Molecular and clinical characterization of IDH associated immune signature in lower-grade gliomas

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Abstract

Background: Mutations in isocitrate dehydrogenase (IDH) affect the development and prognosis of gliomas. We investigated the role of IDH mutations in the regulation of immune phenotype in lower-grade gliomas (LGGs).

Method and patients: A total of 1,008 cases with clinical and IDH mutation data from five cohorts were enrolled. Samples with RNA sequencing data from the Chinese Glioma Genome Atlas (CGGA) were used as training set, whereas RNA data from the Cancer Genome Atlas, Repository for Molecular Brain Neoplasia, GSE16011, and CGGA microarray databases were used for validation. R language tools and bioinformatics analysis were used for gene signature construction and biological function annotation.

Results: We found that IDH mutations caused down-regulation of local immune response as among 332 immune system-related genes, 196(59.0%) were differentially expressed according to IDH mutation status. Nearly 70% of those differentially expressed genes exhibited prognostic value in LGGs. An immune response-based gene signature was constructed that distinguished cases with high- or low-risk of unfavorable prognosis and remained an independent prognostic factor in multivariate analyses in both training and validation cohorts. Samples from high-risk cases exhibited elevated expression of genes involved in immune response and NF-κB pathway activation. Furthermore, we found a strong correlation between the risk score and T cells, macrophage-related immune response, and expression of several prominent immune checkpoints.

Conclusion: Our results indicated that mutant IDH is highly associated with the regulation of the immune microenvironment in LGGs. The observed immune system gene signature, which was sensitive to IDH mutation status, efficiently predicted patient survival.

Introduction

Lower-grade gliomas (LGGs), which include diffuse low-grade and intermediate-grade gliomas, are heterogeneous and infiltrative neoplasms (World Health Organization [WHO] grades II and III).\textsuperscript{1,2} Despite considerable progress in our understanding of the genetic and epigenetic landscapes of LGGs,\textsuperscript{3} there is still no significant progress in the choice of treatment regimens. Currently, maximal safe resection combined with adjuvant radiotherapy and chemotherapy is regarded as the optimal treatment regimen for LGGs that may prolong patients’ overall survival.\textsuperscript{4} However, the same treatment regimens lead to variable survival times.\textsuperscript{5,6} Thus, reliable approaches to identify subsets of patients who are at high risk of death and may be potentially sensitive to additional systemic therapy are needed.

Recent immunotherapy breakthroughs in several cancer types, particularly in melanoma and non-small cell lung cancer,\textsuperscript{7,8} coupled with the increasing understanding of the intricate interactions between glioma and the immune system,\textsuperscript{9,10} have led to the burgeoning field of glioma immunotherapy research.\textsuperscript{11,12} Several immunity-related parameters, such as the number of tumor-infiltrating lymphocytes and presence of PD-L1 expression, have been shown to predict patients’ response to specific kinds of immunotherapy,\textsuperscript{13} highlighting the importance of distinct immune status in glioma management. However, recent tumor immune microenvironment discoveries mainly appeared in the studies of glioblastomas,\textsuperscript{13,14} and there has been no systematic investigation of the immune system composition in patients with LGGs.

Mutations in IDH1 and IDH2 genes encoding different isoforms of isocitrate dehydrogenase (IDH) are the earliest genetic alterations in glioma tumorigenesis that lead to a distinct metabolic profile, hyper-methylated phenotype, and better prognosis in LGGs.\textsuperscript{15} Intriguingly, a recent study reported that distinct immunological tumor characteristics were associated with IDH mutational status.\textsuperscript{16} Hence, we hypothesized that the longer survival time in LGGs with IDH mutations may be in part due to their specific effects on tumor-associated immune system. Here, we analyzed multiple datasets of RNA data, IDH

KEYWORDS
IDH mutation; immunotherapy; immune checkpoints; lower-grade gliomas; signature
mutation status, and immune response evaluations to comprehensively explore the interaction between the presence of IDH mutations and immune response in LGG. Our analysis revealed that LGGs with IDH mutations (IDH<sup>WT</sup>) had a significantly lower level of immune response relative to that in LGGs without IDH mutations (IDH<sup>mut</sup>). Moreover, the signature of immunological genes whose expression is affected by IDH mutations could serve as a vital prognostic biomarker and potential therapeutic target in LGGs, highlighting its potential application in patient management.

