Grade II/III Glioma Microenvironment Mining and Its Prognostic Merit

Jiawei Chen[1,2], Chongxian Hou[3], Peng Wang[1], Yong Yang[1], Dong Zhou[1]

■ OBJECTIVE: **The tumor microenvironment greatly influences tumor formation, invasion, and progression. The ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues) algorithm quantifies stromal and immune components in a tumor, reflecting the tumor microenvironment. This study aimed to explore key prognostic genes in a grade II/III glioma microenvironment.**

■ METHODS: **We obtained stromal/immune scores for the Cancer Genome Atlas (TCGA) grade II/III glioma cohort from the online ESTIMATE portal. The associations of stromal/immune scores with clinicopathologic characteristics and overall survival of patients with grade II/III glioma were assessed by the Mann–Whitney *U* test and the Kaplan–Meier method, respectively. Functional enrichment analysis and protein–protein interaction network assessments were employed to analyze differentially expressed genes (DEGs). The top 7 genes with 5 or more edges in the protein–protein interaction network were selected. For validation, CGGA grade II/III glioma data were analyzed.**

■ RESULTS: **The results showed that elevated stromal/immune/ESTIMATE score was significantly associated with poor survival of patients with TCGA grade II/III glioma. Functional enrichment analysis showed that DEGs were associated with immune cell regulation, extracellular matrix, cytokine activation, and receptor binding. The selected DEGs (interleukin-10, beta-2 microglobulin, C-C motif chemokine ligand 5, cluster of differentiation 74, human leukocyte antigen-DRA, lymphocyte cytosolic protein 2, and myxovirus resistance protein 1) showed prognostic values in patients with grade II/III glioma of the TCGA and CGGA database.**

■ CONCLUSIONS: **Stromal/immune/ESTIMATE scores have prognostic values in patients with grade II/III glioma. The selected DEGs, including interleukin-10, beta-2 microglobulin, C-C motif chemokine ligand 5, cluster of differentiation 74, human leukocyte antigen-DRA, lymphocyte cytosolic protein 2, and myxovirus resistance protein 1, associated with tumor immunity and microenvironment, have prognostic values in grade II/III glioma. Further investigation of these genes could provide novel insights into the tumor microenvironment of glioma.**

INTRODUCTION

Gliomas are the most common type of primary brain tumor in humans. According to the 2016 World Health Organization classification of tumors of the central nervous system, gliomas are further subdivided into 4

Key words
- CGGA
- ESTIMATE algorithm
- Grade II/III glioma
- TCGA
- Tumor microenvironment

Abbreviations and Acronyms
B2M: Beta-2 microglobulin
CAF: Cancer-associated fibroblast
CCL5: C-C motif chemokine ligand 5
CCR: C-C motif chemokine receptor
CD74: Cluster of differentiation 74
CGGA: Chinese Glioma Genome Atlas
CXCL: C-X-C motif chemokine ligand
CXCR: C-X-C motif chemokine receptor
DEG: Differentially expressed gene
ECM: Extracellular matrix
ER: Endoplasmic reticulum
ESTIMATE: Estimation of STromal and Immune cells in MAlignant Tumor tissues
HLA: Human leukocyte antigen
IDH: Isocitrate dehydrogenase
IL-10: Interleukin 10
LCP2: Lymphocyte cytosolic protein 2
MX1: Myxovirus resistance protein 1
PPI: Protein–protein network
TAM: Tumor associated macrophage
TCGA: The Cancer Genome Atlas
TGF-β: Transforming growth factor-β
VEGF: Vascular endothelial growth factor

From the [1]Department of Neurosurgery, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China; [2]Shantou University Medical College, Shantou, Guangdong, China; and [3]Department of Neurosurgery, Faculty of Medicine, Hokkaido University, Sapporo, Japan

To whom correspondence should be addressed: Dong Zhou, M.D. [E-mail: zhoudong5413@163.com]

Citation: World Neurosurg. (2019) 132:e76-e88.
https://doi.org/10.1016/j.wneu.2019.08.253

*Journal homepage: **www.journals.elsevier.com/world-neurosurgery***

*Available online: **www.sciencedirect.com***

grades (I–IV).[1] Grade II/III gliomas are infiltrative tumors, which mostly arise in the cerebral hemispheres of adults, and mainly include diffuse astrocytomas, anaplastic astrocytomas, oligodendrogliomas, and anaplastic oligodendrogliomas.[1,2] Because grade II/III gliomas are highly invasive tumors in the central nervous system, complete resection is unattainable, and recurrence and progression are unavoidable.[3] So far, gliomas have become one of the most life-threatening tumor types in the central nervous system.[4]

The intrinsic genes of cancer cells dictate the development, progression, and infiltration of gliomas.[5] The tumor microenvironment, which consists of immune cells, mesenchymal cells, endothelial cells, inflammatory mediators, and extracellular matrix (ECM) molecules,[6] is of great impact on gene expression in cancer tissues as well as clinical outcomes.[7] The dynamic interaction between the microenvironment and cancer cells promotes tumor growth, proliferation, and protection from immune surveillance and therapy.[8] Immune and stromal cells are 2 major types of non-tumor elements that impact cancer prognosis.

Yoshihara et al.[9] designed an algorithm called ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) to assess tumor purity based on gene expression data. This algorithm analyzes specific gene expression signatures of stromal and immune cells, and calculates stromal and immune scores to predict the infiltration of non-tumor cells. The ESTIMATE algorithm has been applied to assess many cancers such as head and neck squamous cell carcinoma, and melanoma,[10,11] providing new insights into the prediction and investigation of tumors. However, to the best of our knowledge, the ESTIMATE algorithm has not been used to analyze grade II/III gliomas. Meanwhile, the tumor microenvironment of grade II/III gliomas is not well understood. Therefore, we performed data mining in the Cancer Genome Atlas (TCGA) database and used the Chinese Glioma Genome Atlas (CGGA) database for further validation.

MATERIALS AND METHODS

Data Mining from the TCGA Database and ESTIMATE Score Determination

The transcriptome profiles and the corresponding clinical information of patients with grade II/III glioma were obtained from TCGA (https://portal.gdc.cancer.gov/). The edgeR package (version 3.26.2) in R (version 3.6.0; R Foundation for Statistical Computing, Vienna, Austria) was employed to normalize the raw counts of RNA-sequencing data from TCGA. ESTIMATE scores (Immune, Stromal, and ESTIMATE), determined based on the RNA-seqV2 platform, for the TCGA grade II/III glioma cohort were downloaded from the online ESTIMATE portal (http://bioinformatics.mdanderson.org/estimate/).

Data Downloading from the CGGA Database

For validation, the gene expression profiles of grade II/III patients and the corresponding clinical data (survival and outcome) were obtained from the (CGGA) data portal (http://www.cgga.org.cn/).

Differentially Expressed Genes (DEGs) and Heat Map Analysis

Data analysis was performed using the limma package. Fold change >2.0 and $P < 0.01$ were set as cutoffs to screen for DEGs. Heat maps were plotted by R (version 3.6.0).

Protein–Protein Interaction (PPI) Network Construction

The PPI network was retrieved from the STRING database and rebuilt with the Cytoscape software (Version 3.7.1). According to the PPI network, nodes with 5 or more edges were shown in the bar plot and selected into further analysis.

Functional Enrichment Analysis

Functional enrichment analysis of DEGs was performed in R (version 3.6.0) to identify Gene Ontology categories by their biological processes, molecular functions, and cellular components.

Statistics Analysis

Samples with stromal/immune/ESTIMATE scores higher and lower than the median value were assigned to the high- and low-score groups, respectively. Associations of stromal/immune/ESTIMATE scores with clinicopathologic characteristics of patients with grade II/III glioma were analyzed by the Mann–Whitney U test in GraphPad Prism 8.0.2 (GraphPad Software, San Diego, California, USA). The expression levels of DEGs higher and lower than the median value was considered high and low expressions, respectively. Associations of stromal/immune/ESTIMATE scores with overall survival in patients with TCGA with grade II/III gliomas were analyzed by the Kaplan–Meier method in GraphPad Prism 8.0.2. The correlation between the expression levels of DEGs and overall survival of patients with grade II/III glioma was analyzed by the Kaplan–Meier method in GraphPad Prism 8.0.2. $P < 0.05$ was considered statistically significant.

