0.017
ZSCAN25	7q22.1	4.88	0.000	0.017
NCK2	2q12.2	5.11	0.000	0.017

Table 2. Function of differentially expressed CCL through GeneMANIA.

GO ID	Description	*q*
GO:0005125	cytokine activity	0.000
GO:0042379	chemokine receptor binding	0.000
GO:1990869	cellular response to chemokine	0.000
GO:1990868	response to chemokine	0.000
GO:0072676	lymphocyte migration	0.000
GO:0001664	G protein-coupled receptor binding	0.000
GO:1990266	neutrophil migration	0.000
GO:0005126	cytokine receptor binding	0.000
GO:0071621	granulocyte chemotaxis	0.000
GO:0071674	mononuclear cell migration	0.000

Table 3. Enrichment analysis of differentially expressed CC chemokines and their neighboring genes in SKCM through Matescape.

GO	Category	Description	Count	%	Log10(*p*)	Log10(*q*)
hsa04061	KEGG Pathway	Viral protein interaction with cytokine and cytokine receptor	10	100.00	-25.00	-20.83
GO:0002548	GO Biological Processes	monocyte chemotaxis	9	90.00	-24.81	-20.83
GO:0048245	GO Biological Processes	eosinophil chemotaxis	5	50.00	-14.13	-11.43
GO:0006874	GO Biological Processes	cellular calcium ion homeostasis	6	60.00	-8.84	-6.37

Table 4. Key regulated factor of CC chemokines in SKCM through TRRUST.

Key TF	Description	Overlapped Genes	*p*	*q*	List of overlapped genes
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	4	0.000	0.000	CCL4, CCL22, CCL3, CCL5
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	4	0.000	0.000	CCL5, CCL3, CCL4, CCL22

Table 5. The Kinase target networks of CC chemokines in SKCM through LinkedOmics.

CCL	Enriched kinase target	Description	Statistic	FDR (BH)	*p*
CCL3	SYK|Syk	spleen associated tyrosine kinase	0.364	0.040	0.000
	BCL2|Bcl-2	BCL2 apoptosis regulator	-0.357	0.040	0.001
	CASP7|Caspase-7_cleavedD198	caspase 7	0.348	0.040	0.001
CCL4	CASP7|Caspase-7_cleavedD198	caspase 7	0.522	0.000	0.000
	LCK|Lck	LCK proto-oncogene, Src family tyrosine kinase	0.390	0.013	0.000
	SYK|Syk	spleen associated tyrosine kinase	0.365	0.024	0.000
CCL5	CASP7|Caspase-7_cleavedD198	caspase 7	0.546	0.000	0.000
	LCK|Lck	LCK proto-oncogene, Src family tyrosine kinase	0.410	0.005	0.000
	ANXA7|Annexin_VII	annexin A7	0.323	0.098	0.002
CCL8	CASP7|Caspase-7_cleavedD198	caspase 7	0.415	0.008	0.000
	SYK|Syk	spleen associated tyrosine kinase	0.310	0.261	0.003
	CDH1|E-Cadherin	Cadherin1	-0.297	0.268	0.004
CCL14	BCL2L11|Bim	BCL2 like 11	-0.346	0.148	0.001
	SETD2|SETD2	SET domain containing 2, histone lysine methyltransferase	0.296	0.288	0.004
	MAP2K1|MEK1	mitogen-activated protein kinase kinase 1	-0.290	0.288	0.005
CCL18	SERPINE1|PAI-1	serpin family E member 1	0.414	0.009	0.000
	TP53BP1|53BP1	tumor protein p53	-0.384	0.016	0.000
	MAPK14|p38_MAPK	mitogen-activated protein kinase 14	0.372	0.018	0.000
CCL21	SRC|Src_pY416	SRC proto-oncogene, non-receptor tyrosine kinase	-0.327	0.134	0.002
	RPS6KB1|P70s6k	ribosomal protein S6 kinase B1	-0.321	0.134	0.002

	MYH11|MYH11	myosin heavy chain 11	0.315	0.134	0.002
CCL22	MAPK14|p38_MAPK	mitogen-activated protein kinase 14	0.394	0.017	0.000
	BAP1|Bap1-c-4	BRCA1 associated protein 1	-0.383	0.017	0.000
	YWHAZ|14-3-3_zeta	tyrosine-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	0.371	0.019	0.000
CCL23	NDRG1|NDRG1_pT346	N-myc downstream regulated 1	0.291	0.696	0.005
	MAP2K1|MEK1_pS217_S221	mitogen-activated protein kinase kinase 1	0.258	0.696	0.014
	YBX1|YB-1_pS102	Y-box binding protein 1	0.253	0.696	0.015
CCL27	PRKAA1|AMPK_pT172	protein kinase AMP-activated catalytic subunit alpha 1	-0.390	0.025	0.000
	TSC2|Tuberin	TSC complex subunit 2	-0.330	0.132	0.001
	RBM15|RBM15	RNA binding motif protein 15	-0.297	0.267	0.004

Table 6. Correlation of SKCM differential expression of CC chemokine genes with tumor purity and abundance of immune cell infiltration through TIMER.

