

Chemokine and Receptor Family Gene-Based Prognostic Signature for Predicting Clinical Outcomes and Treatment Response in Glioblastoma

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Background: Chemokines and receptor (CCR) genes are closely associated with tumorigenesis and progression. However, their function in the malignant progression of glioblastoma (GBM) is unknown. The present study aims to reveal prognostic factors, molecular subtypes, and prognostic indicators in chemokine genes and receptor genes in GBM.

Methods: In this study, expression profiles in The Cancer Genome Atlas (TCGA), The Chinese Glioma Genome Atlas (CGGA), and University of California Santa Cruz (UCSC xena) were utilized for expression analysis of chemokine/receptor genes in pan-cancer and GBM. Univariate COX models identified chemokine/receptor genes with prognostic value. Chemokine/receptor-related gene types in GBM were determined by consistency clustering analysis. This study constructed a prognostic model (CCR Score) using differentially expressed genes (DEGs) between genotypes. Differences between prognosis, tumor mutation burden (TMB), immune checkpoint genes, tumor microenvironment (TME), and drug sensitivity were explored in CCR Score groups and gene types.

Results: Expression models of chemokine/receptor genes were explored, and two differentially characterized genotypes (*CCR1* and *CCR2*) were identified in pan-cancer and GBM. *CCR1* and *CCR2* exhibited different prognoses, TMB, and TME activity. RTK-RAS (56.2%), PI3K (50.4%), TP53 (30.8%), NOTCH (28.8%), and Hippo (25.2%) pathways had a higher percentage of variants. Next, based on the DEGs in *CCR1* and *CCR2*, we constructed a prognostic model (CCR Score) for predicting the prognosis of GBM patients. The CCR Score showed excellent prognostic and predictive performance, and patients with high CCR Score and *CCR1* showed better immune activity and were more sensitive to immunotherapy. In addition, the CCR Score was significantly correlated with cancer chemotherapy sensitivity.

Conclusion: Overall, we identified the CCR characteristics of GBM patients' prognosis, and the CCR Score helps elucidate the potential link between GBM progression and chemokines/receptors and helps explore the mechanisms of carcinogenesis and therapeutic strategies.

Keywords: glioblastoma; chemokines; chemokines receptor; prognosis; CCR Score

Introduction

Chemokines and receptor family proteins actively influence the immune response in the tumor microenvironment (TME) and are significant regulators of the normal function of immune cells [1]. In tumor cells, chemokines and receptor family proteins exert oncogenic effects and directly influence tumor appreciation and metastasis [1]. Among them, inflammatory CC (*CCL2*, *CCL3*, *CCL5*) and CXC (*CXCL1*, *CXCL2*, *CXCL5*, *CXCL6* and *CXCL8*) have a two-sided role in tumors, where they mainly recruit *CCR2*⁺ monocytes and *CXCR2*⁺ neutrophils in TME, prompting their differentiation into tumor-associated macrophages (TAMs) and tumor-associated neutrophils

granulocytes (TANs) to exert anti-tumor or pro-tumor effects [2–5]. Several studies confirmed that chemokine receptors also play an important role in tumor growth and metastasis, and chemokines directly promote cancer cell value-added metastasis by binding to their receptors and activating pathways related to carcinogenesis [6–8]. For example, *CCL28* activated the MAPK/ERK pathway to promote malignant progression of breast cancer [9]. Understanding the interactions between chemokines and receptors will help develop targeted therapies for cancer [10,11].

Malignant neovascularization in TME and immunosuppression were important reasons why glioblastoma (GBM) was the most aggressive brain tumor [12–15]. Immune heterogeneity of TME in GBM promoted tumor an-

giogenesis and suppressed T cell function, thereby inhibiting immunotherapeutic response [16,17]. Interestingly, CC and CXC family chemokines played an important function in tumor angiogenesis, positively affecting tumor growth and metastatic spread [18,19]. It has been observed that *CCL2*, *CCL11*, *CCL16*, *CCL18*, and *CXCL8* promoted tumor angiogenesis and endothelial cell survival [20]. Additionally, the *CXCL16-CXCR6* axis was necessary for angiogenesis [21]. Whereas anti-angiogenic therapy was an emerging promising treatment in GBM [22], frustratingly, the molecular mechanisms between angiogenesis and chemokines/receptors in GBM are still unclear.

As studies have shown a potential link between chemokine/receptor genes and GBM metastasis, it is necessary to explore their relationship from a new perspective. In this study, we explored chemokine/receptor-related gene types and prognostic molecular features using transcriptome sequencing data and mutation data from GBM patients in public databases. The aim was to reveal the potential function of GBM and chemokine/receptor genes.

Method

Data Set Pre-Processing

High-throughput transcriptome sequencing data of GBM patients (The Cancer Genome Atlas-glioblastoma (TCGA-GBM), The Chinese Glioma Genome Atlas (CGGA)-325, CGGA-693) were obtained from The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>) website and the Chinese Glioma Genome Atlas (CGGA, <http://www.cgga.org.cn/>) database. Samples with missing survival information and survival status were excluded from the online portal Sangerbox (<http://sangerbox.com/home.html>) [23]. Finally, 153, 137, and 153 tumor samples were retained in TCGA-GBM, CGGA-325, and CGGA-693, respectively, for the follow-up study. This study used the TCGA-GBM cohort as the training set and the CGGA-325 and CGGA-693 cohorts as the validation set. Survival data for 32 cancers were obtained from the University of California Santa Cruz (UCSC xena, <http://xena.ucsc.edu/>) database, while data related to single nucleotide variants (SNV) and copy number variations (CNV) were downloaded for GBM patients. The chemokine/receptor gene set was sourced with the study of Ru *et al.* [24].

Pan-Cancer Analysis

In bladder urothelial carcinoma (BLCA); breast invasive carcinoma (BRCA); cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC); cholangiocarcinoma (CHOL); colon adenocarcinoma (COAD); lymphoid neoplasm diffuse large B-cell lymphoma (DLBC); esophageal carcinoma (ESCA); glioblastoma multiforme (GBM); head and neck squamous cell carcinoma (HNSC); kidney chromophobe (KICH); kidney renal clear cell carcinoma (KIRC); kidney renal papillary cell carcinoma (KIRP); acute myeloid leukemia (LAML); lower-grade glioma (LGG); liver hepatocellular carcinoma (LIHC); lung adenocarcinoma (LUAD); lung squamous cell carcinoma (LUSC); mesothelioma (MESO); ovarian serous cystadenocarcinoma (OV); pancreatic adenocarcinoma (PAAD); pheochromocytoma and paraganglioma (PCPG); prostate adenocarcinoma (PRAD); rectum adenocarcinoma (READ); sarcoma (SARC); skin cutaneous melanoma (SKCM); stomach adenocarcinoma (STAD); testicular germ cell tumors (TGCT); thyroid carcinoma (THCA); thymoma (THYM); uterine corpus endometrial carcinoma (UCEC); uterine carcinosarcoma (UCS); uveal melanoma (UVM), the expression of chemokine/receptor genes in tumor tissue and normal tissue was assessed. The correlation between chemokine/receptor gene was calculated by Spearman correlation.

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Prognostic Analysis of Chemokine/Receptor Genes in Pan-Cancer

Univariate COX model analysis of chemokine/receptor genes was performed to identify which affected cancer prognosis based on survival data from the UCSC xena database for pan-cancer. Subsequently, chemokine/receptor genes influencing prognosis were identified in 32 cancers.