RESULTS

Associations of Immune, Stromal, and ESTIMATE Scores with Clinicopathologic Characteristics in Patients with Grade II/III Glioma

Based on the TCGA database, 527 patients with grade II/III glioma were included in the present study. They included 289 (54.84%) male and 238 (45.16%) female patients. A total of 259 (49.15%) cases were grade II glioma and 268 (50.85%) were grade III glioma. As for the isocitrate dehydrogenase (IDH) status, there were 116 (22.01%) wildtype, 383 (72.68%) mutant, and 28 (5.31%) unknown cases (**Table 1**). Based on the CGGA database, 404 patients with grade II/III glioma were included in the present study. They included 229 (56.68%) male and 175 (43.32%) female patients. A total of 173 (42.82%) cases were grade II glioma and 231 (57.18%) were grade III glioma. Regarding the IDH status, there were 91 (22.52%) wildtype, 276 (68.32%) mutant, and 37 (9.16%) unknown cases (**Table 1**). Based on the ESTIMATE algorithm, stromal scores ranged from −1774.12 to 1701.87, immune scores from −1681.08 to 2466.68, and ESTIMATE scores from −3432.57 to 3744.27. The stromal, immune, and ESTIMATE scores of patients with grade III glioma were significantly greater than those of grade II glioma cases ($P < 0.0001$; **Figure 1A–C**). Based on the IDH status, wildtype cases had significantly greater

Table 1. Characteristics of Patients with Grade II/III Glioma in the TCGA and CGGA Databases

	TCGA		CGGA	
Clinicopathologic Characteristics	**No. Patients (n = 527)**	**Percentage**	**No. Patients (n = 404)**	**Percentage**
Age, years				
<60	457	86.72	387	95.79
≥60	70	13.28	17	4.21
Sex				
Male	289	54.84	229	56.68
Female	238	45.16	175	43.32
Grade				
II	259	49.15	173	42.82
III	268	50.85	231	57.18
IDH status				
IDH wildtype	116	22.01	91	22.52
IDH mutant	383	72.68	276	68.32
Unknown	28	5.31	37	9.16

TCGA, The Cancer Genome Atlas; CGGA, Chinese Glioma Genome Atlas; IDH, isocitrate dehydrogenase.

stromal, immune, and ESTIMATE scores compared with IDH mutant cases ($P < 0.0001$; Figure 1D–F).

Associations of Immune, Stromal, and ESTIMATE Scores with Overall Survival in Patients with Grade II/III Glioma

Patients with grade II/III glioma with high stromal scores had significantly worse overall survival compared with those with low stromal scores ($P = 0.0011$; Figure 2A). Patients with grade II/III glioma with high immune scores had significantly worse overall survival compared with those with low stromal scores ($P = 0.0031$; Figure 2B). Patients with grade II/III glioma with high ESTIMATE scores had significantly worse overall survival in comparison with those with low ESTIMATE scores ($P = 0.0144$; Figure 2C).

Associations of Gene Expression with Stromal and Immune Scores in Patients with Grade II/III Glioma

The transcriptome data of all 527 patients with grade II/III glioma were analyzed, and heat maps were generated to show differential gene expression between high and low stromal/immune scores (Supplementary Figures 1 and 2). Based on stromal scores, 1199 genes were up-regulated and 692 down-regulated in the high-score group relative to the low-score group (fold change >2.0, $P < 0.01$). Similarly, 965 genes were up-regulated and 296 genes down-regulated in the high immune score group relative to the low immune score group (fold change >2.0, $P < 0.01$). Furthermore, the Venn plot showed that 887 genes were commonly up-regulated and 277 genes commonly down-regulated in the high score group (Figure 3A–B). These 1164 genes were considered DEGs and extracted for further analysis. A functional enrichment analysis of these 1164 DEGs was performed to explode the potential function of the DEGs. The top 10 Gene Ontology terms in biological process, cellular component, and molecular functions categories suggested that these DEGs were related to immune cell regulation, extracellular matrix, cytokine activation, and receptor binding (Figure 3C).

PPIs Among DEGs

For a better understanding of the interactions among the detected DEGs, a PPI network was obtained from the STRING database (Supplementary Figure 3). This network was made of 181 nodes and 344 edges. The PPI network was rebuilt by the Cytoscape software, and the interleukin-10 (IL-10) and cluster of differentiation 74 (CD74) modules were selected for further assessment (Figure 4A–B). Nodes with 5 or more edges included IL-10, beta-2 microglobulin (B2M), C-C motif chemokine ligand 5 (CCL5), CD74, human leukocyte antigen (HLA)-DRA, lymphocyte cytosolic protein 2 (LCP2), and myxovirus resistance protein 1 (MX1), all of which were considered key nodes and selected for further analysis (Figure 4C).

Associations of IL-10, B2M, CCL5, CD74, HLA-DRA, LCP2, and MX1 Expression Levels with Overall Survival in Patients with Grade II/III Glioma

The Kaplan–Meier method was used to assess the associations of these 7 DEGs with overall survival in patients with grade II/III glioma in the TCGA database. All 7 DEGs significantly predicted worse overall survival based on the log-rank test ($P < 0.05$; Figure 5A–G).