CCL	Variable	Partial.cor	*p*
CCL3	Purity	-0.543	0.000
CCL3	B Cell	0.19	0.000
CCL3	CD8+ T Cell	0.387	0.000
CCL3	CD4+ T Cell	0.244	0.000
CCL3	Macrophage	0.292	0.000
CCL3	Neutrophil	0.48	0.000
CCL3	Dendritic Cell	0.602	0.000
CCL4	Purity	-0.595	0.000
CCL4	B Cell	0.257	0.000
CCL4	CD8+ T Cell	0.671	0.000
CCL4	CD4+ T Cell	0.277	0.000
CCL4	Macrophage	0.343	0.000
CCL4	Neutrophil	0.659	0.000
CCL4	Dendritic Cell	0.712	0.000
CCL5	Purity	-0.645	0.000
CCL5	B Cell	0.259	0.000
CCL5	CD8+ T Cell	0.623	0.000
CCL5	CD4+ T Cell	0.358	0.000
CCL5	Macrophage	0.254	0.000
CCL5	Neutrophil	0.561	0.000
CCL5	Dendritic Cell	0.704	0.000
CCL8	Purity	-0.435	0.000
CCL8	B Cell	0.172	0.000
CCL8	CD8+ T Cell	0.514	0.000
CCL8	CD4+ T Cell	0.223	0.000
CCL8	Macrophage	0.319	0.000
CCL8	Neutrophil	0.639	0.000
CCL8	Dendritic Cell	0.608	0.000

CCL14	Purity	-0.414	0.000
CCL14	B Cell	0.026	0.583
CCL14	CD8+ T Cell	-0.028	0.555
CCL14	CD4+ T Cell	0.212	0.000
CCL14	Macrophage	0.16	0.001
CCL14	Neutrophil	-0.084	0.074
CCL14	Dendritic Cell	0.009	0.853
CCL18	Purity	-0.321	0.000
CCL18	B Cell	0.087	0.065
CCL18	CD8+ T Cell	0.097	0.042
CCL18	CD4+ T Cell	0.083	0.080
CCL18	Macrophage	0.091	0.054
CCL18	Neutrophil	0.057	0.226
CCL18	Dendritic Cell	0.259	0.000
CCL21	Purity	-0.434	0.000
CCL21	B Cell	0.335	0.000
CCL21	CD8+ T Cell	0.126	0.008
CCL21	CD4+ T Cell	0.273	0.000
CCL21	Macrophage	0.133	0.005
CCL21	Neutrophil	0.12	0.011
CCL21	Dendritic Cell	0.173	0.000
CCL22	Purity	-0.647	0.000
CCL22	B Cell	0.249	0.000
CCL22	CD8+ T Cell	0.214	0.000
CCL22	CD4+ T Cell	0.282	0.000
CCL22	Macrophage	0.126	0.007
CCL22	Neutrophil	0.193	0.000
CCL22	Dendritic Cell	0.383	0.000
CCL23	Purity	-0.394	0.000

CCL23	B Cell	0.144	0.002
CCL23	CD8+ T Cell	0.26	0.000
CCL23	CD4+ T Cell	0.181	0.000
CCL23	Macrophage	0.376	0.000
CCL23	Neutrophil	0.352	0.000
CCL23	Dendritic Cell	0.351	0.000
CCL27	Purity	-0.037	0.425
CCL27	B Cell	-0.027	0.565
CCL27	CD8+ T Cell	-0.084	0.081
CCL27	CD4+ T Cell	0.048	0.307
CCL27	Macrophage	-0.026	0.588
CCL27	Neutrophil	-0.04	0.393
CCL27	Dendritic Cell	-0.047	0.320