**Methods**

**Sample and data collection**

We retrospectively collected RNA sequencing (RNA-seq) data and corresponding clinical information regarding 172 patients from the Chinese Glioma Genome Atlas (CGGA) database (www.cgga.org.cn) as the training set. We used messenger RNA microarray data from the Repository for Molecular Brain Neoplasia (REMBRANDT, http://cabig.cancer.gov/solutions/conductresearch/rembrandt/, n = 137), GSE16011 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16011, n = 112), and CGGA database (n = 176) were obtained as validation sets. All glioma tissues were histopathologically confirmed to be grade II or III gliomas. The clinical end point event, overall survival (OS), was calculated from the date of initial diagnosis until death or last follow-up. The details about sample preparation, mRNA measurement, and biomarker detection (IDH mutation status, 1p/19q co-deletion) have been described in detail in our previous study. All patients whose samples were obtained from the CGGA gave written informed consent to participate and the privacy of patients was strictly protected. The study protocol was approved by the ethics committee of the Beijing Tiantan Hospital.

**Bioinformatics analysis**

Gene set enrichment analysis (GSEA, http://www.broadinstitute.org/gsea/index.jsp) was performed to explore whether expression levels of the immune response-related genes were significantly different in samples with or without IDH mutations. Gene ontology (GO) analysis was performed in DAVID (http://david.abcc.ncifcrf.gov/home.jsp) for functional annotation of the genes with increased expression in high risk patients. Significant biological process networks were visualized using Cytoscape. To analyze biological implications of the prognostic signature, functional enrichment analysis was performed using DAVID (david.ncifcrf.gov) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. False discovery rate (FDR) was calculated when multiple comparisons were made.

**Results**

**Correlation of immune phenotype with IDH mutation in LGGs**

Pioneering investigations demonstrated that IDH mutation could serve as an earliest event during lower-grade gliomagenesis. Therefore, we initially utilized RNA-seq and clinical data of 172 patients from the CGGA database to identify the different biological processes and genes according to IDH status. Interestingly, in addition to cell adhesion and blood vessel development, GSEA analyses revealed that IDH<sup>WT</sup> LGGs were also strongly associated with positive regulation of defense response (P = 0.008), immune response (P = 0.012), immune effector process (P = 0.010), and immune system process (P = 0.017) (Fig. 1A). Despite the
Pathogenetic role of IDH mutations in gliomagenesis has been well established, their specific effects on the immune processes in LGGs has not been comprehensively explored. To validate the association between immune system processes and IDH status, 322 immune-related genes were extracted from the Molecular Signatures Database v6.0 (MSigDB) (http://www.broad.mit.edu/gsea/msigdb/index.jsp). Among those immune-related genes, 196 were found to be differentially expressed in IDHWT and IDHMUT LGGs (Fig. 1B, Supplementary Table S1), which was consistent with previous findings of distinct local immune response according to IDH status in gliomas.23,24

**Increased immune system-related risk in IDHWT LGGs**

Considering the different immune status between IDHWT and IDHMUT LGGs, we sought to evaluate the prognostic value of these differentially expressed genes in the training cohort. The univariate Cox regression analyses revealed that 132 out of the 196 differential genes were significantly correlated with the OS.

![Figure 1](image1.png)

**Figure 1.** Functional annotation of isocitrate dehydrogenase (IDH) in the training dataset. A, gene set enrichment analysis indicates a significantly enhanced immune phenotype in the cases of lower-grade glioma (LGG) without IDH mutations. B, heatmap of differentially expressed immune genes in LGG samples from patients with and without IDH mutations (IDHMUT and IDHWT).