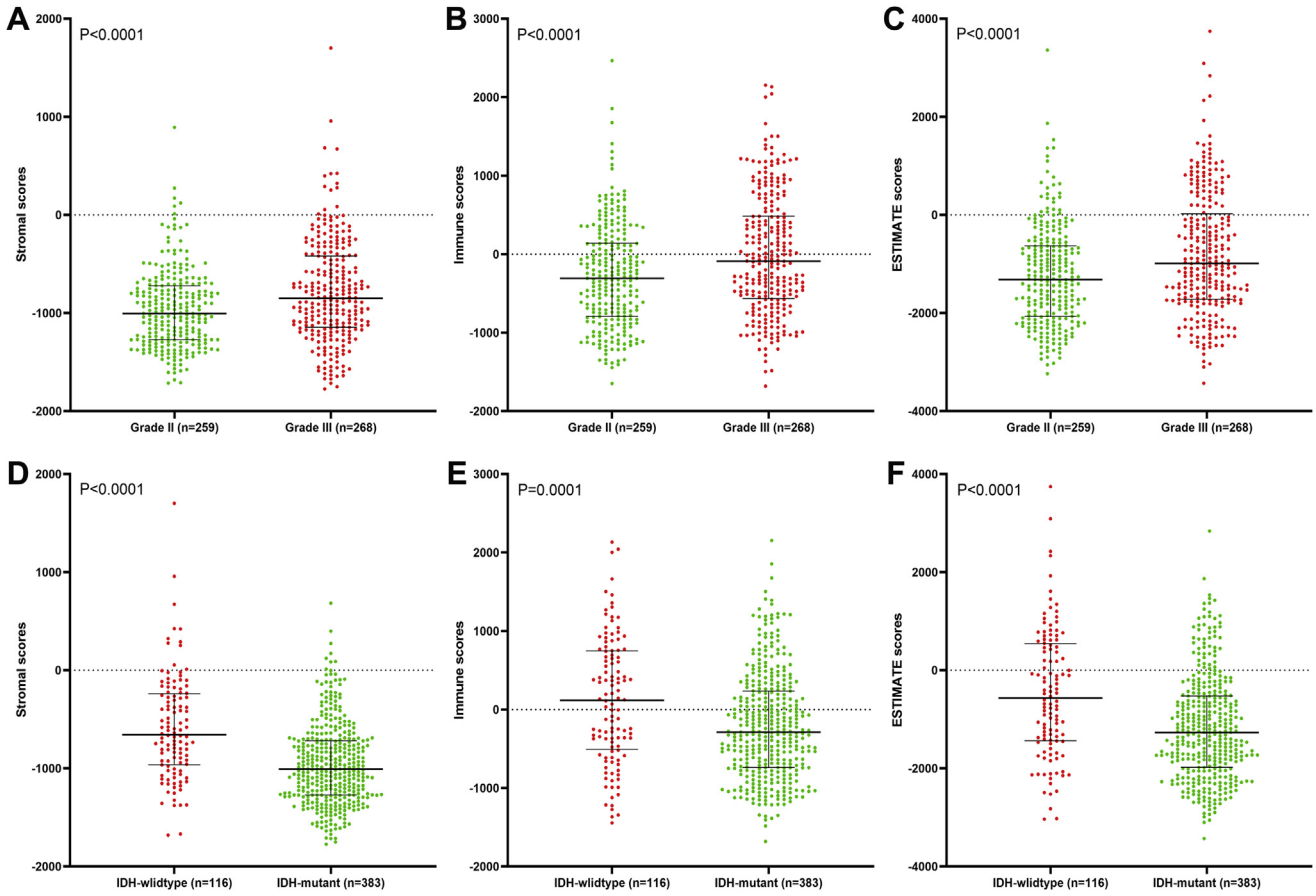


Figure 1. Associations of stromal/immune/Estimation of STromal and Immune cells in MAlignant Tumor tissues (ESTIMATE) scores with clinicopathologic characteristics. Associations of (**A**) stromal, (**B**) immune, and (**C**) ESTIMATE scores with glioma grade. Associations of (**D**) stromal, (**E**) immune, and (**F**) ESTIMATE scores with the isocitrate dehydrogenase (IDH) status of gliomas.

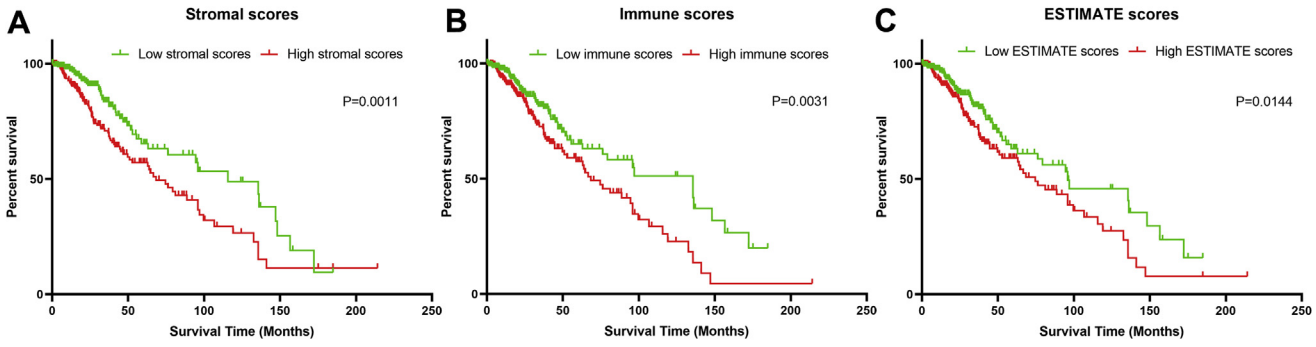


Figure 2. Associations of stromal/immune/Estimation of STromal and Immune cells in MAlignant Tumor tissues (ESTIMATE) scores with overall survival in grade II/III gliomas. Impacts of (**A**) stromal, (**B**) immune, and (**C**) ESTIMATE scores on overall survival in grade II/III gliomas.

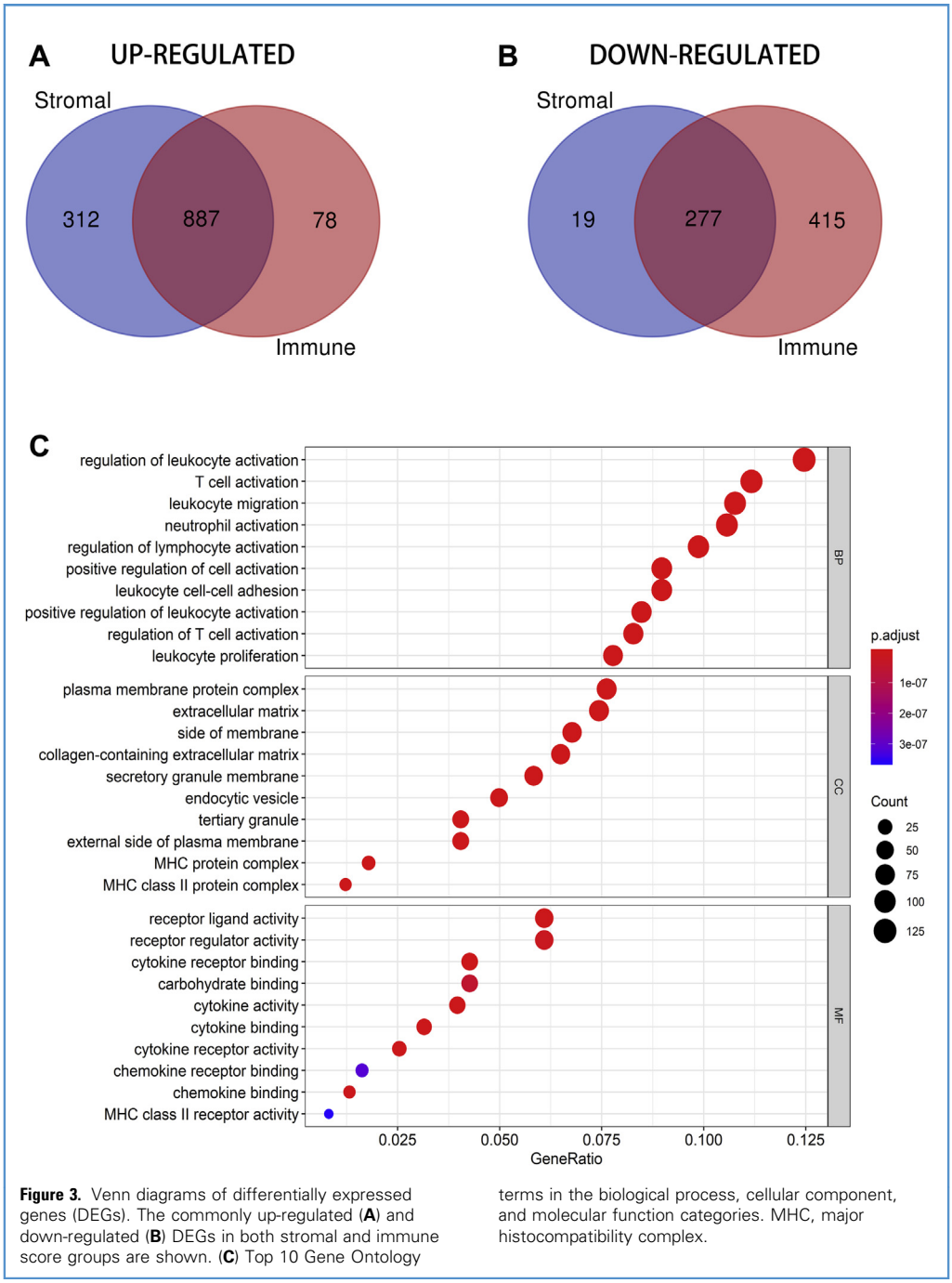


Figure 3. Venn diagrams of differentially expressed genes (DEGs). The commonly up-regulated (**A**) and down-regulated (**B**) DEGs in both stromal and immune score groups are shown. (**C**) Top 10 Gene Ontology terms in the biological process, cellular component, and molecular function categories. MHC, major histocompatibility complex.

Validation in the CGGA Database

We downloaded and analyzed gene expression profiles and the related clinical data of 404 patients with grade II/III glioma from the CGGA database. The 7 DEGs (IL-10, B2M, CCL5, CD74, HLA-DRA, LCP2, and MX1) were confirmed to be significantly associated with prognosis prediction ($P < 0.05$; Figure 5H–N).