Figures

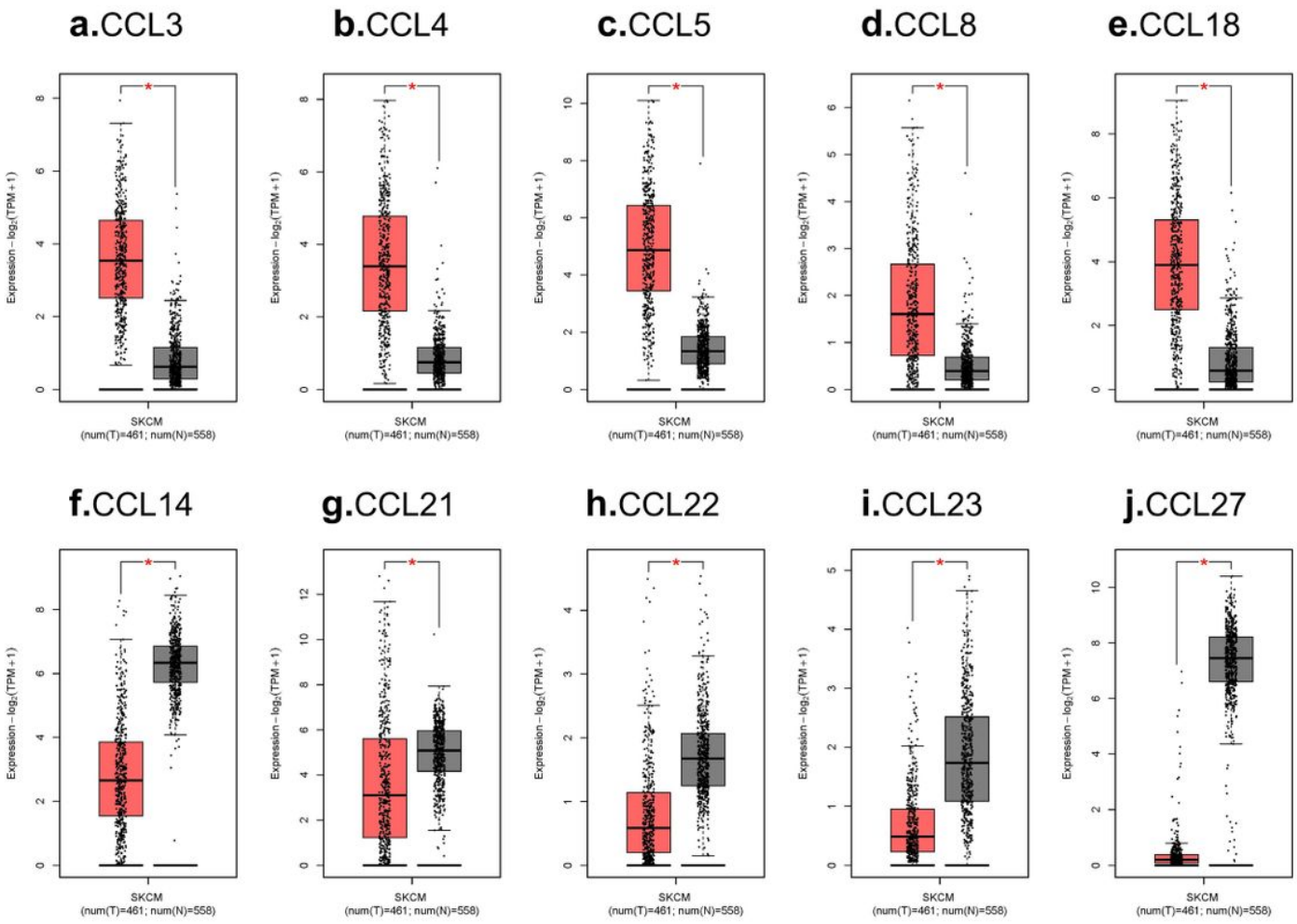

Figure 1

Expression levels of CC chemokine family through GEPIA in SKCM. Red represents tumor tissue and black represents normal tissue. Red* represents CC chemokine expression is statistically significant in SKCM.

Figure 2

Relative levels of CC chemokines in SKCM through GEPIA.

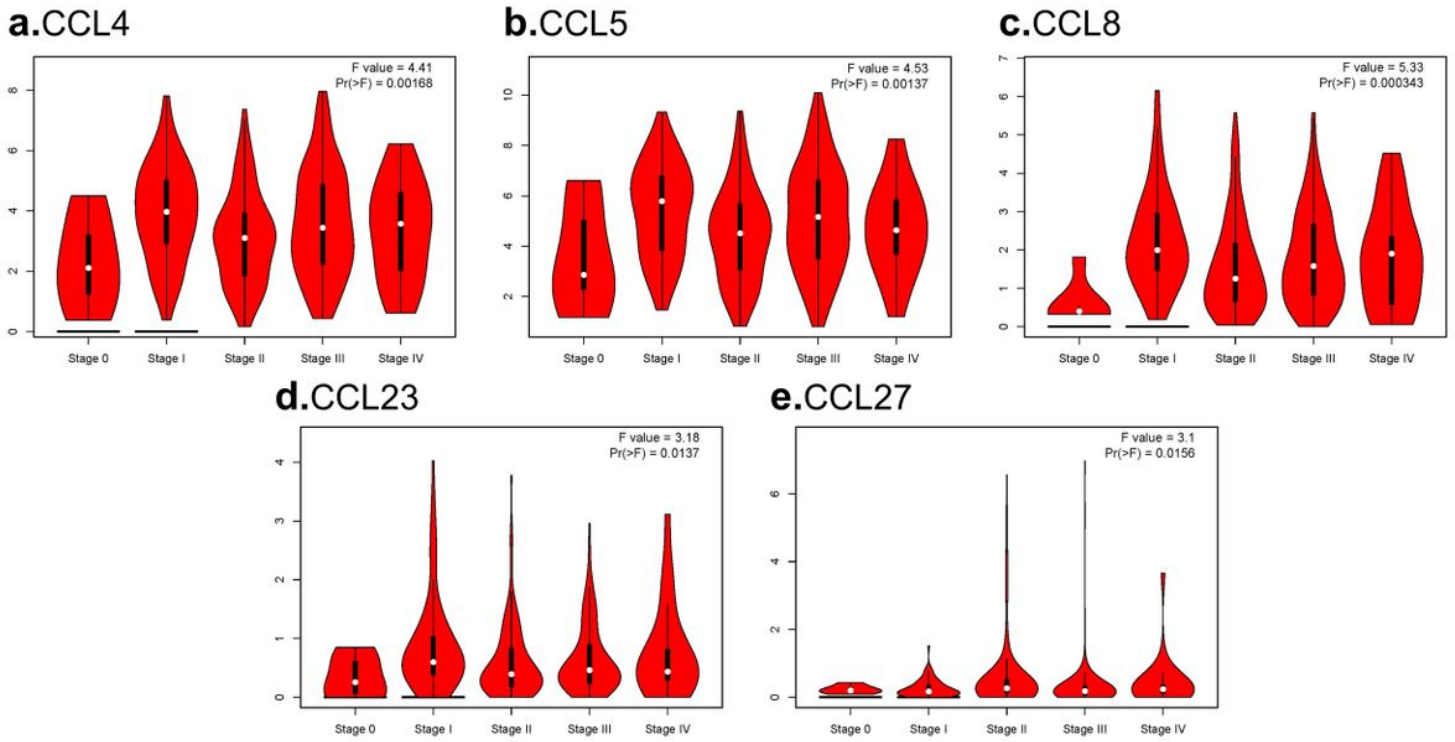

Figure 3

Correlation of differentially expressed CC chemokines with pathological staging of SKCM patients through GEPIA where $p < 0.05$.