Mutational Landscape Analysis of Chemokine/Receptor Genes

Based on SNV data from the TCGA-GBM cohort, we calculated the tumor mutation burden (TMB) of GBM samples using the Maftools software package (version 2.8.05, National University of Singapore, Singapore) [25]. The correlation between TMB and chemokine/receptor gene expression was calculated using the Spearman correlation. Finally, the enrichment of 10 oncogenic pathways in the TCGA-GBM cohort was calculated by the oncogenic pathways function of the Maftools package.

Molecular Landscape Analysis of Chemokine/Receptor Gene-Types

In this study, a consistency matrix was constructed based on the chemokine/receptor gene expression matrix for samples in the TCGA-GBM cohort to identify genetic subtypes associated with chemokine/receptor gene expression in GBM, referring to the method of Wilkerson *et al.* [26]. For the clustering results, validation was performed in the CGGA325 cohort. For patients with chemokines and receptor (CCR) genotypes, prognostic differences were assessed using Kaplan-Meier (K-M) survival curves. CNV locus differences in GBM were evaluated by the gisticOncoPlot function in the software package Maftools, and this study demonstrates the mutation frequency of genes at loci with mutations top10 in GBM.

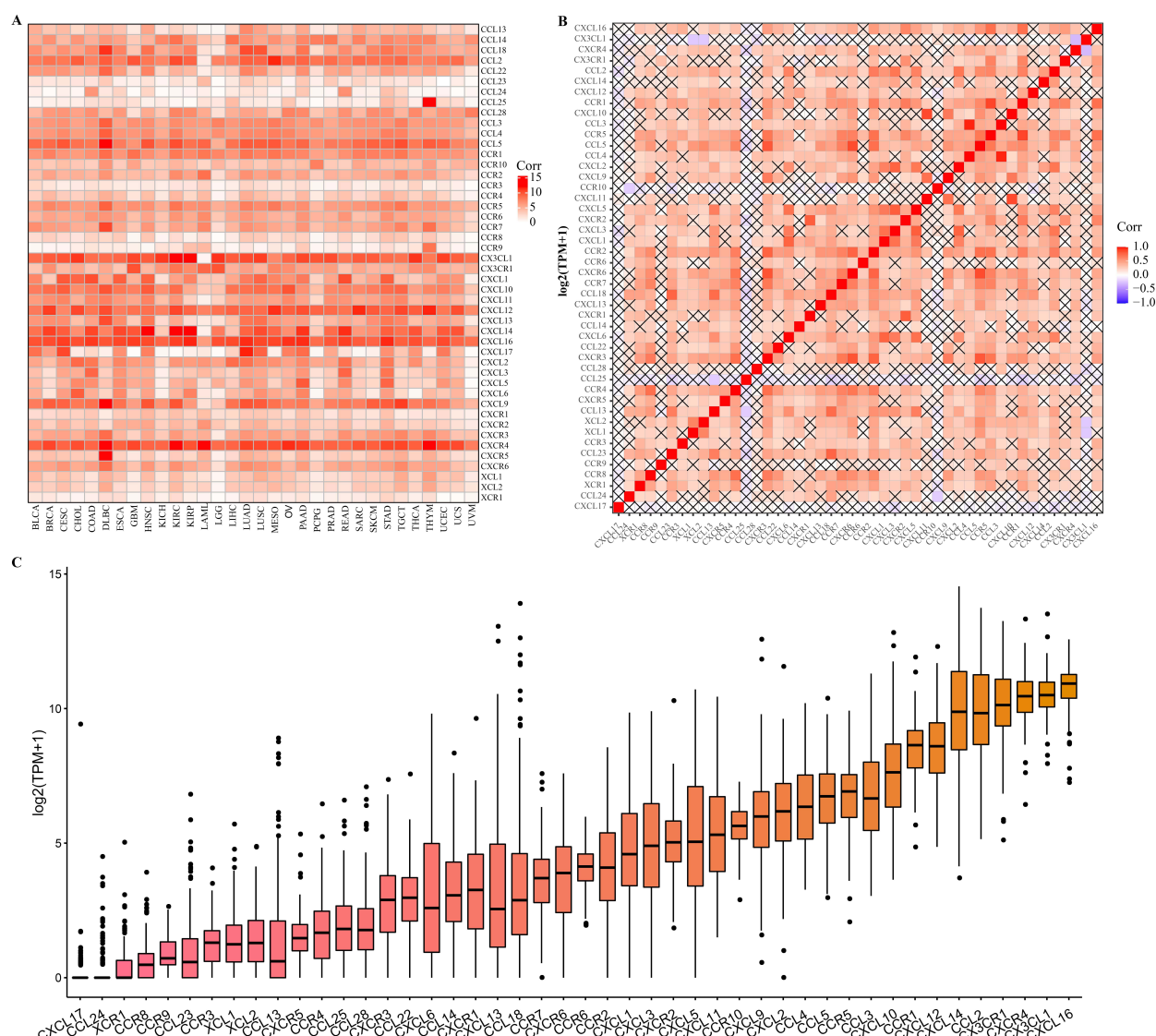


Fig. 1. Expression analysis of chemokine/receptor gene in pan-cancer. (A) Expression levels of chemokine/receptor genes in pan-cancer. The color represents the expression level; the darker the color, the higher the expression level. (B) Spearman correlation between chemokine/receptor gene expression. The × in the figure represents that the correlation between two genes is not significant, the red color represents that there is a positive correlation between two genes, and the blue color represents that there is a negative correlation between two genes. The darker the color, the higher the correlation. (C) Expression levels of chemokine/receptor genes in the The Cancer Genome Atlas-glioblastoma (TCGA-GBM) cohort.

Immune Landscape Analysis of CCR Gene-Types

In the TCGA-GBM cohort, the anti-cancer immune status of GBM patients was assessed in Tracking Tumor Immunophenotype (TIP, <http://bioacc.hrbmu.edu.cn/TIP/>), an online database for assessing tumor immune phenotypes, which can be used to assess cancer patients' immune status by assessing seven immune cycles (Step 1: release of cancer cell antigens; Step 2: cancer antigen presentation; Step 3: priming and activation; Step 4: trafficking of immune cells to tumors; Step 5: infiltration of immune cells into tumors; Step 6: recognition of cancer cells by T cells; Step 7: killing of cancer cells) to determine their immune signature gene expression levels in the anti-cancer immune

status [27]. Using the ESTIMATE method, we assessed patients' StromalScore, ImmuneScore, and ESTIMATEScore in CCR1 and CCR2 [28]. Finally, in the study of Charoentong *et al.* [29] downloading 28-signatures predicting immune checkpoint blockade response, the enrichment score of 28 signatures was assessed using the single sample gene set enrichment analysis (ssGSEA) method to assess the immune scores of immune cells in CCR1 and CCR2 [30].