DISCUSSION

In the present study, we determined genes associated with the tumor microenvironment that contribute significantly to overall survival in patients with grade II/III glioma based on the TCGA database. With the ESTIMATE algorithm, we determined stromal, immune, and ESTIMATE scores, which were significantly lower in patients with grade II glioma compared with grade III glioma

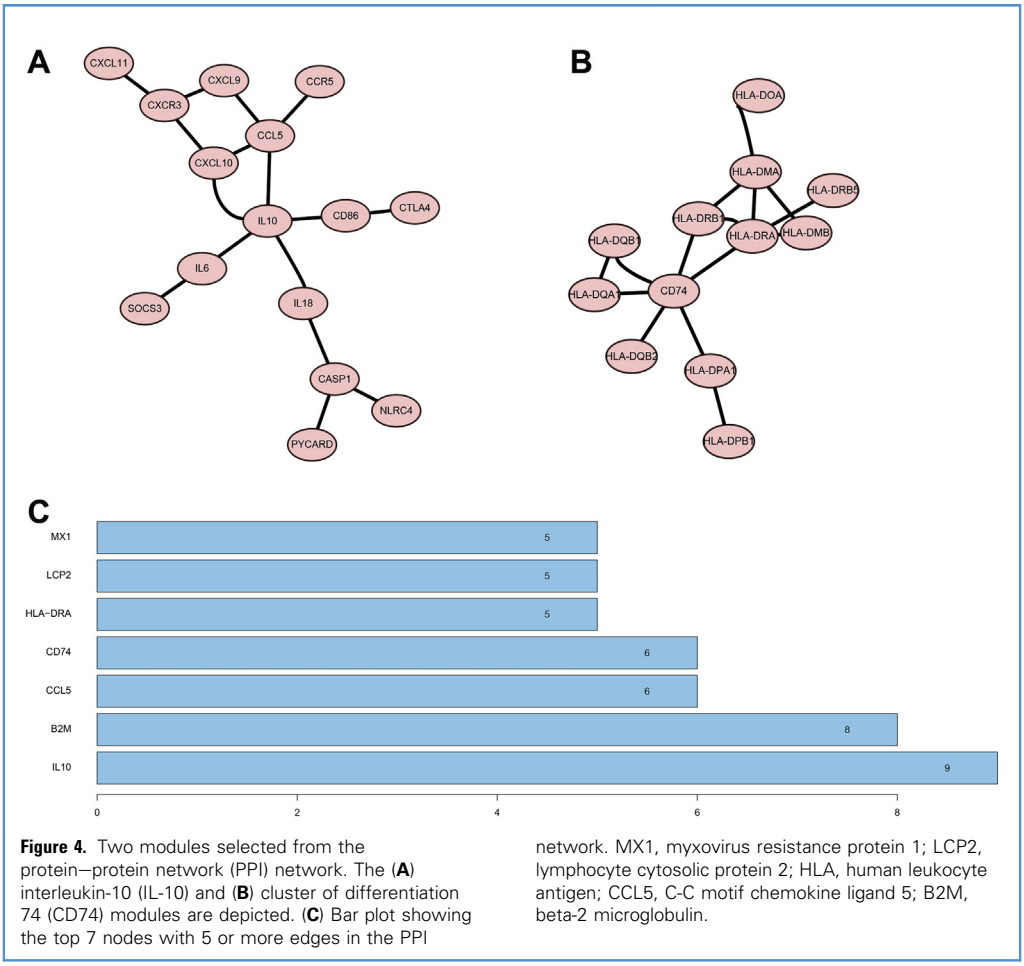
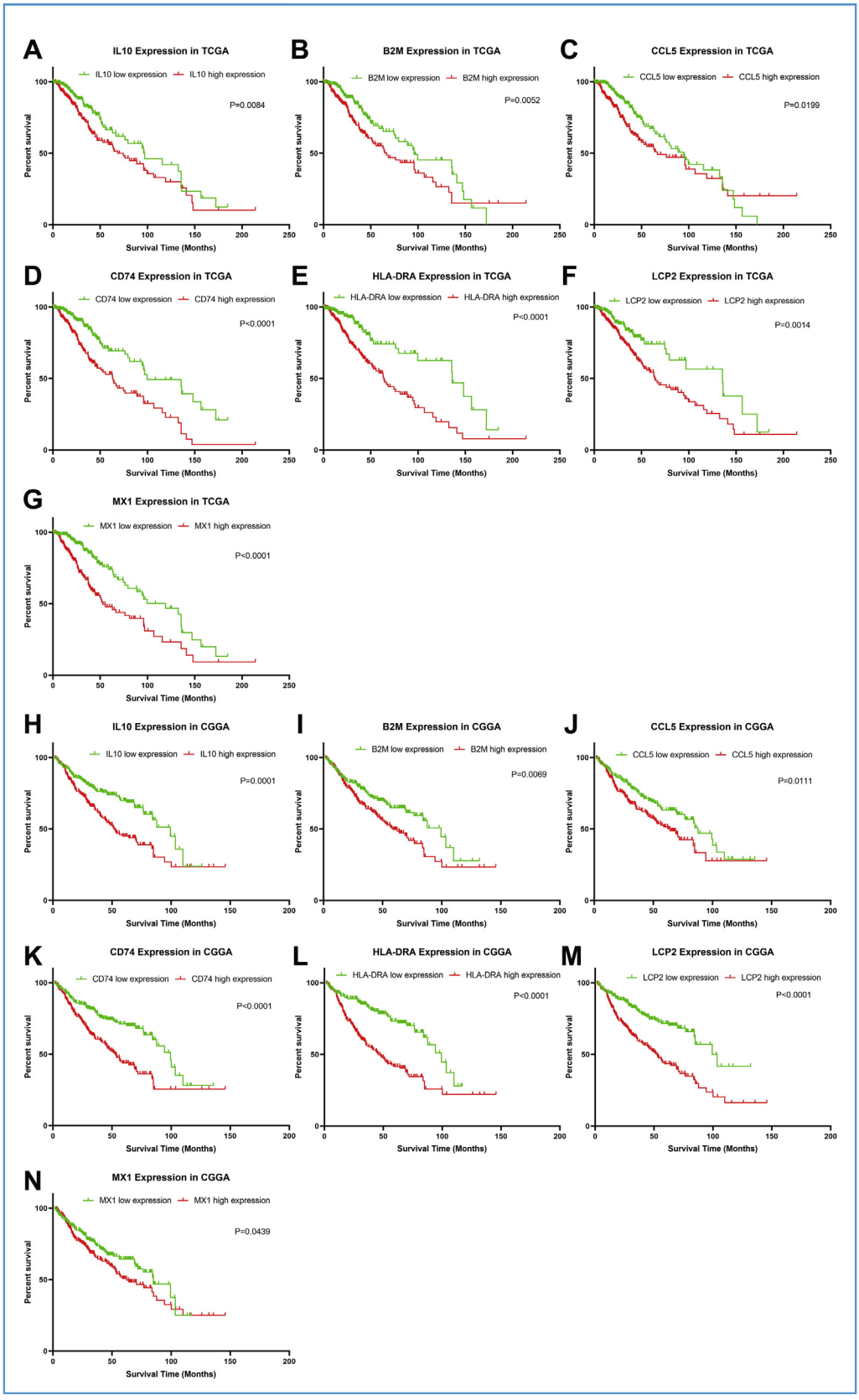


Figure 4. Two modules selected from the protein—protein network (PPI) network. The (**A**) interleukin-10 (IL-10) and (**B**) cluster of differentiation 74 (CD74) modules are depicted. (**C**) Bar plot showing the top 7 nodes with 5 or more edges in the PPI network. MX1, myxovirus resistance protein 1; LCP2, lymphocyte cytosolic protein 2; HLA, human leukocyte antigen; CCL5, C-C motif chemokine ligand 5; B2M, beta-2 microglobulin.

cases. IDH mutant cases had significantly lower stromal, immune, and ESTIMATE scores compared with wild-type cases. Higher stromal, immune, and ESTIMATE scores were correlated with worse overall survival in patients with grade II/III glioma. After RNA-sequencing data analysis, we extracted 1164 genes commonly up-regulated or down-regulated in the high score group. Then, functional enrichment analysis demonstrated that these 1164 genes contributed to immune cell regulation, extracellular matrix, cytokine or receptor binding, and activation. Furthermore, we constructed a PPI network comprising 181 nodes and 344 edges, and selected 7 genes (IL-10, B2M, CCL5, CD74, HLA-DRA, LCP2, and MX1) and 2 modules. Survival analysis based on these 7 genes revealed that all of them had potential prognostic values in patients with grade II/III glioma in the TCGA database. For cross-validation, analysis of the same 7 genes based on the CGGA database was performed, with similar results.