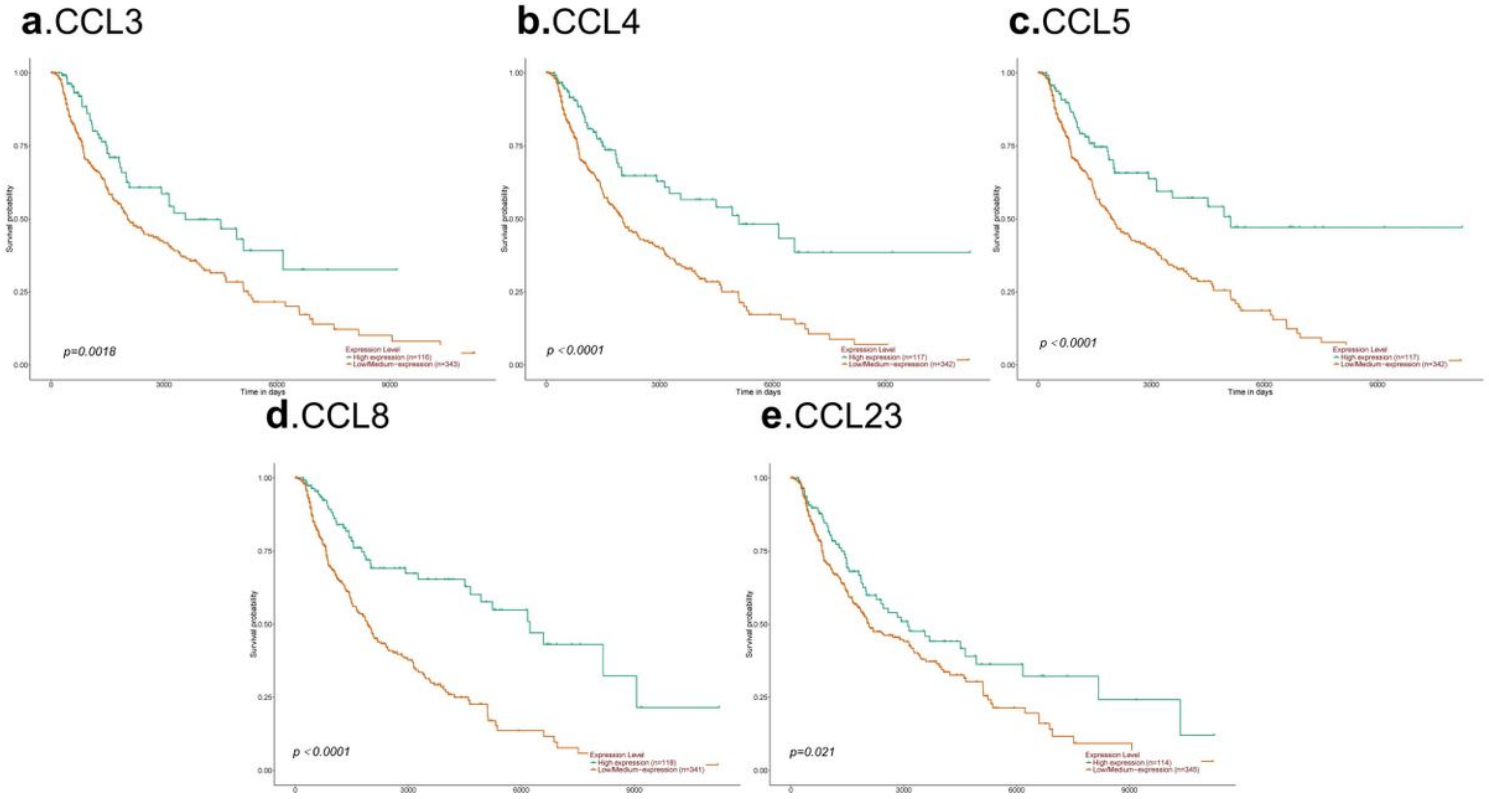

Figure 4

Association of differentially expressed CC chemokine expression with SKCM patients prognosis through UALCAN. The green line represents high levels of CC chemokine expression and the red line represents low levels of CC chemokine expression. The graphs show the CC chemokines that are statistically significant for SKCM prognosis, where we chose $p < 0.05$.