CCR Score Construction and Validation

In the TCGA-GBM and CGGA325 cohorts, differential analysis of CCR1 and CCR2 was performed using the R package limma (version 3.42.2, Bioinformatics Di-

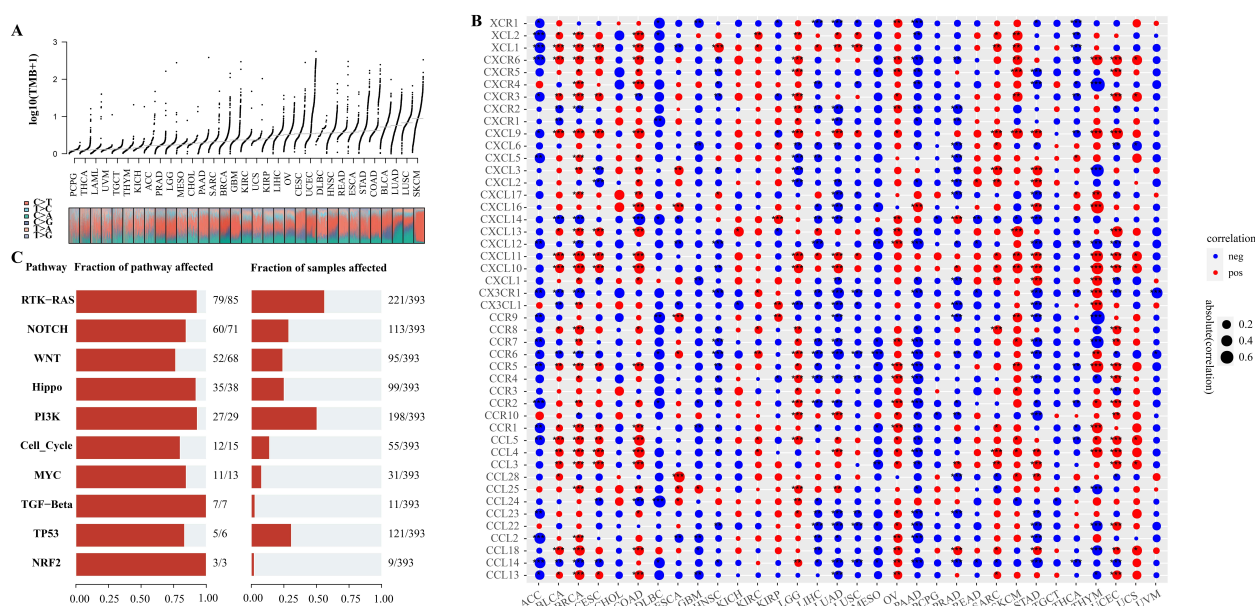


Fig. 3. Mutational landscape of chemokine/receptor genes. (A) Tumor mutation burden (TMB) statistics in pan-cancer. (B) Correlation of TMB with chemokine/receptor gene expression. (C) Variations of oncogenic pathways in the TCGA-GBM cohort. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the CCR Score by Spearman correlation ($p < 0.05$). Score significantly associated pathways ($p < 0.05$, $|\text{cor}| > 0.6$). In addition, we compared the ssGSEA Score of 28 signatures predicting immune checkpoint blockade responses.

Association between Immunological Characteristics of CCR Score Groups

Tumor-reactive T cell signature (TRS) is a newly discovered biomarker for determining tumor responsiveness and plays an important role in predicting immunotherapy response in tumor patients [35]. TRS scores of patients in *CCR1*, *CCR2*, and CCR Score groups were calculated using the ssGSEA method. We also compared the TMB differences between patients in the *CCR1*, *CCR2*, and CCR Score subgroups. In addition, T-cell-inflamed GEP signatures predicting the sensitivity of cancer individuals to pembrolizumab treatment and immunoreactive-related Th1/IFN γ gene signatures were from previous study, T-cell-inflamed GEP score and Th1/IFN γ gene signatures score were contained by the ssGSEA method [36,37].

CCR Score Predicts GBM Drug Sensitivity Analysis

In this study, sensitivity information for 51 conventional therapeutic agents for GBM was obtained from the website of The Genomics of Drug Sensitivity in Cancer (GDSC, <https://www.cancerrxgene.org/>) [38]. The half maximal inhibitory concentration (IC_{50}) representing the drug response was calculated in the TCGA-GBM cohort by the R package pRRhetic (version 0.5, University of Chicago, Chicago, IL, USA) [39].

Results

Expression Analysis of Chemokine/Receptor Gene in Pan-Cancer and Prognostic Significance

The heatmap of 46-chemokine/receptor gene expression levels in 32 cancers in TCGA was presented in Fig. 1A, which showed that 46-chemokine/receptor genes demonstrated different expression patterns in pan-cancer, showing tumor heterogeneity. Next, we showed the Spearman correlation between chemokine and receptor gene expression levels in the TCGA-GBM cohort. We found a positive correlation between most gene expressions (Fig. 1B). The expression levels of chemokine/receptor genes are shown in Fig. 1C. Further, in the pan-cancer univariate COX model analysis, *CCL2*, *CCL5*, *CCL13*, *CXCL5*, *CXCL6*, *CXCL13*, *CCR6*, *CCR7*, *CCR8* significantly affected cancer patients' overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) (Fig. 2). We also focused on the association of 9-prognosis-related genes of 33 cancers in the TCGA database. We observed that 9-prognosis-related genes significantly influenced the prognosis of GBM patients (Supplementary Fig. 1).

Mutational Landscape of Chemokine/Receptor Genes

We calculated the TMB for cancer patients from the SNV data of 33 cancers in the TCGA database and observed that C>T and C>A mutations are the most common in pan-cancer (Fig. 3A). Spearman correlation between TMB and chemokine/receptor gene expression for 33 cancers in TCGA is demonstrated in Fig. 3B. A negative correlation between GBM and the majority of chemokine/receptor