The microenvironment of gliomas contains different types of cells, including stromal, endothelial, immune, and other cells, which regulate and support tumor development and are important for chemoresistance.[12] These cells, together with the ECM, cytokines, growth factors, and specific conditions, including hypoxia, constitute the glioma microenvironment.[13] The endothelial cell associated with an increased expression of vascular endothelial growth factor (VEGF) can lead to vascular alterations and promote hypoxia, acidosis, and nutrient deprivation.[14] Tumor-associated macrophages, a kind of tumor-associated immunosuppressive cells, have 2 phenotypes: the M1 phenotype, which is proinflammatory and antitumoral; and the M2 phenotype, which is anti-inflammatory and protumoral. Most of the macrophages recruited into TME become M2 phenotype, induce T-cell anergy, secrete ECM components, and stimulate angiogenesis to induce the modification of stromal and blood vessel architecture and enhance tumorigenicity and drug resistance.[15,16] ECM, an important part of tumor microenvironment, is not only an extracellular scaffold, but a dynamic compartment onto which components are continuously deposited, degraded, or remodeled. The overexpression of ECM contributes to the deregulation of cell proliferation, differentiation, death, and invasion.[17,18] Glioma tissue appears to be stiffer compared with the non-tumoral tissue because of an increase in the fluid pressure, cell compression, and tumor cellular contractility, thereby promoting glioma stiffness.[15,19] These alterations break the vessel integrity and impede the recruitment of inflammatory cells and the delivery of macromolecules, including chemotherapeutic agents.


A
IL10 Expression in TCGA
IL10 low expression
IL10 high expression
P=0.0084
B
B2M Expression in TCGA
B2M low expression
B2M high expression
P=0.0052
C
CCL5 Expression in TCGA
CCL5 low expression
CCL5 high expression
P=0.0199
D
CD74 Expression in TCGA
CD74 low expression
CD74 high expression
P<0.0001
E
HLA-DRA Expression in TCGA
HLA-DRA low expression
HLA-DRA high expression
P<0.0001
F
LCP2 Expression in TCGA
LCP2 low expression
LCP2 high expression
P=0.0014
G
MX1 Expression in TCGA
MX1 low expression
MX1 high expression
P<0.0001
H
IL10 Expression in CGGA
IL10 low expression
IL10 high expression
P=0.0001
I
B2M Expression in CGGA
B2M low expression
B2M high expression
P=0.0069
J
CCL5 Expression in CGGA
CCL5 low expression
CCL5 high expression
P=0.0111
K
CD74 Expression in CGGA
CD74 low expression
CD74 high expression
P<0.0001
L
HLA-DRA Expression in CGGA
HLA-DRA low expression
HLA-DRA high expression
P<0.0001
M
LCP2 Expression in CGGA
LCP2 low expression
LCP2 high expression
P<0.0001
N
MX1 Expression in CGGA
MX1 low expression
MX1 high expression
P=0.0439
Percent survival
Survival Time (Months)

Moreover, ECM stiffness regulates the cancer stem cell plasticity and the expression of markers.[20,21] In various types of cancer, especially gliomas, a hypoxic microenvironment is very common and strongly associated with chemoresistance through various factors such as functions of glioma stem cells, apoptosis, and angiogenesis.[12,22] Tumor progression is greatly regulated by the surrounding environment, like a Darwinian selection of the favorite clones, which confers the selected phenotypes greater adaptation abilities.[23,24] Therefore, glioma cells and their microenvironment affect each other and evolve simultaneously.

In the present study, 7 genes (IL-10, B2M, CCL5, CD74, HLA-DRA, LCP2, and MX1) were selected among 1164 DEGs, according to the PPI network. We mainly focused on the IL-10 module (CCL5 included), which is related to the chemokine family, and the CD74 module (HLA-DRA included), which is highly relevant to the HLA family.

B2M, LCP2, and MX1

B2M, an invariant component of major histocompatibility complex class I molecules expressed by most nucleated cells, is involved in immunoregulation. Liu et al.[25] detected B2M protein expression levels in pancreatic ductal adenocarcinoma and found that its overexpression is associated with poor prognosis, tumor invasion, and metastasis. It was reported that B2M mutation or aberrant expression impairs human leukocyte antigen (HLA)-mediated cancer cell recognition in several hematologic and solid neoplasms.[26] Loss of heterozygosity in B2M and HLA class I regions was associated with shorter survival in patients with glioblastoma, but the relevant mechanism was not well understood.[27] Lymphocyte cytosolic protein 2 (LCP2) is a substrate of the T-cell antigen receptor—activated protein tyrosine kinase pathway. LCP2 was identified as a hub gene and regulatory factor of glioblastoma multiforme through TCGA datamining.[28] It was reported that a splice variant of LCP2 causes severe immune imbalance and dysregulation, providing an opportunity for immunosuppression.[29] Myxovirus resistance protein 1 (MX1) is a member of the MX family of proteins, which are antiviral factors of the innate immune system. Wang et al.[30] revealed that MX2, another member of the MX protein family, suppresses cell proliferation, migration, and invasion via the extracellular signal-regulated kinase/P38/NF-κB signaling pathway. High expression of MX1 is associated with high levels of tumor-infiltrating lymphocytes in HER2-positive breast cancer, and the prognostic value of tumor-infiltrating lymphocytes has been determined in breast cancer.[31]

IL-10 Module

This module mainly consists of IL-6 and IL-18 of the interleukin family, and C-X-C motif chemokine ligand (CXCL)3, CXCL9, CXCL10, CXCL11, CCL5, and C-C motif chemokine receptor (CCR)5 belonging to the chemokine family. IL-10, an anti-inflammatory cytokine, affects immunoregulation and inflammation. Solid tumors escape from the host immunity via production of suppressive mediators, including IL-10, transforming growth factor β (TGF-β), and VEGF-A.[32] These mediators suppress dendritic cells, inhibit T-cell activation and penetration into the tumor stroma, and enhance regulatory T-cell function.[33] IL-10 is considered an immunosuppressive factor because of its association with different populations of immune suppressive and regulatory cells as well as its inhibitory effects on antigen presentation and immune cell activation.[34] For example, several studies suggested that greater expression of serum IL-10 is associated with greater recurrence rate and a worse prognosis in colorectal and ovarian cancers.[35,36] Batchu et al.[37] suggested that IL-10 could help pancreatic cancer cells shield themselves from immune surveillance, and IL-10 blockade in the microenvironment might reverse tumor-derived immunosuppression. IL-10 was associated with a deficient anti-tumor immune response, increased TGF-β levels, and inhibited STAT3 signaling in astrocytes.[38-40] IL-8 was involved in enhancing vascular permeability and neovascularization by both autocrine and paracrine modes in the form of vasculogenic mimicry through the IL-8/C-X-C motif chemokine receptor (CXCR)1/2 signaling pathway, which promoted glioma cell proliferation and invasion.[41,42] The chemokines CCL2 and CXCL10 recruit inflammatory cells to tumor sites, whereas proinflammatory cytokines, including IL-6 and TNF-α, create a proinflammatory microenvironment, thereby promoting the malignant transformation and progression of low-grade glioma.[43] CCL2 can trigger the release of IL-6 from microglia, which, in turn, promotes the invasiveness of glioma cells.[44] CCR3, CCR4, CCR5, CCR8, CXCR3, and CXCR4, alongside other members of the chemokine family, are important chemotactic factors involved in the regulation of leukocyte migration.[45] T-cell related intratumoral infiltration is inhibited by C-X-C motif chemokine ligand 12 (CXCL12), a pivotal member of the chemokine family.[46] Zhao et al. revealed crucial roles for CCL5/CCR5 in regulating glioblastoma generation, proliferation, and invasion.[47] Another study reported that the TGF-β1/CXCL12/CXCR7 autocrine axis promotes epithelial—mesenchymal transition and cancer stem-like cell formation, further enhancing chemoresistance in lung cancer.[48] Accumulating evidence indicates that tumor-infiltrating B cells are involved in cancer initiation and progression through a subset of B cells that inhibit antitumor immune responses.[49] Probable suppressive mechanisms include enhancement of inhibitory ligand expression, signal transducer and activator of transcription 3 (STAT3) phosphorylation, and IL-10 and TGF-β production.[50]

Figure 5. Associations of overall survival of patients with grade II/III glioma in the Cancer Genome Atlas cohort and the expression levels of selected differentially expressed genes (DEGs). (**A**) interleukin-10 (IL-10), (**B**) beta-2 microglobulin (B2M), (**C**) C-C motif chemokine ligand 5 (CCL5), (**D**) cluster of differentiation 74 (CD74), (**E**) human leukocyte antigen (HLA)-DRA, (**F**) lymphocyte cytosolic protein 2 (LCP2), and (**G**) myxovirus resistance protein 1 (MX1) were assessed. Validation of the selected DEGs, including (**H**) IL-10, (**I**) B2M, (**J**) CCL5, (**K**) CD74, (**L**) HLA-DRA, (**M**) LCP2, and (**N**) MX1 in the Chinese Glioma Genome Atlas cohort.