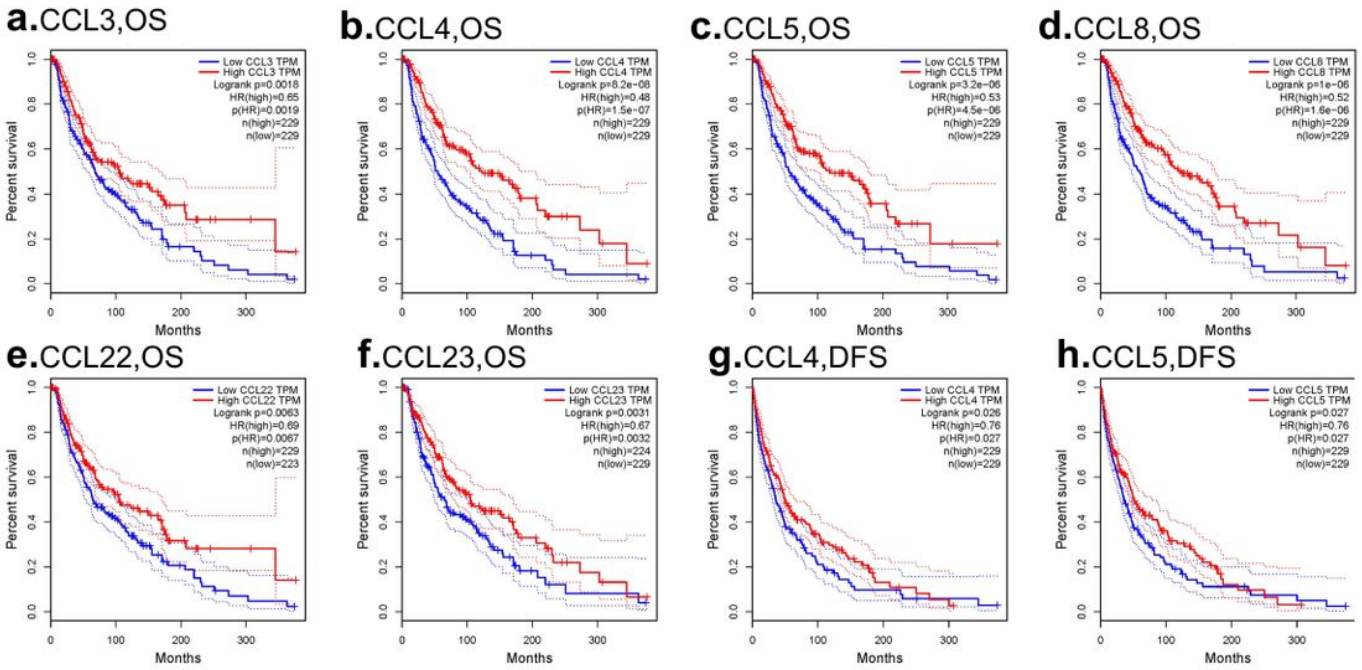

Figure 5

Prognostic value of different expression of CC chemokines in the OS curve and the DFS curve of SKCM patients through GEPIA. The graphs show the CC chemokines that are statistically significant for SKCM prognosis, in which we chose $p < 0.05$. OS: overall survival. DFS: disease free survival.

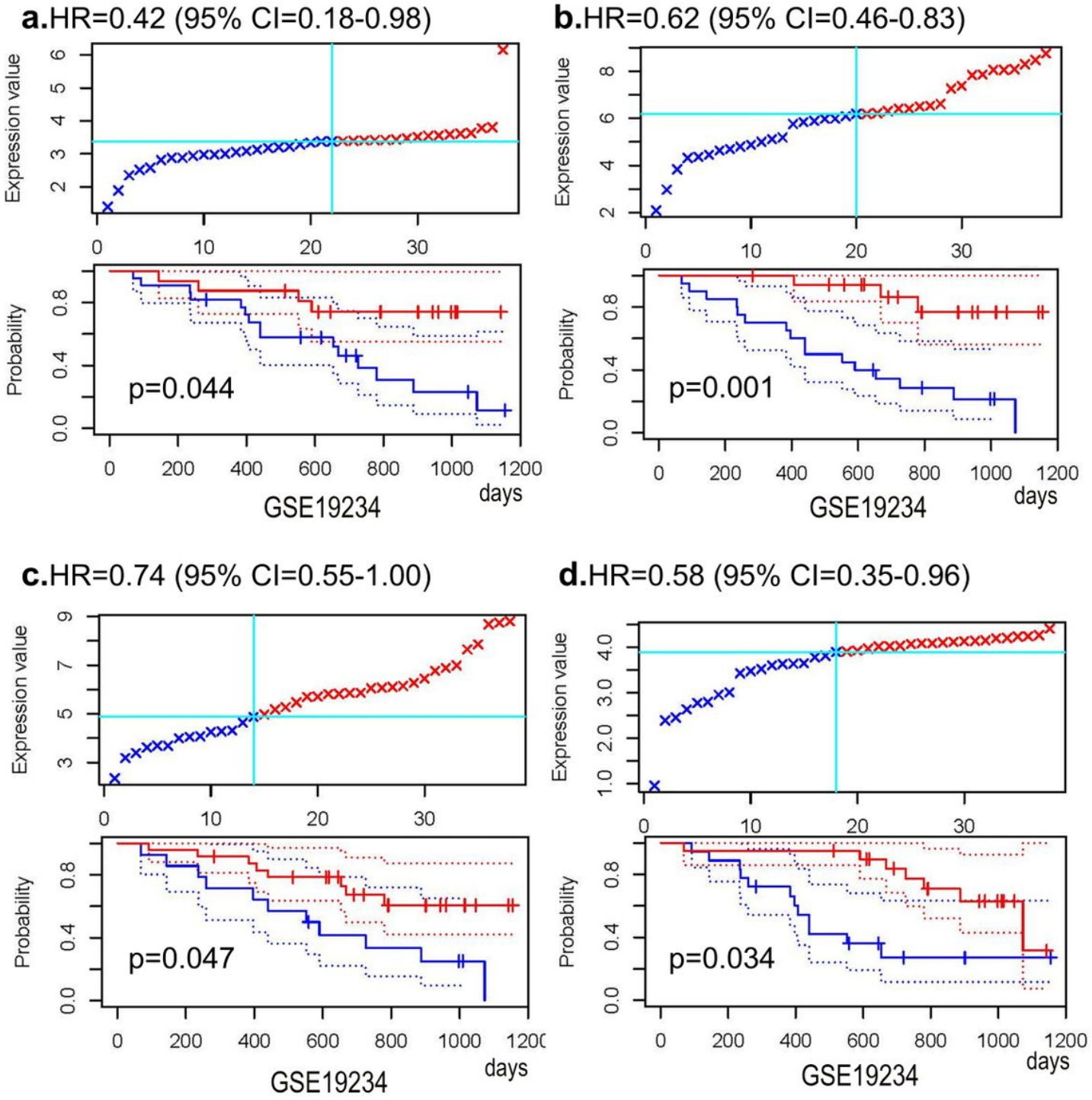

Figure 6

Prognostic value of different expression of CC chemokines in the overall survival curve of SKCM patients through Prognscan. The graphs show the CC chemokines that are statistically significant for SKCM prognosis, in which we chose $p < 0.05$.