![Figure 2](image2.png)

**Figure 2.** Identification of prognostic immune system genes. A, univariate Cox regression analysis identified 131 immune system genes significantly associated with the overall survival. B, ten-fold cross-validation for tuning parameter selection in the LASSO model. The LASSO coefficient profiles of the 131 immune genes: minimum criteria (C), 1 SE of the minimum criteria (D) and the Coef value for each of the 11 selected genes (E).
Among these, 114 (86.4%) genes upregulated in IDH\textsuperscript{WT} group were associated with a hazard ratio > 1 for death, suggesting an enhanced immune system-related risk in IDH\textsuperscript{WT} LGGs (Fig. 2A). To further generate an IDH associated immune signature for prognostication, we then applied a LASSO Cox regression model to select the genes with the largest prognostic value. According to the minimum criteria and one SE of the minimum criteria, 11 genes (\textit{APLN}, \textit{BCL10}, \textit{CCRL1}, \textit{CD276}, \textit{CEBPG}, \textit{COLEC12}, \textit{HAMP}, \textit{HDAC4}, \textit{LIG1}, \textit{MADCAM1}, and \textit{SECTM1}) and 5 genes (\textit{BCL10}, \textit{CD276}, \textit{SECTM1}, \textit{HDAC4}, \textit{MADCAM1}) were identified, respectively (Fig. 2B, C, D, E).

Using parallel analyses, similar clinical and biological functions were revealed in these two selected gene sets, which indicated that either of those two criteria of gene selection was appropriate. Two heatmaps were used to investigate the correlations between the 11 genes in CGGA and TCGA cohorts (Fig. 3A, B), respectively. The correlation patterns of CGGA and TCGA cohorts were similar, indicating that correlations between the selected genes were relatively stable in different databases. Subsequently, the expression levels of these genes and corresponding Coef were used to calculate the risk score. Patients in the training cohort were assigned into two groups (high-risk and low-risk groups) according to the median risk score. As shown in Fig. 3C, E, patients in the high-risk group had significantly shorter OS than low-risk counterparts.

To determine whether the risk score had similar prognostic value in different populations, we applied the same formula to three different cohorts from the TCGA, REMBRANDT, and GSE16011 databases as external validation sets. As expected, patients in the low-risk group had significantly longer OS than patients in the high-risk group (Fig. 3D, F; Supplementary Fig. S1A–D). The prognostic value of the risk score was also validated in the CGGA microarray cohort (Supplementary Fig. S1E, F), indicating the applicability of our risk score in different platforms. Moreover, we observed that the majority of the selected genes could be used to stratify patients into high-risk and low-risk groups in both CGGA and TCGA cohorts (Supplementary Fig. S1G, H).

To further investigate whether the risk score could serve as an independent predictor of prognosis in LGGs, uni- and multivariate Cox regression analyses were applied to the CGGA cohort. After adjusting for the clinical and pathological factors such as age, sex, WHO grade, histology, IDH, 1p/19q, and radiotherapy, the risk score remained an independent prognostic factor (Table 2 and Supplementary Table S3). Furthermore, the risk score was also validated as an independent factor after multivariate Cox regression analysis of the TCGA cohort (Supplementary Table S3), confirming its robustness to predict LGG prognosis independently.

Associations between the risk score and clinicopathological factors

To assess the association between the risk groups classified on the basis of our risk score and previous widely accepted

Figure 3. Survival analysis for the gene signature. A, B, correlations between the 11 prognostic immune genes in the Chinese Glioma Genome Atlas (CGGA) and the Cancer Genome Atlas (TCGA) cohorts, respectively. The dichotomized risk score allowed the segmentation of patients into high- and low-risk groups in both CGGA (11 genes, C, 5 genes, E) and TCGA (11 genes, D, 5 genes, F) cohorts.
prognostic and predictive factors in LGG (IDH status, 1p/19q status, and WHO grade), we evaluated statistical difference of the latter parameters between high and low-risk groups. In both CGGA and TCGA databases (Table 1, Supplementary Table S2), those clinicopathological factors were significantly different in patients from high and low-risk groups. Moreover, the majority of 11 genes were also identified to be differentially expressed between cases stratified by the IDH status, 1p/19q status, or WHO grade (Supplementary Fig. S2). These results implied that the prognostic and predictive value of those clinicopathological factors may partially derive from their roles in the regulation of LGG immune status.

