AQP1 as a novel biomarker to predict prognosis and tumor immunity in glioma patients

Abstract

Background: Glioma is a kind of nervous system cancer with a low overall survival rate. Aquaporin 1 (AQP1) is linked to a number of cancers. Its prognostic relevance and immunological consequences in gliomas, however, are unclear.

Objectives: Our objective was to thoroughly examine the modified expression of AQP1, its prognostic significance, and its correlation with immune cells and markers to discover innovative molecular immunotherapy strategies for glioma patients.

Methods: RNA sequencing data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases were used. In addition, we used real-time reverse transcription polymerase chain reaction (RT-PCR) and Western Blot methods to monitor AQP1 expression in glioma tissues.

Results: AQP1 expression was greater in gliomas than in traumatized brain tissues. The increased AQP1 expression in gliomas was additionally confirmed through immunohistochemical analysis in the Human Protein Atlas (HPA) repository. An elevated level of AQP1 expression was identified as a separate determinant of the overall survival (OS) and prognosis of individuals with glioma. AQP1 expression was shown to be tightly linked to the tumor immune milieu, immune checkpoint blockade (ICB) and temozolomide drug reaction. In conclusion, the 50 genes that show coexpression with AQP1 indicate that the predominant functions and pathways are related to anterior pattern specification, pattern specification, regionalization, high-density lipoprotein particles, protein–lipid complexes, glycosaminoglycan binding, DNA-binding transcription repressor specific activation to RNA polymerase II, DNA-binding transcription repressor activity, nitrogen metabolism, alpha-linolenic acid metabolism, and fat digestion and absorption.

Conclusions: The results indicate that AQP1 could serve as both a predictive marker and a potential treatment target in glioma.

Keywords: AQP1; glioma; biomarker; poor prognosis; tumor immunity

Introduction

Glioma refers to a diverse collection of brain tumors that possess distinct biological and clinical features [1]. The current conventional treatment of glioma is limited to maximum surgical excision [2], postoperative radiotherapy [3] and temozolomide chemotherapy [4]. Nevertheless, the prognosis for glioma survival remains uncertain. Earlier research has indicated that immune-associated genes can be trusted for assessing the predictive significance of gliomas [5–7]. Hence, the development of a reliable prognostic indicator for further investigation into the onset of glioma is possible. Furthermore, the discovery of novel molecular targets holds practical importance in the advancement of new treatments and the enhancement of early detection and prediction of glioma.

Aquaporins (AQPs) are membrane proteins composed of small transmembrane channels that primarily facilitate the transportation of substances like water and glycerol across biolmms [8, 9]. Up to this point, AQP1 stands as the initial among 13 mammal AQPs that have been recognized. It has been reported that AQP1 expression is closely linked to the advancement of cancer, and AQP1 is elevated in several types of cancer, such as those affecting the colon, stomach, and ovaries [10–12]. Moreover, the cytoplasmic expression of AQP1 is higher in lymph node metastasis compared to the corresponding primary tumors. Additionally, AQP1 serves as a standalone prognostic factor, where elevated expression suggests an unfavorable prognosis [13, 14]. Some other scientists propose that AQP1 could be utilized as a potential indicator for predicting the outcome of breast cancer [15, 16]. Remarkably, heightened AQP1 manifestation in lung adenocarcinoma (LUAD) exhibited a strong correlation with a substantial number of macrophages, neutrophils, and dendritic cells [17].
Nevertheless, the precise status of AQP1 expression and its significance in gliomas remains undisclosed. To investigate discrepancies in AQP1 levels and its prognostic significance among glioma patients, we analyzed data obtained from the TCGA and GEO repositories. Furthermore, we investigated the relationship between the level of AQP1 expression and several clinicopathological characteristics of glioma, such as the World Health Organization (WHO), the status of IDH mutation, and the status of 1p/19q co-deletion. We additionally assessed the functional and biological significance of AQP1 in the development of glioma by conducting Gene Ontology (GO) and Kyoto Genome Encyclopedia (KEGG) analyses. To validate the bioinformatics prediction of AQP1 expression, glioma clinical samples were assessed for AQP1 mRNA expression levels using RT-qPCR. Using these discoveries, we created a prognostic model for estimating the overall survival (OS) of glioma patients by considering AQP1 expression and associated traits. Our primary focus was to explore the correlation between the expression of AQP1 and immune cells, as well as immunological markers, within the field of immunology. This research aimed to acquire fresh perspectives on molecular immunotherapy. To sum up, the results of this study can illuminate AQP1’s novel role in the development of glioma and offer a distinct molecular target for therapy.

Materials and methods

Database resource

Information regarding gene expression and clinical characteristics of glioma patients was acquired from the TCGA database (https://portal.gdc.cancer.gov). The TCGA_GBM and TCGA_LGG project STAR were utilized to gather and analyze the data, extracting the transcripts per million (TPM) in RNA-seq format. External data validation was conducted using GSE66354, which comprised of 13 samples of normal tissue and 136 samples of brain tumor tissue, obtained from the GEO database. We obtained genetic and phenotypic data on glioma patients, along with WHO classifications and IDH mutation status, from the University of California database. However, this particular dataset did not include any information on mutations. Xena (https://xenabrowser.net/datapages/) serves as the TCGA genotype – organization, transforming RNA sequencing data of expression level TPM into a standardized format. Furthermore, GTEx provides extracted data on normal tissues.

Evaluation of the Human Protein Atlas

The HPA, available at https://www.proteinatlas.org/, gathers data on the distribution of proteins throughout the human body. The analysis of 27, 397 antibodies’ proteome reveals distinct proteins originating from 17, 291 various human tissue types. We utilized it for evaluating the levels of AQP1 expression in glioma and healthy brain tissue.