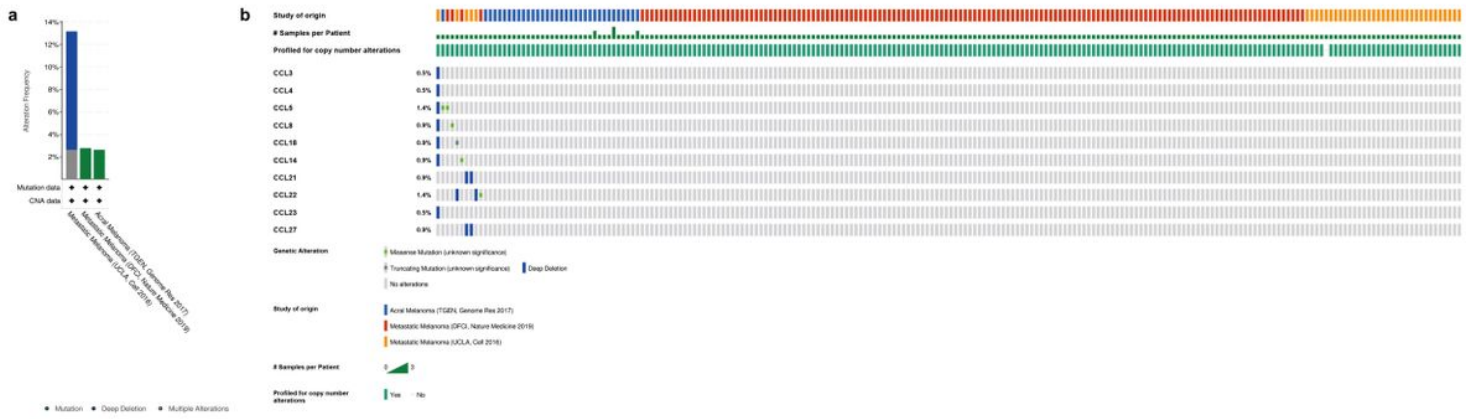

Figure 7

Genetic alterations of differentially expressed CC chemokines in SKCM patients through cBioPortal.

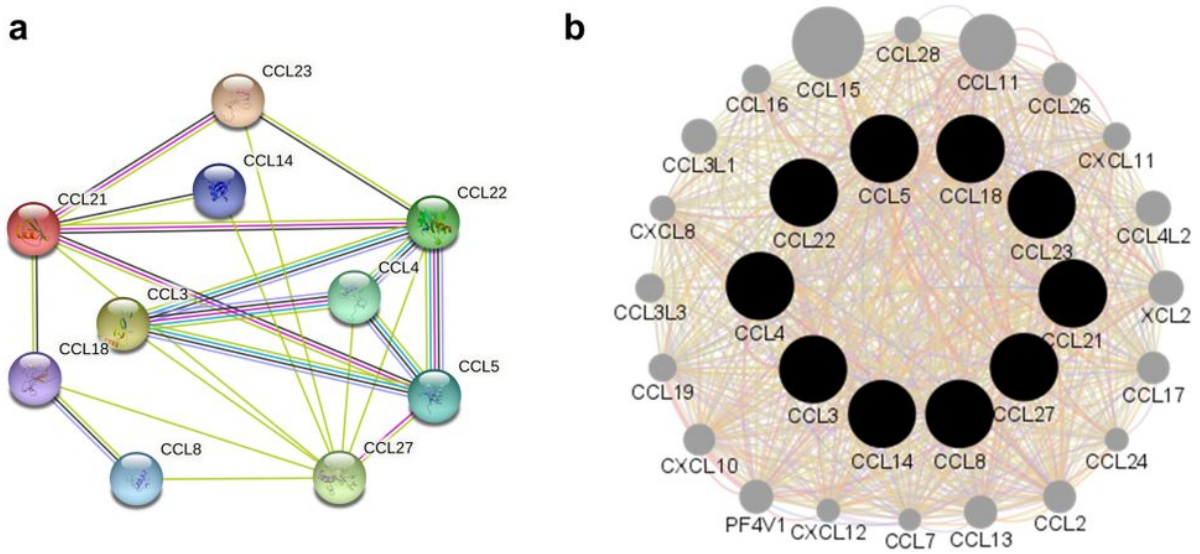

Figure 8

Protein-protein interaction network of differentially expressed CC chemokines through STRING and GeneMANIA. (a) STRING. **(b)** GeneMANIA.