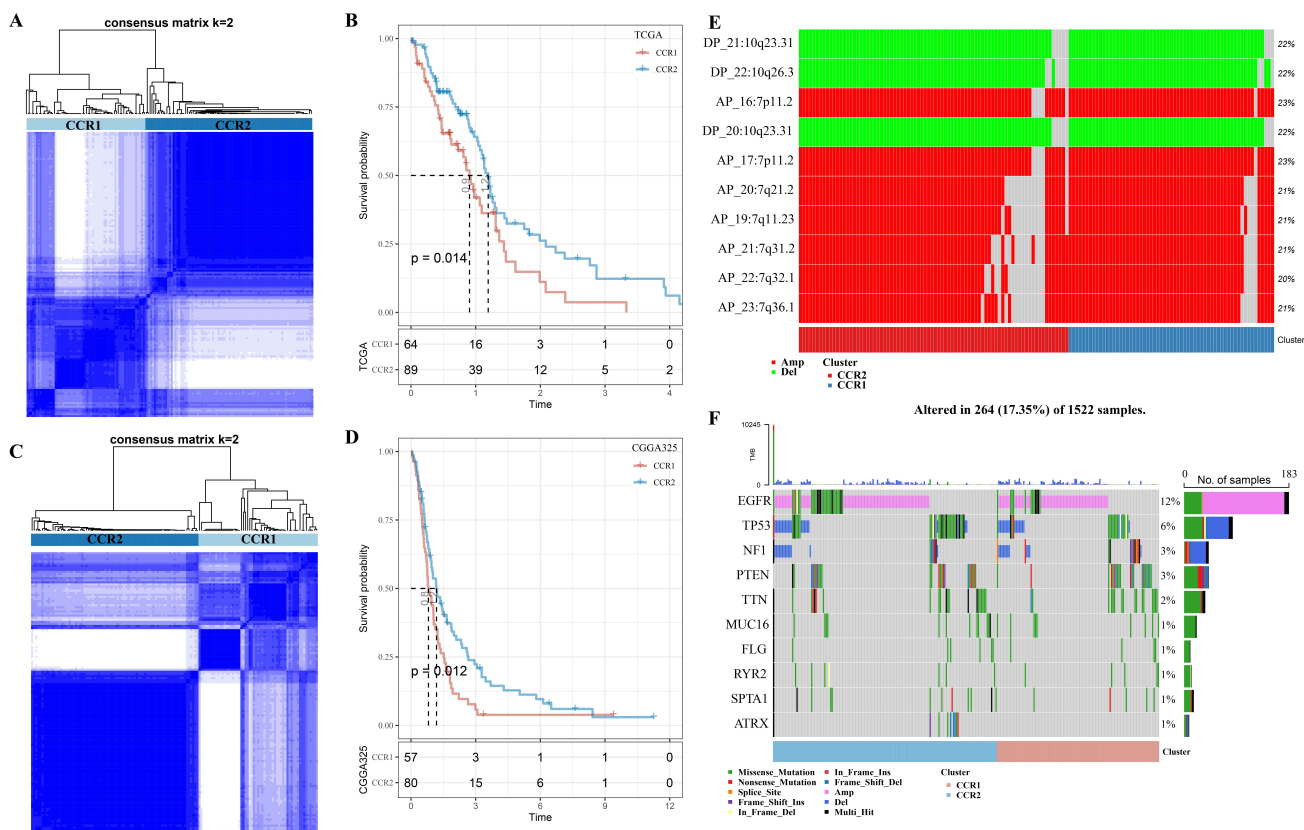


Fig. 4. Molecular landscape of chemokine/receptor in gene-types. (A) Consistent clustering heat map of *CCR1* and *CCR2* in TCGA-GBM cohorts. (B) Kaplan-Meier (K-M) survival curves of *CCR1* and *CCR2* samples in TCGA-GBM cohorts. (C) Consistent clustering heat map of *CCR1* and *CCR2* in CGGA325 cohorts. (D) K-M survival curves of *CCR1* and *CCR2* samples in CGGA325 cohorts. (E) Top 10 copy number variations (CNV) loci for mutation frequency. (F) Top 10 genes for mutation frequency.

genes could be observed from the heatmap. Finally, we calculated the variant enrichment of 10 oncogenic pathways in the TCGA-GBM cohort. The results showed high mutation rates in NRF2 (100%), TGF-Beta (100%), PI3K (93.1%), RTK-RAS (92.9%), and Hippo (92.1%) pathways. The rates of mutations in RTK-RAS (56.2%), PI3K (50.4%), TP53 (30.8%), NOTCH (28.8%), and Hippo (25.2%) pathways were higher in GBM patients (Fig. 3C).

Molecular Landscape of Chemokine/Receptor in Gene-Types

Consistent clustering of chemokine/receptor genes on tumor samples in the TCGA-GBM cohort showed that GBM samples were classified into *CCR1* and *CCR2* genotypes (Fig. 4A). K-M survival curves showed a significant difference in OS between *CCR1* and *CCR2* patients, with *CCR2* patients having a better prognosis (Fig. 4B). Further, we validated the *CCR1* and *CCR2* genetic subtypes in the CGGA325 cohort. It was observed that the tumor samples in the CGGA325 cohort could also be classified into *CCR1* and *CCR2* genotypes, and the OS of GBM patients in *CCR2* was superior to that of *CCR1* (Fig. 4C,D). These results suggest that gene types related to chemokine/receptor gene expression exist in GBM, and

these could complement the existing molecular typing of GBM, providing new insights into diagnosing GBM. Next, in the TCGA-GBM cohort, we showed the CNV statistics of the top 10 loci in *CCR1* and *CCR2* patients, and the results showed a concordance of CNV for 10 loci in *CCR1* and *CCR2*, with *AP_16:7p11.2*, *AP_17:7p11.2*, *AP_20:7q21.2*, *AP_19:7q11.23*, and *AP_21:7q31.2*, *AP_22:7q32.1*, *AP_23:7q36.1* being amplified and *DP_21:10q23.31*, *DP_22:10q26.3*, *DP_20:10q23.31* being deleted (Fig. 4E). In addition, we also found that the top 10 genes with mutation frequency in GBM were *EGFR*, *TP53*, *NF1*, *PTEN*, *TTN*, *MUC16*, *FLG*, *RYR2*, *SPTA1*, *ATRX*, and Missense_Mutation and amplification were the most common mutation types (Fig. 4F).

Immune Landscape of CCR Gene-Types

To investigate the immune landscape in *CCR1* and *CCR2*, we assessed the anti-cancer immune status of the tumor immune cycle in GBM patients in TIP, and we observed that *CCR1* patients were more in an immune activation state (Fig. 5A). Also, *CCR1* had a higher Immunoactivity score (Fig. 5B). Further, ESTIMATE results showed that *CCR1* patients had higher StromalScore, ImmuneScore, and ESTIMATEScore, indicating that *CCR1* had higher im-

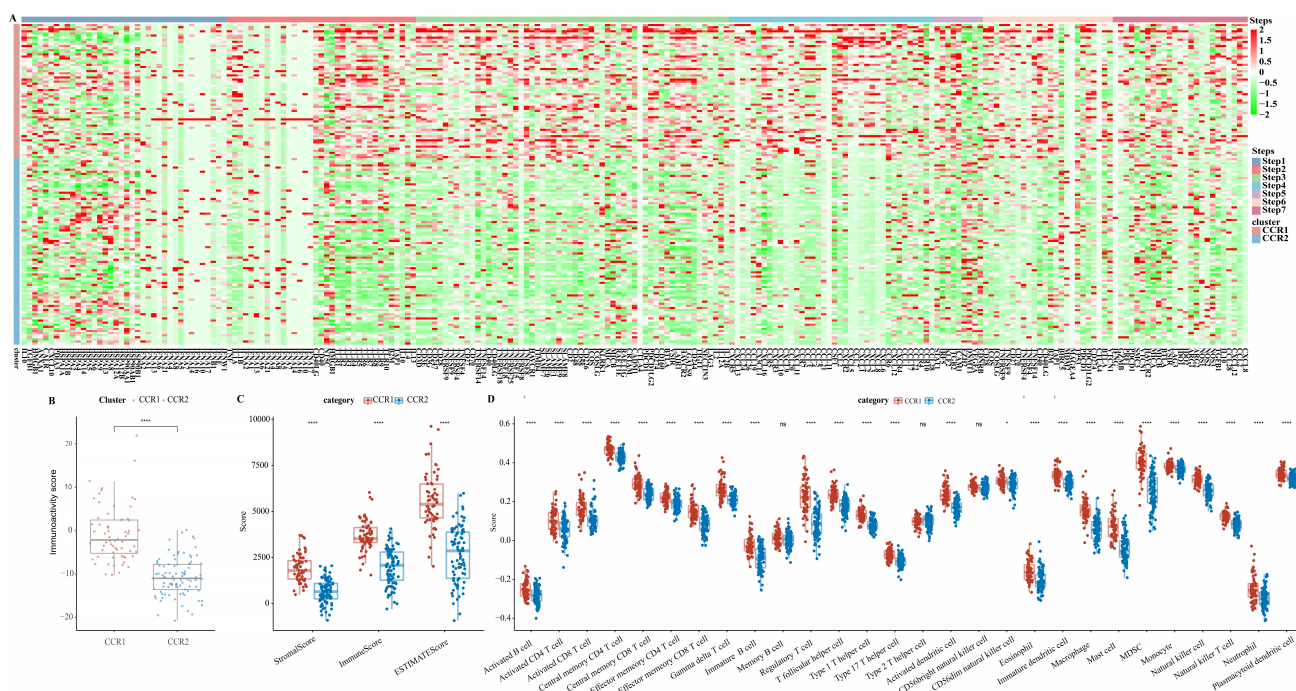


Fig. 5. Immune landscape of chemokines and receptor (CCR) gene-types. (A) Tumor immune cycle activity of glioblastoma (GBM) samples. (B) Immunoactivity score. (C) ESTIMATE results. (D) ImmuneScore of 28-immune cells. * $p < 0.05$, *** $p < 0.0001$, ns, no statistical difference.