CD74 Module

This module is strongly associated with the HLA family, because it includes HLA-DP, HLA-DQ, and HLA-DR. Cluster of differentiation 74 (CD74) is an HLA-DR antigen-associated invariant chain, belonging to the HLA class II system. HLA class II molecules are transmembrane glycoproteins primarily expressed on antigen-presenting cells, including macrophages, dendritic cells and B lymphocytes. Rangel et al.[51] revealed that ovarian cancer overexpresses HLA-DRA, as a result of inflammatory events in the tumor microenvironment, and cancer cells reduce the production of functional major histocompatibility complex class II molecules by some compensatory mechanisms, leading to reduced immunogenicity and enhanced cancer growth. CD74 increases the complexity of the tumor cell HLA peptidome and is associated with improved prognosis in patients with brain metastasis.[52] Macrophage migration inhibitory factor is important in tumorigenesis due to its overexpression in tumor cells.[53] A recent report demonstrated that macrophages with high HLA-DR expression might potentially represent interferon-γ—activated cells with antitumor activity, since interferon-γ specifically induces HLA-DR expression on macrophages.[54] Meanwhile, the macrophage migration inhibitory factor/CD74/CD44 signaling pathway was found to inhibit interferon-γ in microglia through phosphorylation of microglial extracellular signal-regulated kinase 1 and 2, and promote tumorigenesis in brain tumor.[55]

Growing evidence indicates that tumor immunity begins as cancer-associated inflammation, which exists long before visible tumor formation.[56] Cancer-associated inflammation currently is considered a double-edged sword during tumor generation and development; it can promote or inhibit tumorigenesis depending on the microenvironment, type of cancer, and phase of inflammation. Tumor growth results in the destruction of intrinsic tissue architecture and stress generation. Such stress is exemplified by the hypoxic response, which induces hypoxia-inducible factor-1α, activates cancer-associated fibroblasts (CAFs), and aggregates chemokines to help form an immunosuppressive microenvironment.[57] CAFs have been shown not only to support tumor growth but also to recruit immune cells and modulate anti-tumor immunity. The 2-way regulation of CAFs is dependent on the context and tumor type.[58] CAFs produce CXCL12, CXCL13, CXCL19, CCL25, and IL-7 to recruit lymphocytes and regulate their differentiation.[57,59] Mariathasan et al.[60] found that CAFs also prevent the infiltration of immune cells into the tumor stroma and trap them within the collagen-rich peritumoral stroma, using TGF-β—neutralizing antibodies. In addition, endoplasmic reticulum (ER) stress is exhibited in various types of cancer. ER stress is induced by saturated fatty acids and cholesterol, as well as various metabolic disorders.[61] It activates JNK-AP-1 and IKK-NF-κB signaling pathways and stimulates proinflammatory cytokine secretion.[62] Cubillos-Ruiz et al.[63] demonstrated that ER stress within dendritic cells is an immunosuppressive factor. Tumor-associated macrophages (TAMs), the most crucial myeloid cells in the tumor environment, can easily adapt to various conditions.[64] In the initial phase of inflammation, TAMs produce cytokines such as IL-1β, tumor necrosis factor, and IL-6, which promote tumor growth, and VEGF, which enhances tumor angiogenesis. In the late phase, TAMs support tumor development by producing immunosuppressive cytokines, programed-death ligand 1, TGF-β, and IL-10.[65]

To the best of our knowledge, the prognostic values of the aforementioned 7 genes in patients with grade II/III glioma and the tumor microenvironment remain poorly understood. Accumulating evidence supports that these genes play important roles in immunity regulation, extracellular matrix, cytokine activation, and receptor binding. The interaction between the tumor and the microenvironment regulates tumor evolution, which deeply affects immunosuppression, immune evasion, chemo- or radioresistance, and survival outcome in tumor patients. The present study comprehensively analyzed the interactions of microenvironmental and genetic factors in the following order: gene expression, immune and stromal scores, and prognostic immunity-related genes. Based on the present analysis, immunity-related genes with prognostic potential were identified, and a more comprehensive understanding of the tumor microenvironment was achieved.

CONCLUSIONS

Overall, the ESTIMATE algorithm is of great importance in tumor microenvironment datamining. Stromal, immune, and ESTIMATE scores have prognostic values in patients with grade II/III glioma. The DEGs IL10, B2M, CCL5, CD74, HLA-DRA, LCP2, and MX1, which are associated with tumor immunity and the microenvironment, have prognostic values in patients with grade II/III glioma. Finally, further investigation of these genes could provide new insights into the tumor microenvironment of gliomas.

REFERENCES

1. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131:803-820.
2. Ceccarelli M, Barthel FP, Malta TM, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell*. 2016;164:550-563.
3. Cancer Genome Atlas Research Network, Brat DJ, Verhaak RG, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N Engl J Med*. 2015;372:2481-2498.
4. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS. CBTRUS Statistical Report: primary brain and other central nervous system tumors diagnosed in the United States in 2011—2015. *Neuro Oncol*. 2018;20(suppl 4):iv1-iv86.
5. Wang Q, Hu B, Hu X, et al. Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. *Cancer Cell*. 2017;32:42-56 e6.
6. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012;21:309-322.
7. Senbabaoglu Y, Gejman RS, Winer AG, et al. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. *Genome Biol*. 2016;17:231.
8. Sonnenschein C, Soto AM. Theories of carcinogenesis: an emerging perspective. *Semin Cancer Biol*. 2008;18:372-377.
9. Yoshihara K, Shahmoradgoli M, Martinez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. 2013;4:2612.

10. Lin CY, Kwon H, Rangel Rivera GO, Li X, Chung D, Li Z. Sex differences in using systemic inflammatory markers to prognosticate patients with head and neck squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2018;27:1176-1185.

11. Yang S, Liu T, Nan H, et al. Comprehensive analysis of prognostic immune-related genes in the tumor microenvironment of cutaneous melanoma [e-pub ahead of print]. *J Cell Physiol*. 2019. https://doi.org/10.1002/jcp.29018.

12. Li G, Qin Z, Chen Z, Xie L, Wang R, Zhao H. Tumor microenvironment in treatment of glioma. *Open Med (Wars)*. 2017;12:247-251.

13. Seano G. Targeting the perivascular niche in brain tumors. *Curr Opin Oncol*. 2018;30:54-60.

14. Da Ros M, De Gregorio V, Iorio AL, et al. Glioblastoma chemoresistance: the double play by microenvironment and blood–brain barrier. *Int J Mol Sci*. 2018;19. https://doi.org/10.3390/ijms19102879.

15. Lau EY, Ho NP, Lee TK. Cancer stem cells and their microenvironment: biology and therapeutic implications. *Stem Cells Int*. 2017;2017:3714190.

16. Jinushi M, Chiba S, Yoshiyama H, et al. Tumor-associated macrophages regulate tumorigenicity and anticancer drug responses of cancer stem/initiating cells. *Proc Natl Acad Sci U S A*. 2011;108:12425-12430.