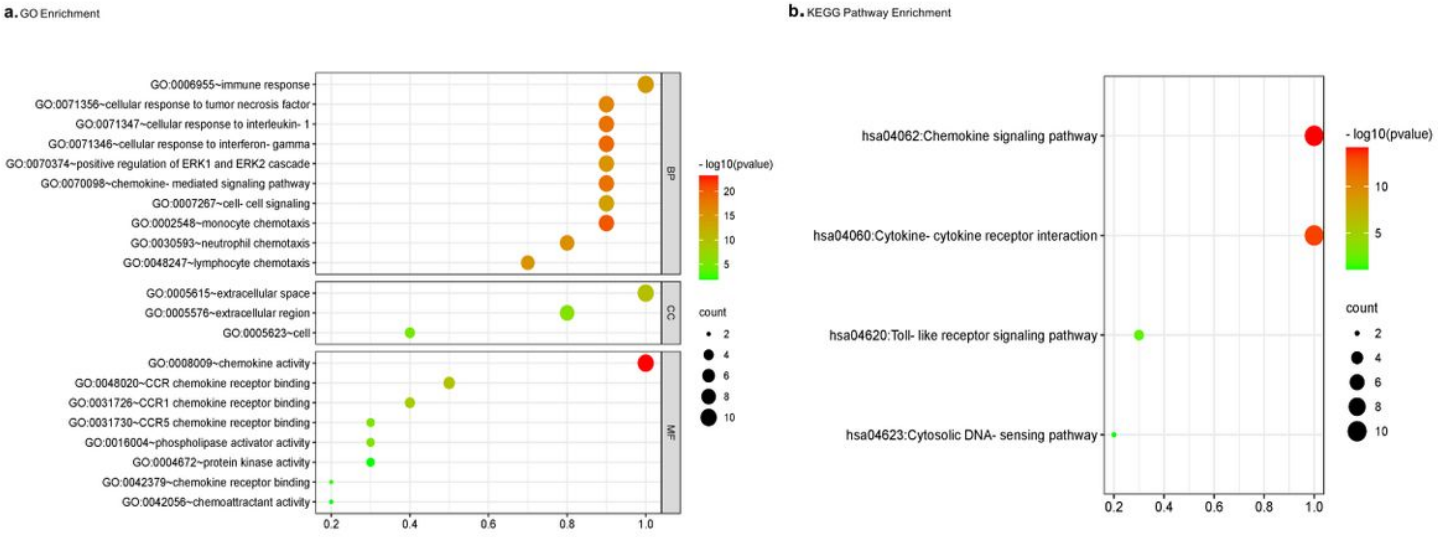

Figure 9

Enrichment analysis of differentially expressed CC chemokines and their neighboring genes in SKCM through Omicshare. (a-d) Bar graphs of GO enrichment in cellular component terms, biological process terms, and molecular function terms. **(e)** Bar graph of KEGG enrichment terms.

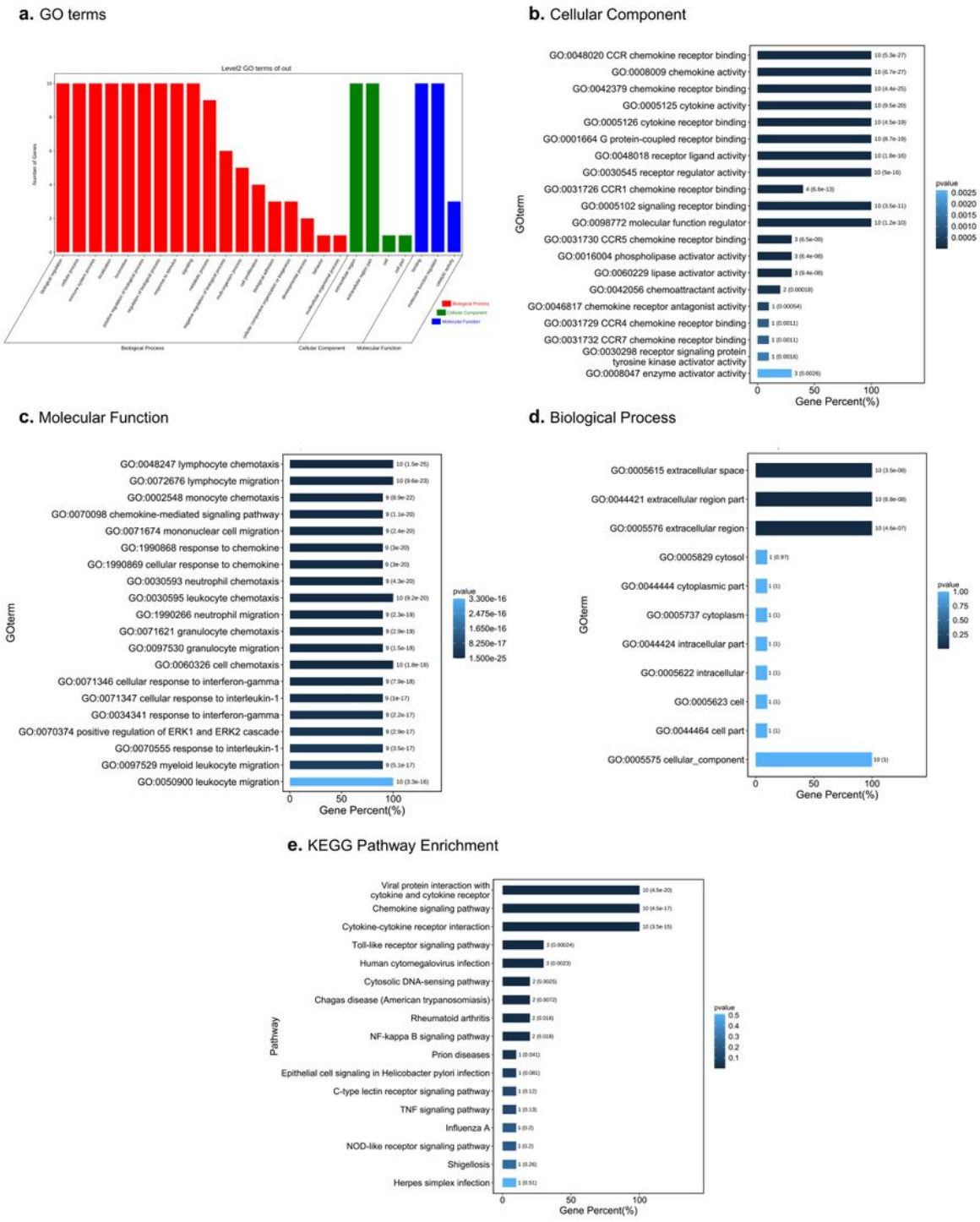

Figure 10

Enrichment analysis of differentially expressed CC chemokines and their neighboring genes in SKCM through David 6.8. (a) Bar graphs of GO enrichment in cellular component terms, biological process terms, and molecular function terms. **(b)** Bar graph of KEGG enrichment terms.