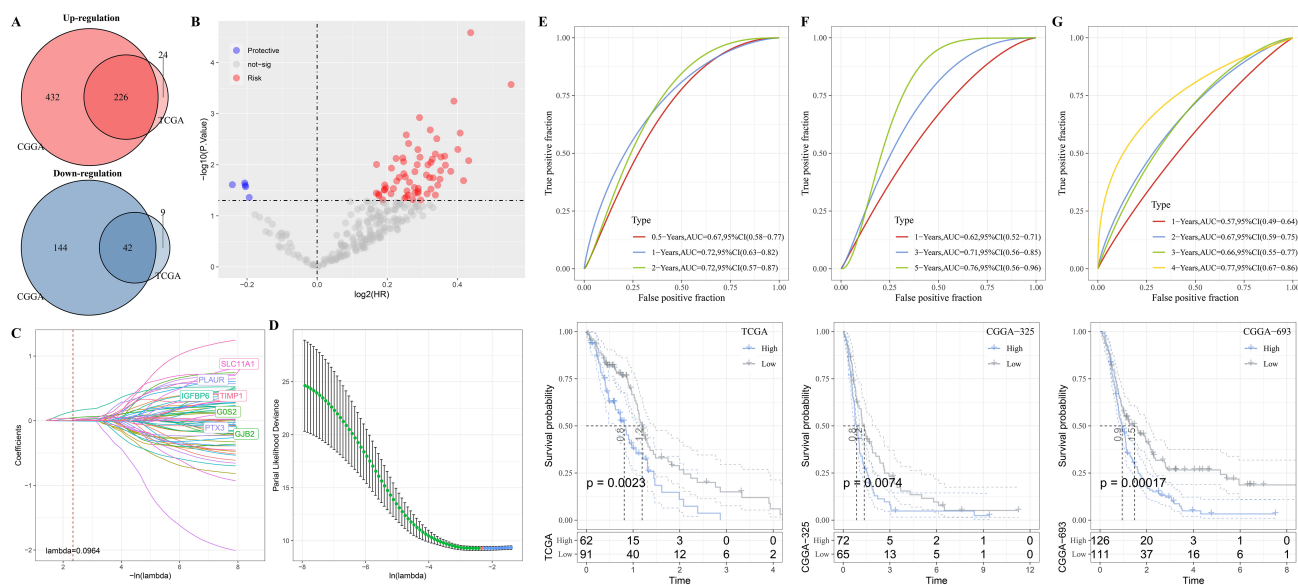
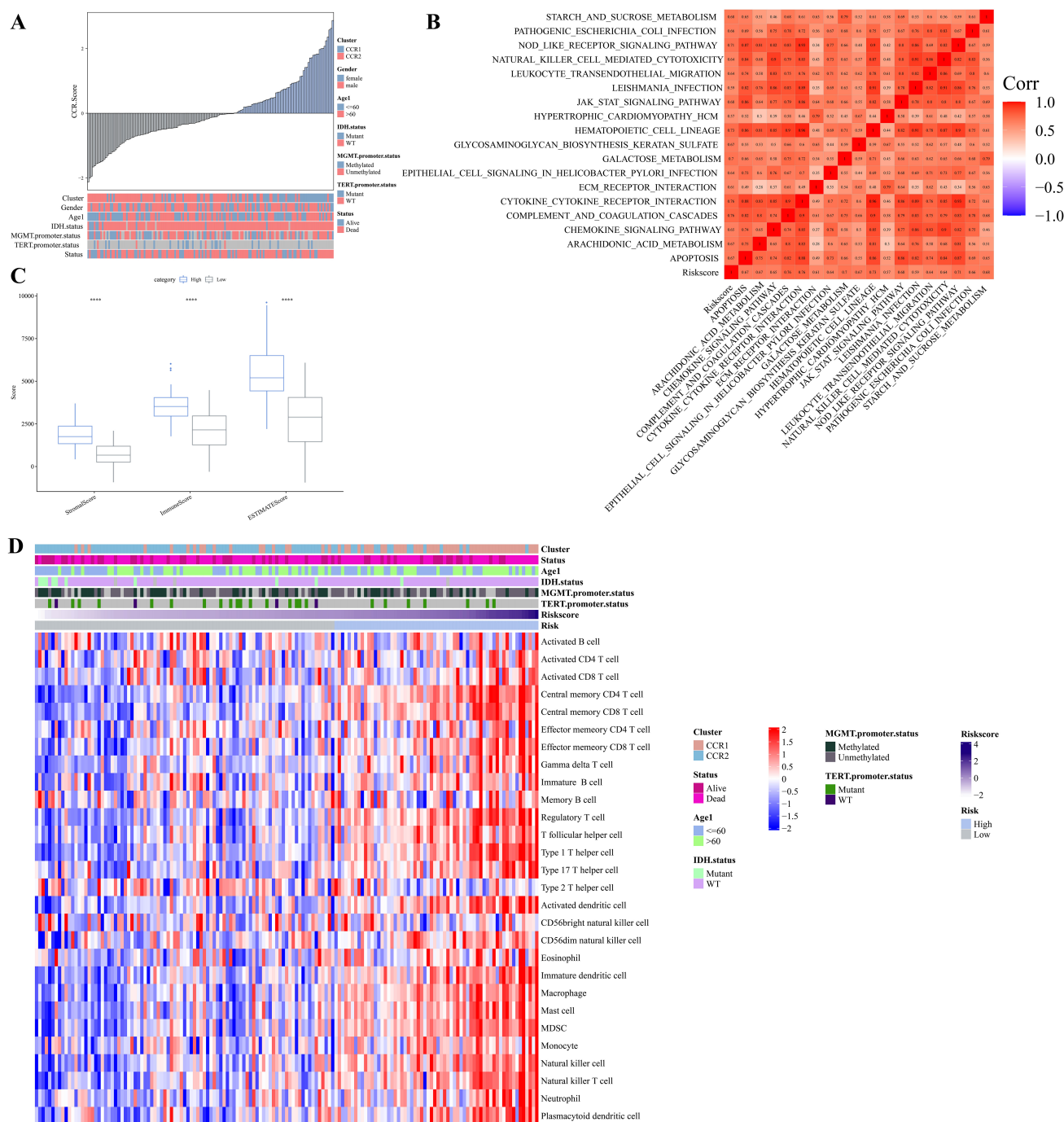


Fig. 6. Construction and validation of CCR Score. (A) Venn diagram of overlapping differentially expressed genes (DEGs). (B) Univariate COX model analysis of overlapping DEGs, 69 GBM prognosis-related DEGs existed among them. The blue dots were protective factors, and the red dots were risk factors. (C) Trajectory of change in independent variables. (D) The confidence interval of lambda. (E–G) K-M survival curves and ROC curves in the CCR Score subgroup of TCGA-GBM, CGGA325, and CGGA693 cohorts.

immune infiltration (Fig. 5C). Finally, the expression levels of immune cell characteristic genes in GBM were assessed by the ssGSEA method in 28, and the results showed that most of the immune cells in *CCR1* had higher ImmuneScores than *CCR2* (Fig. 5D).