**In silico functional analysis of the risk score**

In order to gain new insights into the biological role of the obtained risk score, we carried out GO analysis. The genes with a P-value < 0.05 and Pearson correlation coefficient > 0.5 were considered to be strongly correlated with the risk score. Heatmaps for risk score-associated genes were generated taking into account clinical and molecular factors, such as sex, age, WHO grade, histology, IDH mutation, 1p/19q and ATRX status (Fig. 4A, Supplementary Figs. S3A, S4A, B). Then, we used GO analysis to explore the underlying biological functions of the genes with increased expression in the high-risk group. This analysis revealed that the genes relevant to the risk score in both CGGA and TCGA databases were mostly involved in the immune response (GO terms, such as immune response, leukocyte migration, positive regulation of macrophage differentiation, positive regulation of T cell proliferation, T cell receptor signaling pathway, and inflammatory response) and NF-κB pathway activation (e.g., positive regulation of NF-κB, transcription factor activity, apoptotic

**Table 1. Clinical characteristics for patients diagnosed with lower grade glioma in CGGA cohort.**

|                   | Risk Score (11) | Risk Score (5) |
|-------------------|-----------------|----------------|
|                   | Low Risk (n = 86) | High Risk (n = 86) | P value | Low Risk (n = 86) | High Risk (n = 86) | P value |
| Age               |                 |                 |         |                 |                 |         |
| ≥45 vs. <45       |                 |                 | 0.181   |                 |                 |         |
| Sex               |                 |                 |         |                 |                 |         |
| Female            |                 |                 | 0.999   |                 |                 |         |
| Male              |                 |                 |         |                 |                 |         |
| WHO grade         |                 |                 |         |                 |                 |         |
| WHO II            |                 |                 | <0.001  |                 |                 |         |
| WHO III           |                 |                 |         |                 |                 |         |
| Histology         |                 |                 |         |                 |                 |         |
| O/OA              |                 |                 | <0.001  |                 |                 |         |
| A                 |                 |                 | 0.458   |                 |                 |         |
| IDH Status        |                 |                 |         |                 |                 |         |
| MUT               |                 |                 | <0.001  |                 |                 |         |
| WT                |                 |                 | 0.001   |                 |                 |         |
| 1p/19q Status     |                 |                 |         |                 |                 |         |
| Non-codel         |                 |                 | <0.001  |                 |                 |         |
| Codel             |                 |                 |         |                 |                 |         |
| Radiotherapy      |                 |                 |         |                 |                 |         |
| Yes               |                 |                 | 0.051   |                 |                 |         |
| No                |                 |                 |         |                 |                 |         |
| NA                |                 |                 |         |                 |                 |         |

**Table 2. Cox regression analysis of clinical characteristics and 11-gene based Risk Score in CGGA cohort.**

|       | Univariate analysis |       | Multivariate analysis |
|-------|---------------------|-------|-----------------------|
|       | HR | 95%CI               | P value | HR | 95%CI               | P value |
| Age   |    |                     |         |    |                     |         |
| ≥45 vs. <45 | 2.900 | 1.659—5.068 | <0.001 | 2.537 | 1.350—4.771 | 0.004 |
| Sex   |    |                     |         |    |                     |         |
| Male vs. Female | 1.078 | 0.608—1.908 | 0.798 |    |                     |         |
| WHO Grade | 6.036 | 3.270—11.142 | <0.001 | 2.973 | 1.508—5.861 | 0.002 |
| History | non-O vs. O | 3.078 | 1.747—5.422 | <0.001 |    |                     |         |
| IDH   |    |                     |         |    |                     |         |
| MUT vs. WT | 0.252 | 0.142—0.445 | <0.001 |    |                     |         |
| 1p/19q | Codel vs. non-codel | 0.188 | 0.084—0.420 | <0.001 | 0.458 | 0.185—1.133 | 0.091 |
| Radiotherapy | Yes vs. No | 0.536 | 0.276—1.040 | 0.065 | 0.392 | 0.188—0.815 | 0.012 |
| Risk Score | High vs. Low | 14.160 | 6.226—32.204 | <0.001 | 7.818 | 3.030—20.172 | <0.001 |
signaling pathway, and negative regulation of apoptotic process) (Fig. 4B, Supplementary Figs. S3B and S4C, D). Similar assessments of genetic alterations in each of the selected genes were also conducted (Supplementary Table S4).