17. Huijbers IJ, Iravani M, Popov S, et al. A role for fibrillar collagen deposition and the collagen internalization receptor endo180 in glioma invasion. *PLoS One*. 2010;5:e9808.

18. Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb Perspect Biol*. 2011;3. https://doi.org/10.1101/cshperspect.a005058.

19. Miroshnikova YA, Mouw JK, Barnes JM, et al. Tissue mechanics promote IDH1-dependent HIF1alpha-tenascin C feedback to regulate glioblastoma aggression. *Nat Cell Biol*. 2016;18:1336-1345.

20. Wong CC, Tse AP, Huang YP, et al. Lysyl oxidase-like 2 is critical to tumor microenvironment and metastatic niche formation in hepatocellular carcinoma. *Hepatology*. 2014;60:1645-1658.

21. Pang MF, Siedlik MJ, Han S, Stallings-Mann M, Radisky DC, Nelson CM. Tissue stiffness and hypoxia modulate the integrin-linked kinase ILK to control breast cancer stem-like cells. *Cancer Res*. 2016;76:5277-5287.

22. Persano L, Rampazzo E, Basso G, Viola G. Glioblastoma cancer stem cells: role of the microenvironment and therapeutic targeting. *Biochem Pharmacol*. 2013;85:612-622.

23. Gerlinger M, Swanton C. How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. *Br J Cancer*. 2010;103:1139-1143.

24. Loeb LA. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat Rev Cancer*. 2011;11:450-457.

25. Liu C, Yang Z, Li D, et al. Overexpression of B2M and loss of ALK7 expression are associated with invasion, metastasis, and poor-prognosis of the pancreatic ductal adenocarcinoma. *Cancer Biomark*. 2015;15:735-743.

26. Pizzi M, Boi M, Bertoni F, Inghirami G. Emerging therapies provide new opportunities to reshape the multifaceted interactions between the immune system and lymphoma cells. *Leukemia*. 2016;30:1805-1815.

27. Yeung JT, Hamilton RL, Ohnishi K, et al. LOH in the HLA class I region at 6p21 is associated with shorter survival in newly diagnosed adult glioblastoma. *Clin Cancer Res*. 2013;19:1816-1826.

28. Li Y, Min W, Li M, et al. Identification of hub genes and regulatory factors of glioblastoma multiforme subgroups by RNA-seq data analysis. *Int J Mol Med*. 2016;38:1170-1178.

29. Siggs OM, Miosge LA, Daley SR, et al. Quantitative reduction of the TCR adapter protein SLP-76 unbalances immunity and immune regulation. *J Immunol*. 2015;194:2587-2595.

30. Wang H, Guan Q, Nan Y, Ma Q, Zhong Y. Overexpression of human MX2 gene suppresses cell proliferation, migration, and invasion via ERK/P38/NF-κB pathway in glioblastoma cells. *J Cell Biochem*. 2019;120:18762-18770.

31. Lee SJ, Hwang CS, Kim YK, et al. Expression of myxovirus resistance A (MxA) Is associated with tumor-infiltrating lymphocytes in human epidermal growth factor receptor 2 (HER2)-positive breast cancers. *Cancer Res Treat*. 2017;49:313-321.

32. Burkholder B, Huang RY, Burgess R, et al. Tumor-induced perturbations of cytokines and immune cell networks. *Biochim Biophys Acta*. 2014;1845:182-201.

33. Takeuchi Y, Nishikawa H. Roles of regulatory T cells in cancer immunity. *Int Immunol*. 2016;28:401-409.

34. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol*. 2010;10:170-181.

35. Zhang L, Liu W, Wang X, Wang X, Sun H. Prognostic value of serum IL-8 and IL-10 in patients with ovarian cancer undergoing chemotherapy. *Oncol Lett*. 2019;17:2365-2369.

36. Li B, Wang F, Ma C, Hao T, Geng L, Jiang H. Predictive value of IL-18 and IL-10 in the prognosis of patients with colorectal cancer. *Oncol Lett*. 2019;18:713-719.

37. Batchu RB, Gruzdyn OV, Mahmud EM, et al. Inhibition of Interleukin-10 in the tumor microenvironment can restore mesothelin chimeric antigen receptor T cell activity in pancreatic cancer in vitro. *Surgery*. 2018;163:627-632.

38. Orihuela R, McPherson CA, Harry GJ. Microglial M1/M2 polarization and metabolic states. *Br J Pharmacol*. 2016;173:649-665.

39. Li W, Graeber MB. The molecular profile of microglia under the influence of glioma. *Neuro Oncol*. 2012;14:958-978.

40. Szulzewsky F, Pelz A, Feng X, et al. Glioma-associated microglia/macrophages display an expression profile different from M1 and M2 polarization and highly express Gpnmb and Spp1. *PLoS One*. 2015;10:e0116644.

41. Dwyer J, Hebda JK, Le Guelte A, et al. Glioblastoma cell-secreted interleukin-8 induces brain endothelial cell permeability via CXCR2. *PLoS One*. 2012;7:e45562.

42. Sharma I, Singh A, Siraj F, Saxena S. IL-8/CXCR1/2 signalling promotes tumor cell proliferation, invasion and vascular mimicry in glioblastoma. *J Biomed Sci*. 2018;25:62.

43. Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. *Nat Neurosci*. 2016;19:20-27.

44. Saederup N, Cardona AE, Croft K, et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS One*. 2010;5:e13693.

45. Molon B, Ugel S, Del Pozzo F, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med*. 2011;208:1949-1962.

46. Oelkrug C, Ramage JM. Enhancement of T cell recruitment and infiltration into tumours. *Clin Exp Immunol*. 2014;178:1-8.

47. Zhao L, Wang Y, Xue Y, Lv W, Zhang Y, He S. Critical roles of chemokine receptor CCR5 in regulating glioblastoma proliferation and invasion. *Acta Biochim Biophys Sin (Shanghai)*. 2015;47:890-898.

48. Wu YC, Tang SJ, Sun GH, Sun KH. CXCR7 mediates TGFbeta1-promoted EMT and tumor-initiating features in lung cancer. *Oncogene*. 2016;35:2123-2132.

49. Chang JH, Jiang Y, Pillarisetty VG. Role of immune cells in pancreatic cancer from bench to clinical application: an updated review. *Medicine (Baltimore)*. 2016;95:e5541.

50. Horikawa M, Minard-Colin V, Matsushita T, Tedder TF. Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice. *J Clin Invest*. 2011;121:4268-4280.

51. Rangel LB, Agarwal R, Sherman-Baust CA, et al. Anomalous expression of the HLA-DR alpha and beta chains in ovarian and other cancers. *Cancer Biol Ther*. 2004;3:1021-1027.

52. Zeiner PS, Zinke J, Kowalewski DJ, et al. CD74 regulates complexity of tumor cell HLA class II peptidome in brain metastasis and is a positive prognostic marker for patient survival. *Acta Neuropathol Commun*. 2018;6:18.

53. Conroy H, Mawhinney L, Donnelly SC. Inflammation and cancer: macrophage migration inhibitory factor (MIF)—the potential missing link. *QJM*. 2010;103:831-836.

54. Muller E, Speth M, Christopoulos PF, et al. Both type I and type II interferons can activate

antitumor M1 macrophages when combined with TLR stimulation. *Front Immunol*. 2018;9:2520.

55. Ghoochani A, Schwarz MA, Yakubov E, et al. MIF-CD74 signaling impedes microglial M1 polarization and facilitates brain tumorigenesis. *Oncogene*. 2016;35:6246-6261.

56. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140:883-899.

57. Cruz FM, Colbert JD, Merino E, Kriegsman BA, Rock KL. The biology and underlying mechanisms of cross-presentation of exogenous antigens on MHC-I molecules. *Annu Rev Immunol*. 2017;35:149-176.

58. Lakins MA, Ghorani E, Munir H, Martins CP, Shields JD. Cancer-associated fibroblasts induce antigen-specific deletion of CD8 (+) T cells to protect tumour cells. *Nat Commun*. 2018;9:948.