Construction and Validation of CCR Score

In the TCGA-GBM and CGGA325 cohorts, 268 overlapping DEGs in *CCR1*_VS_*CCR2* ($\log_2FC > 1$, $FDR < 0.05$) contained 226 upregulated DEGs and 42 downregulated DEGs (Fig. 6A). CCR Score was constructed for predicting GBM prognosis. Univariate COX regression model



analysis combined with survival information of patients in TCGA-GBM revealed that 69 DEGs were significantly associated with GBM prognosis, 5 of the DEGs were protection factors, and 64 DEGs were risk factors (Fig. 6B) (Table 1) ($p < 0.05$). In the LASSO COX model, the model fit was optimal at $\lambda = 0.0964$ when *PLAUR*, *PTX3*, *SLC11A1*, *TIMP1*, *IGFBP6*, *G0S2*, and *GJB2* were

included (Fig. 6C,D). We calculated the regression coefficients of these 7 genes by the multifactorial COX model, and finally, we established the CCR Score to predict the prognosis of GBM, $\text{CCR Score} = -0.045 \times \text{PLAUR} + 0.046 \times \text{PTX3} + 0.103 \times \text{SLC11A1} - 0.006 \times \text{TIMP1} + 0.229 \times \text{IGFBP6} + 0.089 \times \text{G0S2} + 0.06 \times \text{GJB2}$. GBM patients in the TCGA-GBM, CGGA325, and CGGA693 cohorts were

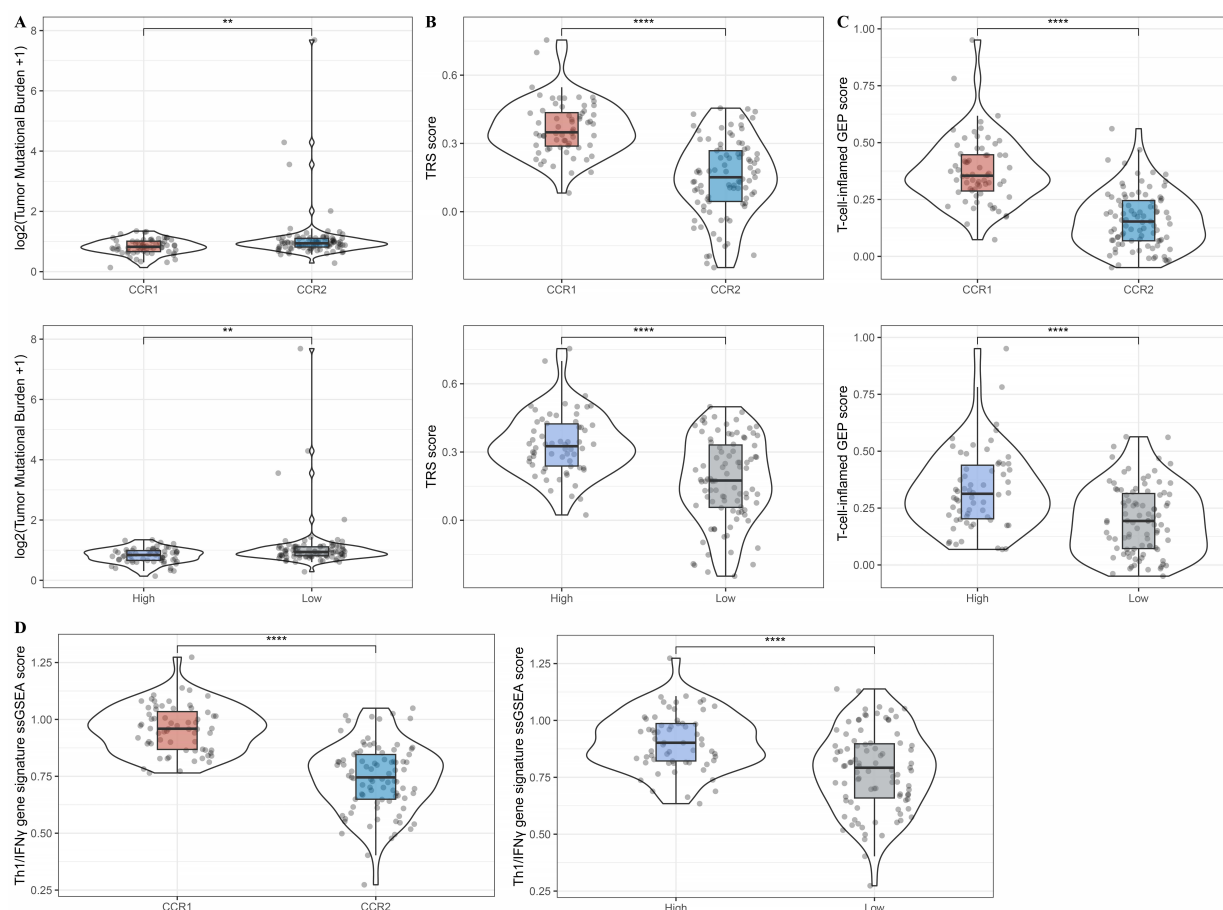


Fig. 8. Immunological characterization of the CCR Score groups. (A) TMB levels of patients in the CCR1/CCR2 and high/low CCR Score groups. (B) Tumor-reactive T cell signature (TRS) score of patients in the CCR1/CCR2 and high/low CCR Score groups. (C) T-cell-inflamed GEP score of patients in the CCR1/CCR2 and high/low CCR Score groups. (D) Th1/IFN gene signature single sample gene set enrichment analysis (ssGSEA) score of patients in the CCR1/CCR2 and high/low CCR Score groups. ** $p < 0.01$, **** $p < 0.0001$.

classified into high-CCR Score and low-CCR Score groups. K-M survival curves and ROC curves were conducted to assess the prognostic, predictive performance of the CCR Score. We found that GBM patients with high CCR Scores in the three cohorts had significantly worse OS than those with low CCR Scores, and the CCR Score predicted 0.5-year, 1-year, and 2-year survival in the TCGA-GBM cohort with AUC values of 0.67, 0.72, and 0.72, respectively. 1-year, 3-year, and 5-year AUC values in CGGA325 were 0.62, 0.71, and 0.76, respectively. The AUC values for 1, 2, 3, and 4 years in CGGA693 were 0.57, 0.67, 0.66, and 0.77, respectively (Fig. 6E–G). Overall, the CCR Score showed good AUC values in predicting GBM prognosis. Therefore, the CCR Score was reliable in predicting GBM prognosis.

Differences between the Clinical and Biological Functions of the CCR Score

In Fig. 7A, we found the association between CCR Score distribution and CCR gene-types, Gender, Age, IDH.status, MGMT promoter status, TERT promoter sta-

tus, status by heat map. We found that the CCR Score was higher in CCR1 patients. 18 pathways in GBM were shown to be significantly and positively correlated with the CCR Score in GSVA analysis ($p < 0.05$, $|\text{cor}| > 0.6$) (Fig. 7B). ESTIMATE results showed that patients with high CCR Scores had StromalScore, ImmuneScore, and ESTIMATEScore, which were significantly higher in patients with high CCR Scores than in those with low CCR Scores (Fig. 7C). Similarly, we found higher ImmuneScores for 28 immune cells in patients with high CCR Scores (Fig. 7D).

Immunological Characterization of the CCR Score Groups

The TMB status of patients was systematically evaluated in the CCR1, CCR2, and CCR Score subgroups, and we observed that patients with low CCR Score and CCR2 had significantly higher TMB (Fig. 8A). The results of the TRS analysis showed that high CCR Score and CCR1 patients had higher TRS scores, indicating that the