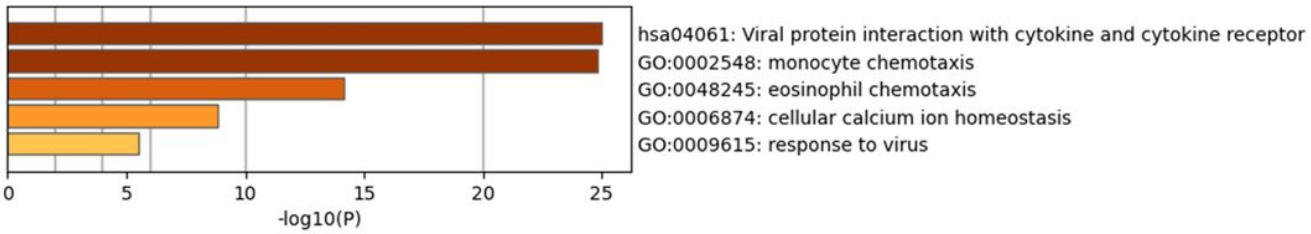

Figure 11

Enrichment analysis of differentially expressed CC chemokines and their neighboring genes in SKCM through Matescape. Bar graphs of GO enrichment in cellular component terms, biological process terms, molecular function terms and KEGG enrichment terms.

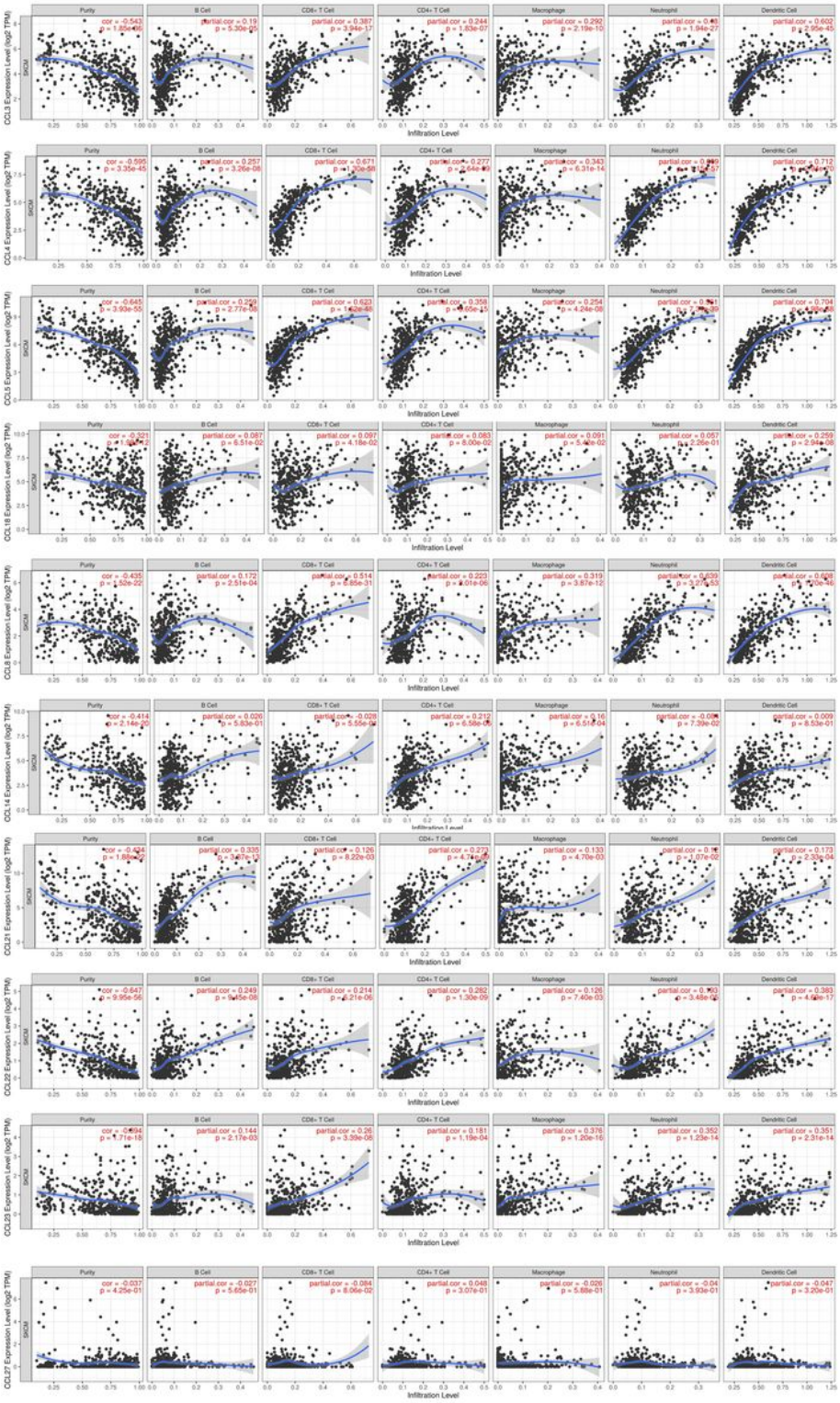

Figure 12

The correlation between different expressed CC chemokines and immune cell infiltration in SKCM through TIMER. The correlation between the abundance of immune cell and the expression of **(a)** CCL3, **(b)** CCL4, **(c)** CCL5, **(d)** CCL8, **(e)** CCL11, **(f)** CCL14, **(g)** CCL18, **(h)** CCL21, **(i)** CCL23, **(j)** CCL27 in SKCM.

Supplementary Files

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

- supplementaryfigure1.jpg
- supplementaryfigure2.jpg
- supplementaryfigure3.jpg
- supplementaryfigure4.jpg
- supplementaryfigure5.jpg
- supplementaryfigure6.jpg
- supplementaryfigure7.jpg
- supplementaryfigure8.jpg
- supplementaryfigure9.jpg