**Relationship between the risk score and immune response**

To understand better the relationship between the risk score and immune response, previously described seven inflammatory and one immune checkpoint related metagenes were considered. We found that B7 family and most metagenes (HCK, interferon, LCK, MHC-I, MHC-II, STAT1) were positively correlated with the risk score except for IgG in both CGGA and TCGA cohorts (Fig. 5A, Supplementary Fig. S5A, B, C). These results, together with the GO analysis, indicated that the high-risk score was mostly based on genes involved in the activity of macrophages and T cell signaling transduction suppressors, but not in genes involved in B lineage-related immune responses. Targeting immune checkpoint regulators has been shown to exert antitumor effects by reversing their immunosuppressive effects. Thus, we correlated the risk score with expression levels of important immune checkpoints in gliomas. Circos plots demonstrated that the risk score tightly correlated with expression levels of CD80, PD-L1, PD-L2, and TIM-3 in both CGGA and TCGA cohorts (Fig. 5B, C).

**Discussion**

In the present study, we analyzed RNA data and IDH mutation status in a total of 1008 LGG cases from five different cohorts.
and developed a prognostic risk score based on differential expression levels of immune response-related genes in samples from IDH\textsuperscript{WT} and IDH\textsuperscript{MUT} patients.

LGG is an intrinsic brain tumor characterized by infiltrative growth. None of the existing treatment options is currently curative, which renders LGG inevitably lethal. Over the past few years, the successful application of the modulation of the CTLA-4 and PD1 checkpoints in melanoma and non-small cell lung cancer generated a possible hope for glioma immunotherapy. Detailed studies of the interactions between the tumor and immune microenvironment have been widely conducted in those cancers, and they hold great promise in terms of future treatment design and prognosis improvement.\textsuperscript{28,29} However, such studies have rarely been applied to LGG. In this study, by using large sample datasets from different populations and platforms, we carried out a comprehensive analysis of how IDH mutations influence tumor immune microenvironment and, consequently, impact prognosis in LGGs. We found that IDH\textsuperscript{MUT} LGGs were associated with significantly weaker immune response compared to that in IDH\textsuperscript{WT} counterparts. Then, we established a prognostic signature based on immune system genes affected by IDH mutations and validated it in multiple independent cohorts. Additionally, the identified signature, or risk score, was associated with changes in the level of some prominent immune checkpoints, suggesting their synergistic role in regulating immune microenvironment.

Great efforts have been made in elucidating the mechanisms by which IDH mutations affect the development of gliomas and their characteristics.\textsuperscript{30} However, we do not yet fully understand their role in influencing tumor immune microenvironment. Recently, many studies have been carried out to investigate the impact of molecular processes on the intensity of the immune response in various tumors, because such knowledge would facilitate the prediction of the response to immunotherapy.\textsuperscript{31,32} In gliomas, the relationship between IDH mutations and expression levels immune checkpoint proteins has only started to be unveiled.\textsuperscript{16} In this study, we extended this area of research to the whole immune microenvironment landscape and found that IDH\textsuperscript{WT} LGGs were characterized by enhanced immune response. In addition, more than half of immune system-related genes were found to be differentially expressed based on the IDH mutation status, including 155 and 41 that were upregulated in IDH\textsuperscript{WT} and IDH\textsuperscript{MUT}, respectively. Existing studies have partially explored the role of these upregulated genes in IDH\textsuperscript{WT} cases in gliomas. For instance, CCL2 and IL10 have been identified as chemotactic factors for promoting the proliferation of microglia/macrophages toward an immune suppressive phenotype and eventually contributing to malignancy progression.\textsuperscript{33,34} IL6, an important Th2-type cytokine, has been reported to generate an inflammatory microenvironment that enables maintenance of glial stem cell properties. On the basis of the aforementioned findings, we speculate that the IDH\textsuperscript{WT} gliomas might create an immunosuppressive environment for promoting glioma growth, progression, invasion, and angiogenesis.