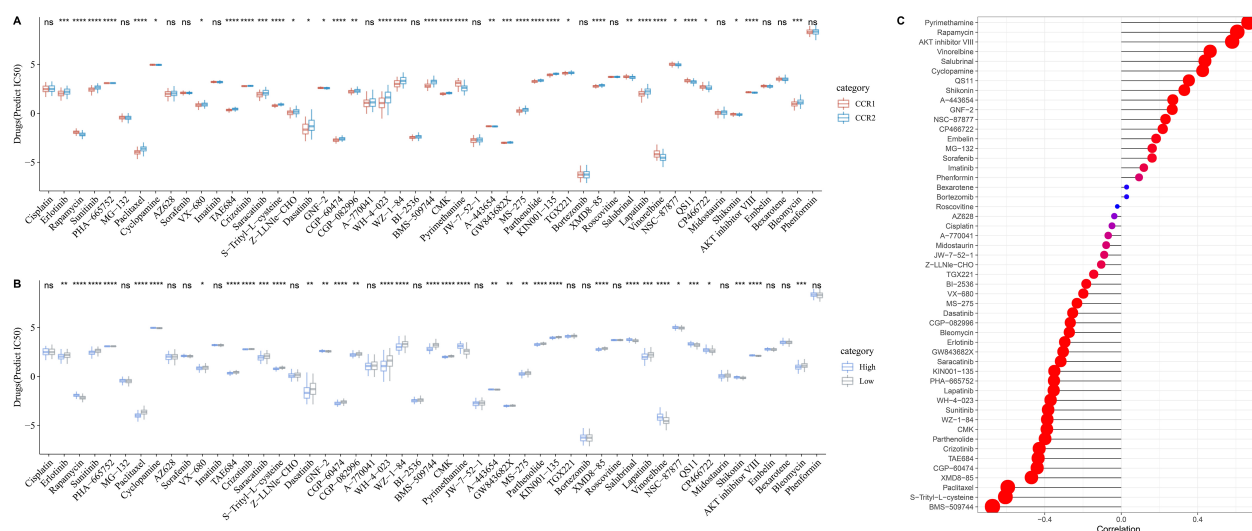


Fig. 9. CCR Score could predict GBM drug response. (A,B) The half maximal inhibitory concentration (IC₅₀) of oncology therapeutics in CCR genotypes and CCR Score groups. (C) Correlation of CCR Score with IC₅₀. The points to the right of line 0 in the figure show a positive correlation between the IC₅₀ value of the drug and the CCR Score. The point to the left of line 0 shows a negative correlation between the IC₅₀ value of the drug and the CCR Score. The size of the dots represents the magnitude of the correlation. The red and blue colors represent the significance of the statistical analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, no statistical difference.

patients therein showed better adaptation to immunotherapy (Fig. 8B). The results of the TRS analysis revealed that patients with high CCR Scores and *CCR1* had higher TRS scores, indicating that patients among them showed better adaptation to immunotherapy (Fig. 8B). Notably, patients with high CCR and *CCR1* also showed better T-cell-inflamed GEP scores and Th1/IFN gene signature ssGSEA scores (Fig. 8C,D). These results further suggest that high CCR Scores and *CCR1* patients have an activated immune state and are more sensitive to immunotherapy.

CCR Score could Predict GBM Drug Response

We further investigated whether the CCR Score can predict drug response in GBM patients. We found that in the TCGA-GBM cohort, both *CCR1* and *CCR2* patients and patients in the CCR Score subgroup responded to Erlotinib, Rapamycin, Sunitinib, PHA-66752, Paclitaxel, Cyclopamine, VX-680, TAE684, Imatinib Saracatinib, S-Trityl-L-cysteine, Z-LNle-CHO, Dasatinib, GNF-2, CGP-60474, CGP-082996, WH-4-023, WZ-1-84, BMS-509744, CMK, Pyriethamine, A-443654, GW843682X, MS-275, Parthenolide, KIN001-135, TGX221, XMD8-85. Salubrinal, Lapatinib, Vinorelbine, NSC87877, QS11, CP466722, Shikonin, AKT inhibitor VIII, and Bleomycin drugs produced responses (Fig. 9A,B). The CCR Score was significantly correlated with these drugs by Spearman correlation (Fig. 9C). These results suggest that the CCR Score correlates with sensitivity to the above drugs.

Discussion

In this study, we performed a comprehensive bioinformatics analysis of chemokine/receptor gene expression, prognosis, immunological features, and genomic characteristics in pan-cancer, especially GBM. Our data suggested that chemokine/receptor genes had different expression patterns in tumors with strong tumor heterogeneity. Our data suggested that chemokines and receptor genes performed a key role in the immune activity and therapeutic response of GBM.

The comparison of mutations in RTK-RAS, PI3K, TP53, NOTCH, and Hippo pathways was higher in GBM samples. Previous study found that the RTK-RAS signaling pathway exhibited high-frequency alterations in pan-cancer, especially in malignant subgroups [40]. PI3K was a common dysregulated signaling pathway in numerous cancers, with amplification, inactivation, or loss of PTEN activity, and PI3K mutations and amplification ultimately causing drug resistance in cancer patients and leading to clinical treatment failure [41]. A recently identified glioma with recognized limitations was characterized, among other things, by a high frequency of TP53 mutations [42], which caused an inflammatory response in GBM, leading to poor prognosis and treatment response [43]. Notch1-4, Dll1-3-4, and Jagged1-2 in the NOTCH pathway played important functions in maintaining the central nervous system, and their high-frequency mutations led to brain tumor development, eventually forming GBM [44]. In contrast, the Hippo pathway was a major pathway affecting GBM chemoresistance and tumor immune response [45]. In GBM, we iden-

Table 1. Results of univariate COX analysis of differentially expressed genes (DEGs).

Gene ID	p-value	HR	Low 95% CI	High 95% CI
<i>CCL2</i>	0.024891	1.142966	1.017009	1.284523
<i>BCL2A1</i>	0.01364	1.213309	1.040514	1.4148
<i>CSTA</i>	0.033145	1.221276	1.016114	1.467861
<i>SI00A8</i>	0.007525	1.169027	1.042526	1.310879
<i>HK3</i>	0.009006	1.252442	1.057788	1.482915
<i>SI00A9</i>	0.031533	1.14245	1.011857	1.289897
<i>CD300E</i>	0.008321	1.349449	1.080142	1.6859
<i>SLAMF8</i>	0.045007	1.189053	1.003863	1.408407
<i>ALOX5</i>	0.048969	1.227339	1.000926	1.504968
<i>SI00A11</i>	0.039104	1.263398	1.011756	1.577628
<i>OSCAR</i>	0.020461	1.335868	1.04572	1.706521
<i>CXCL5</i>	0.01165	1.158325	1.033323	1.298449
<i>IER3</i>	0.036229	1.212999	1.012467	1.453249
<i>PLAUR</i>	0.000268	1.467468	1.193924	1.803684
<i>GPR84</i>	0.029921	1.252422	1.022134	1.534595
<i>LILRA5</i>	0.018063	1.257633	1.039983	1.520832
<i>RETN</i>	0.007777	1.255723	1.061866	1.484971
<i>AQP9</i>	0.00871	1.186395	1.044164	1.348
<i>SERPINA1</i>	0.027883	1.212106	1.021124	1.438807
<i>CCL5</i>	0.010186	1.288734	1.062017	1.563849
<i>FAM20A</i>	0.017826	1.249421	1.039214	1.502148
<i>NFKBIZ</i>	0.00501	1.320828	1.087549	1.604146
<i>SI00A4</i>	0.032617	1.194509	1.014815	1.406022
<i>ICAM1</i>	0.007057	1.27761	1.069041	1.52687
<i>FCGR2B</i>	0.00615	1.263476	1.068824	1.493579
<i>MAN1A1</i>	0.036969	1.242896	1.013235	1.524611
<i>JAK3</i>	0.025121	1.269273	1.030213	1.563807
<i>CTSZ</i>	0.013443	1.302387	1.056232	1.605908
<i>TREM1</i>	0.014956	1.187025	1.033922	1.362801
<i>CLEC2B</i>	0.03587	1.220486	1.013231	1.470135
<i>LTBP2</i>	0.013039	1.276763	1.052791	1.548383
<i>DPYD</i>	0.043024	1.193069	1.005556	1.415548
<i>TNFAIP2</i>	0.010044	1.244905	1.053668	1.470852
<i>RARRES1</i>	0.007363	1.184533	1.046549	1.340709
<i>BIRC3</i>	0.003086	1.266999	1.083183	1.482007
<i>THBD</i>	0.002392	1.326841	1.105472	1.592538
<i>SOCS3</i>	0.002081	1.250683	1.084662	1.442115
<i>PTX3</i>	0.001192	1.224474	1.083324	1.384016
<i>CXCL1</i>	0.036875	1.157165	1.008934	1.327172
<i>CD163</i>	0.04019	1.129951	1.005483	1.269828
<i>IL32</i>	0.019151	1.274782	1.040448	1.561892
<i>CARD16</i>	0.048035	1.207783	1.001633	1.456361
<i>SLC11A1</i>	0.006782	1.287107	1.072161	1.545144
<i>SPP1</i>	0.017343	1.190549	1.03122	1.374496
<i>RDH10</i>	0.03915	1.197142	1.008995	1.420373
<i>SERPING1</i>	0.029031	1.221496	1.020657	1.461855
<i>THBS1</i>	0.028396	1.145625	1.014482	1.293721
<i>CXCL13</i>	0.037416	1.12932	1.007109	1.266361
<i>CHI3L1</i>	0.009878	1.124669	1.02864	1.229662
<i>TIMP1</i>	0.000567	1.31061	1.12376	1.528526

Table 1. Continued.