59. Shalapour S, Lin XJ, Bastian IN, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature*. 2017;551:340-345.

60. Mariathasan S, Turley SJ, Nickles D, et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018;554:544-548.

61. Nakagawa H, Umemura A, Taniguchi K, et al. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell*. 2014;26:331-343.

62. Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agostinis P. ER stress-induced inflammation: does it aid or impede disease progression? *Trends Mol Med*. 2012;18:589-598.

63. Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, et al. ER Stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. *Cell*. 2015;161:1527-1538.

64. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14:399-416.

65. Kuang DM, Zhao Q, Peng C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *J Exp Med*. 2009;206:1327-1337.

Conflict of interest statement: This study was financially supported by the Natural Science Foundation of Guangdong Province of China, China (No.2018A0303130236), the National Natural Science Foundation of China, China (No.81901250), and the High-level Hospital Construction Project (No.DFJH201924).

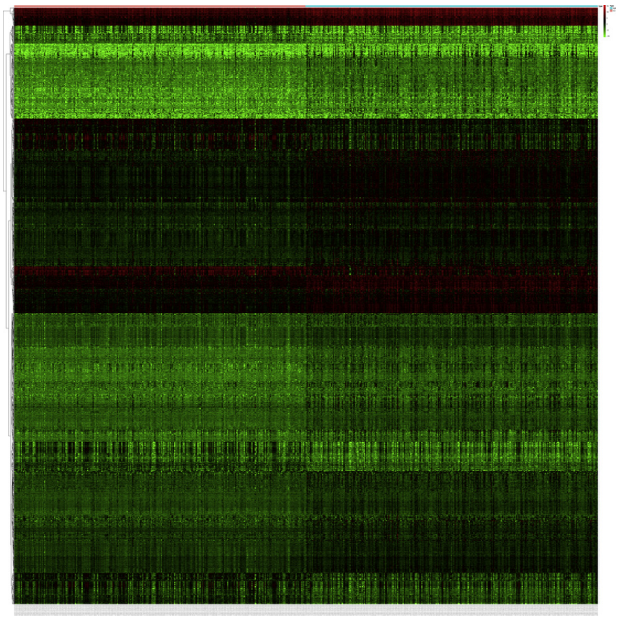
Received 31 July 2019; accepted 30 August 2019

Citation: World Neurosurg. (2019) 132:e76-e88.
https://doi.org/10.1016/j.wneu.2019.08.253

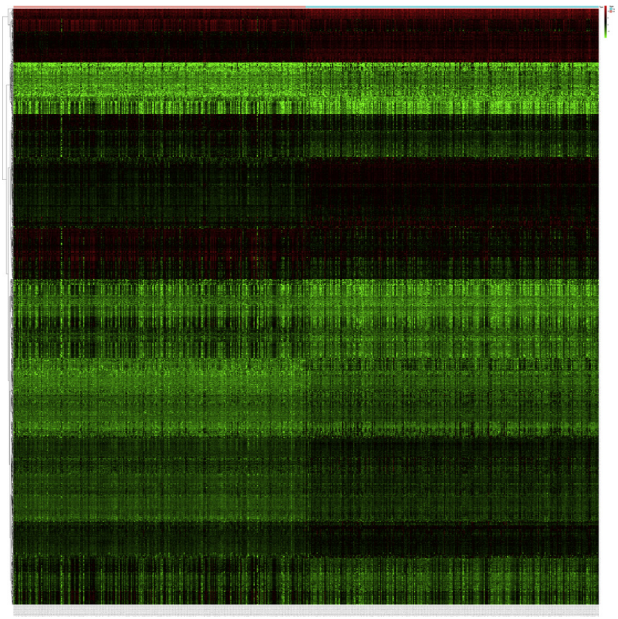
Journal homepage: ***www.journals.elsevier.com/world-neurosurgery***

Available online: ***www.sciencedirect.com***

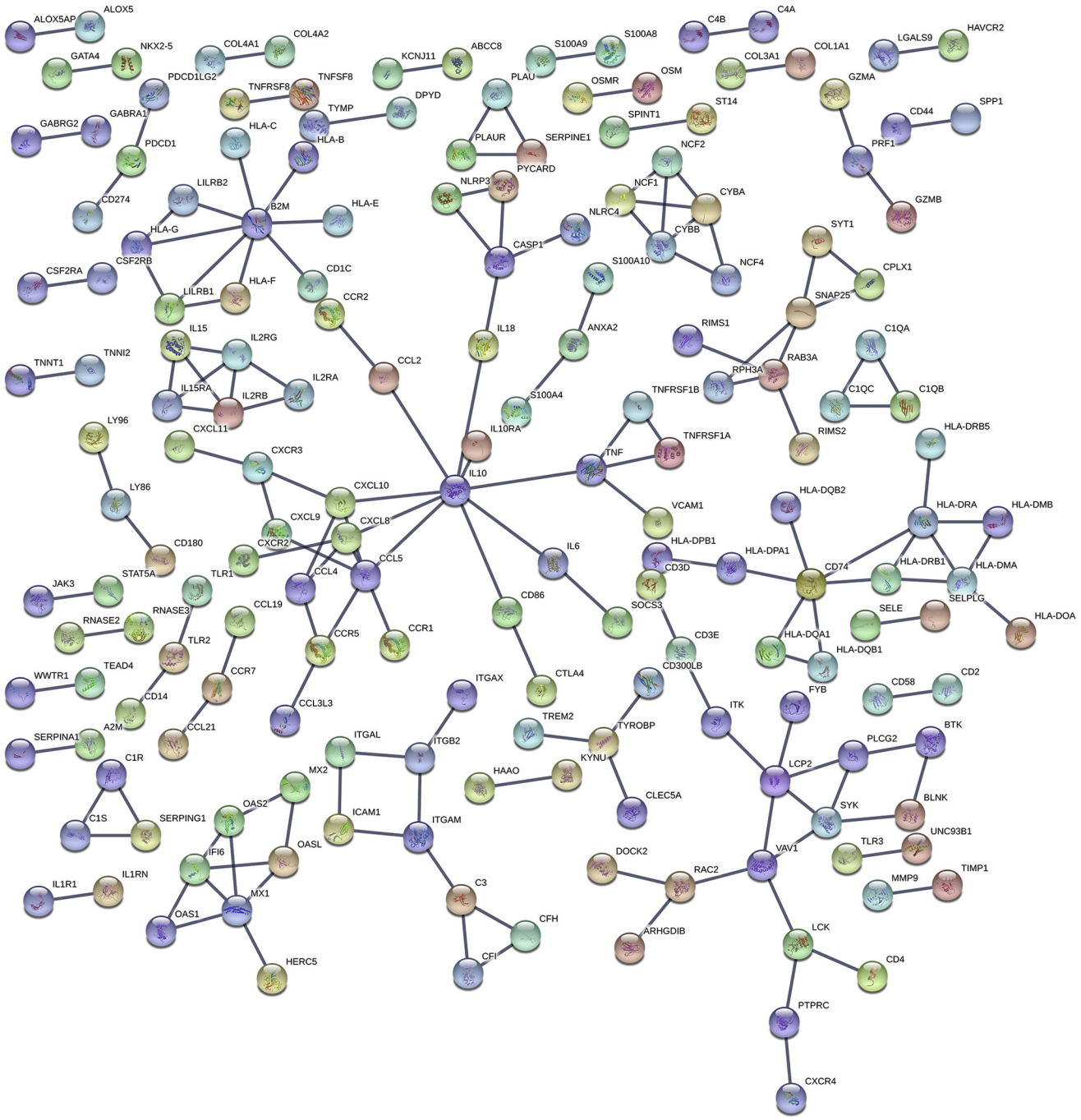
SUPPLEMENTARY DATA



Supplementary Figure 1. Heat map of differentially expressed genes associated with stromal scores. Genes with elevated expression are shown in *red*, and those with reduced expression in *green*; genes with unchanged expression are shown in *black*.



Supplementary Figure 2. Heat map of differentially expressed genes associated with immune scores. Genes with elevated expression are shown in *red*, and those with reduced expression in *green*; genes with unchanged expression are shown in *black*.



Supplementary Figure 3. Protein-protein network of commonly up-regulated and down-regulated differentially expressed genes in both stromal and immune score groups, generated with the STRING database.