We found that nearly 70% of those differentially expressed immune system genes exhibited prognostic value in LGGs. This result implies that the prognostic value of IDH mutations may partially derive from the pivotal role of IDH in regulating the balance between tumor and immune microenvironments. Recently, extensive efforts have been applied to construct gene expression signatures representative of tumor immune status, and their potential clinical relevance has been examined in several cancers.\textsuperscript{35} In the present study, we used the LASSO Cox regression model to establish a novel signature based on immune system gene expression profiles that could reliably distinguish LGG patients with high and low risk of unfavorable survival in multiple data sets. Remarkably, even after Cox regression analyses, this signature retained its power to independently predict the prognosis in internal and external validation sets, thereby adding a complementary prognostic value to molecular and clinical characteristics. Bioinformatic analysis of this signature revealed that there is a natural synergy between the immune response and NF-κB pathway activation in promoting glioma invasion, angiogenesis, and proliferation growth, which collectively lead to significant effects on patient survival. Importantly, this signature provides an immune perspective on the clarification of the mechanism determining the clinical outcome in LGGs. Further studies of the applicability of this signature as a new component in LGG classification may lead to the development of a more comprehensive LGG prognostic and therapeutic system.

Even though the latest edition of WHO Classification has demonstrated an improvement in current sub-classification of LGGs,\textsuperscript{36} we know relatively little how individual treatment regimens prolong survival time. According to the results from the RTOG 9802 randomized trial,\textsuperscript{4} maximal safe resection combined with chemoradiation has been established as a new standard treatment as it was confirmed to provide survival benefit in LGG. However, the concern about long-term neurocognitive function decline raises the dilemma of treatment options. Therefore, efforts to establish the criteria for the selection of candidates who are likely to benefit from this standard treatment are currently underway. Recently, with the application of molecular parameters in a randomized clinical trial, a subgroup of patients who harbor oligodendrogial lesions with IDH mutations and 1p19q co-deletions has been shown to benefit from the combination of surgery and adjuvant chemoradiation.\textsuperscript{4} In contrast, patients with astrocytic gliomas devoid of those genetic signatures did not show significant improvement of the OS.\textsuperscript{4} Interestingly, those patients were mainly in our high risk score group. Growing evidence suggests that excessive immune response (e.g., pre-existing T-cell infiltration, expression of checkpoint proteins within the tumor, and high mutational burden) prior to the treatment predicted the subsequent response to immune treatment in various tumors.\textsuperscript{37,38} Taken together, gene signature identified by us may provide a rational approach in immune-based therapy of LGG. Furthermore, we also revealed that the signature positively correlated with the expression levels of checkpoint proteins and inflammatory response (e.g., T cell activation and macrophage-related immune response) that determine the local immunosuppressive microenvironment within the glioma. Therefore, successful immunotherapy needs to consider
immune system interactions in order to generate a more robust antitumor immune response.\textsuperscript{39,40} Our study provides a panoramic view of the tumor immune microenvironment that will facilitate the design of the combinatorial treatment strategy in LGG. However, a prospective clinical trial will be needed to validate our findings. Additionally, there remains a need for promising approaches to facilitate the penetration of the blood-brain barrier of therapeutic antibodies to improve clinical efficacy.

In conclusion, our study showed that IDH plays a crucial role in the regulation of immune microenvironment in LGG. Immune system gene signature sensitive to IDH mutation status can effectively predict patient survival and may add a prognostic value to current glioma classification. Moreover, this signature will be a useful predictive tool to identify patients who might benefit from immunotherapy.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Ethical approval**

The gliomas tissue collection was approved by the Institutional Review Board of Beijing Tiantan Hospital affiliated to Capital Medical University.

**Acknowledgments**

The authors conducting this work represent the Chinese Glioma Cooperative Group (CGCG).

**Funding**

This work was supported by Beijing Postdoctoral Research Foundation (2016ZZ-37).

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