Gene ID	p-value	HR	Low 95% CI	High 95% CI
<i>PDZK1IP1</i>	0.009959	1.218884	1.048549	1.416889
<i>CLCF1</i>	0.015818	1.241329	1.04143	1.479597
<i>MMP19</i>	0.021938	1.200444	1.026781	1.403481
<i>LIF</i>	0.048249	1.138484	1.000999	1.294852
<i>IGFBP6</i>	2.59E-05	1.354768	1.176049	1.560646
<i>IBSP</i>	0.035883	1.12424	1.007748	1.254199
<i>MEDAG</i>	0.029274	1.16292	1.015337	1.331956
<i>SNX10</i>	0.007114	1.225604	1.056848	1.421306
<i>MXRA5</i>	0.005044	1.220464	1.061816	1.402815
<i>LOX</i>	0.020818	1.170537	1.024222	1.337754
<i>LGALS3</i>	0.017661	1.176229	1.028634	1.345002
<i>G0S2</i>	0.003838	1.198218	1.059974	1.354492
<i>COL8A1</i>	0.030769	1.141991	1.012375	1.288203
<i>GJB2</i>	0.002605	1.192806	1.063471	1.337871
<i>HES6</i>	0.024542	0.845709	0.730773	0.978723
<i>OLIG2</i>	0.022724	0.866629	0.766208	0.980212
<i>NEU4</i>	0.025077	0.867521	0.766093	0.982378
<i>OLIG1</i>	0.026946	0.868147	0.765933	0.984002
<i>HES5</i>	0.043624	0.874333	0.7674	0.996165

tified two genotypes (CCR1 and CCR2) by consistent clustering. To our knowledge, this was the first study of cancer genetic subtyping in GBM based on chemokine/receptor genes. Interestingly, *CCR1* and *CCR2* showed different immune activity and prognostic differences. Patients with *CCR1* have more wear in a state of immune activation and have a worse OS. As these pathways mutate, causing tumor development, inflammatory infiltration in the tumor increases. Therefore, we hypothesized that the frequency of RTK-RAS, PI3K, TP53, NOTCH, and Hippo variants was higher in patients in CCR1, leading to poor prognosis.

With the availability of high-throughput sequencing technology, numerous biomarkers have been found to serve as chemotherapy and immunotherapy sensitivity in GBM. The present study was the first to construct a molecular signature for predicting prognosis, chemotherapy response, and immunotherapy sensitivity in GBM by DEGs between molecular subtypes related to chemokine/receptor genes. Patients with *CCR1* and those with high CCR Scores were highly overlapped, and both showed extremely poor prognoses. This suggested that the CCR Score could predict unfavorable survival outcomes in GBM. In addition, the CCR Score performed a major function in assessing immune cell activity in the tumor microenvironment, which was a highly heterogeneous tumor with a unique tumor microenvironment status resulting in GBM to exhibit extreme heterogeneity within and between the tumor microenvironment [46]. Understanding the tumor microenvironmental state of GBM would help to understand the immunological features of the CNS [46]. The CCR Score helps researchers deepen their understanding of the immune features of GBM.

The CCR Score contains *PLAUR*, *PTX3*, *SLC11A1*, *TIMP1*, *IGFBP6*, *G0S2*, *GJB2*. All these genes were functionally associated with immune infiltrating cells in GBM. High expression of *PLAUR* was associated with monocyte infiltration in TME of GBM, and important immune response markers (*PD-L2/CD276/B7-H3*) were closely associated and were essential genes for resolving the highly heterogeneous TME [47]. Recent findings found that *PTX3* affected macrophage infiltration abundance, migratory activity, and anti-inflammatory polarization, including through cellular communication, and that high *PTX3* expression induced an immunosuppressive microenvironment in GBM, leading to adverse clinical outcomes [48]. *SLC11A1* was a biomarker that predicted *PD-L1/PD-1* axis gene expression in GBM [49]. *TIMP1* was significantly overexpressed in infiltrating immune cells and accelerated GBM progression, and *Sp1-TIMP1* could predict the abundance of immune cell infiltration in GBM sites to assess their tumor progression [50]. GBM patients with significantly upregulated IDH mutations increased the difficulty of exploring GBM mechanisms [51]. *G0S2* and *GJB2* were considered potential therapeutic markers for GBM [52,53]. Consistently, the genes included in the CCR Score were strongly associated with GBM immunity, progression, and prognostic treatment. Our study further confirms the existing findings. However, this study still has limitations. First, although we validated the predictive performance of the CCR Score in three datasets, subsequent multicenter large and clinical samples are needed to validate the prognostic value of the CCR Score. Second, the molecular subtypes identified in this study need to be validated in clinical studies for their differences from traditional pathologic typing and clinical value. Finally, the prognostic genes identified in this study need to be followed up with *in vivo* and *in vitro* tests to explore their functions in GBM.

Conclusion

Overall, our description was the first to distinguish new gene types in GBM based on chemokine/receptor genes, which could complement the existing clinicopathological typing and deepen the understanding of GBM mechanisms. We also constructed a CCR Score that predicts poor prognosis in GBM, which performed a novel role in GBM prognosis, immune activity, and drug treatment susceptibility, but the limitations of this study remained. The data were obtained from public databases, and there was a lack of large-sample multicenter trials to validate the gene types of our findings and the clinical applicability of the CCR Score. In addition, *in vivo* and *in vitro* studies are necessary to validate the gene types and clinical validity of the CCR Score.

Abbreviations

GBM, glioblastoma; TCGA, The Cancer Genome Atlas; CGGA, The Chinese Glioma Genome Atlas; UCSC xena, University of California Santa Cruz; DEGs, differentially expressed genes; TME, tumor microenvironment; TMB, tumor mutation burden; TAMs, tumor-associated macrophages; TANs, tumor-associated neutrophils granulocytes; SNV, single nucleotide variants; CNV, copy number variations; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, lower-grade glioma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; K-M, Kaplan-Meier; ssGSEA, single sample gene set enrichment analysis; GSVA, Gene Set Variation Analysis; TRS, tumor-reactive T cell signature; IC₅₀, the half maximal inhibitory concentration; DSS, disease-specific survival; PFI, progression-free interval.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding authors. Available at CMJ (Jiangsy1112@zju.edu.cn).

Author Contributions

MWL, SYQ, GHW, JW, DYM, WZ and CMJ contributed to the study conception and design. Material preparation, data collection and analysis were performed by MWL, SYQ and GHW. The first draft of the manuscript was written by JW, DYM, WZ and CMJ. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.391>.

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