Analysis survival of AQP1 in glioma

To examine the prognostic importance of AQP1 mRNA expression in gliomas, the clinical features and gene expression data from the TCGA database were employed. Based on their median AQP1 expression level, the patients were categorized into two groups: one comprising individuals with high expression and the other consisting of those with low expression. Using the R packages ‘survival’ and ‘survminer’, we utilized survival analysis and Cox proportional risk modelling to assess the predictive significance of AQP1.

Analysis of Cox regression using both univariate and multiple variables

Using univariate Cox regression analysis, we examined the impact of varying levels of AQP1 expression on OS in patients with glioma. Moreover, the study employed multivariate Cox regression analysis to assess whether AQP1 served as an autonomous prognostic indicator for overall survival in glioma patients. AQP1 was found to be statistically significant in Cox regression analysis with a significance level of p <0.05.

Examining the relationship between the expression of AQP1 and the infiltration of immune cells in tumors through correlation analysis

ssGSEA was employed to assess the infiltration of immune cells in glioma tissue. We evaluated the level of infiltration of 24 different immune cell types in gliomas by utilizing the ‘GSVA’ package in R along with immunological datasets. Furthermore, utilizing the R packages ‘ggplot2’ (V3.3.6), ‘limma’, and ‘pheatmap’, we conducted Spearman correlation analysis to examine the co-expression of AQP1 with immune checkpoints including histocompatibility complex (MHC), chemokine, Immunostimulator, immune receptors, and inhibitors.

Examining the variation in gene expression between glioma patients with low and high levels of AQP1

The R package ‘limma’ was utilized to perform an analysis using an unpaired Student’s t-test, aiming to identify differentially expressed genes (DEGs) by comparing the expression profiles (HTSeq-TPM) of high and low AQP1 mRNA expression groups. DEGs were identified based on statistical analysis using thresholds of |log2Fold Change| >1.5 and p <0.05.

Perform GO and KEGG enrichment analyses

The functional enrichment investigation was carried out with the use of the R package ‘clusterProfiler’, which entailed evaluating biological processes (BP), cellular components (CC), and molecular functions (MF) utilizing GO analysis and KEGG pathway analysis. The statistical significance of enriched ontological concepts was determined using a significance threshold of p<0.05.
Clinical sample

Between 2018 and 2023, Jiujiang Foundation Attached Nursing home in Jiangxi Profession derived 16 saucy glioma samples stranger a fair to middling develop into of patients. Everlastinglly continent action underwent a surgical attitude ordain physically administered chemotherapy or flare cure-yon. Administer brains kinsfolk was a false immigrant of six patients who had undergone unnerving intellectual surgery. Arbitrary RNA was extracted alien all samples examined beastlike subjected to glacial in detersive nitrogen midget, marked by the Medicine roborant Judgement Ship aboard of the Combined Facility of Jiujiangy Code of practice in Jiangxi Profession (approval no. jiuher-a-2023-0103).

RNA extraction and RT-qPCR

RT-qPCR was used to detect the levels of AQP1 mRNA expression in 6 cases of traumatic brain injury and 16 cases of glioma. Jiangxi Province’s Affiliated Hospital of Jiujiang Medical Department of Neurosurgery provided a total of 22 tissue samples. To summarize, total RNA was extracted from glioma and traumatic brain tissue using the TRIzol reagen(t TAKARA, 9109, Japan) following the guidelines provided by the reagent supplier. Next, cDNA was synthesized from 1,000 ng of total RNA using the Primirip TMRT kit (TAKARA, RR047A, Japan) to obtain complementary deoxyribonucleic acid (cDNA). Using TB Green® Premix Ex Taq™ II (TAKARA, RR820A, Japan) as a standardized control, qRT-PCR detected the expression of AQP1 mRNA, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) utilized. Primers are: AQP1: F: 5′CTGGGATCAGATCCGG-3′, R: 5′ATCCCCAGCCAGTGTGTC-3′. The forward primer sequence for GAPDH is 5′-GGACGCAATGCCCTCATTT-3′ and the reverse primer sequence is 5′-GCTGTTGTCATAATTCT-3′.

Assay for Western blot

Proteins from six tissues (three healthy and three cancerous) were obtained using the Radio-Immunoprecipitation Assay (RIPA) procedure, and their concentration was assessed using the BCA method. To achieve electrophoretic separation using 12 % sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the concentration of the protein was raised to 4 μg/μL. The proteins are deposited to the polyvinylidene difluoride (PVDF) membrane after electrophoresis. Afterwards, the protein was immersed in tris buffered saline-Tween (TBST) for a duration of 5 min, washed, and subsequently sealed for 15 min at ambient temperature using a fast-acting sealing solution. At 4 °C overnight, the system was subsequently sealed with AQP1 (ab300463, 1:1,000) and GAPDH (Proteintech 60004-1-lg, 1:50,000) primary antibodies. After that, the initial antibody was rinsed, and the subsequent antibody marked with horse-radish peroxidase (HRP) (1:10,000) was introduced and left at ambient temperature for 1 h, then proceeded with three 5 min TBST rinses. Before being developed in the dark with highly sensitive enhanced chemiluminescence (ECL), the excess liquid on the film is soaked up using filter paper. In the end, the protein bands were analyzed to ascertain the proportional manifestation of AQP1, while utilizing GAPDH as an internal reference.

Statistical analysis

The software paraphernalia ‘ggplot2’ in the Resting tongue was hand-me-down to ponder the conveyance of AQP1 in glioblastoma and common body. To interpret the stance between clinicopathologic phiz and AQP1 childbirth, the Kruskal–Wallis, Wilcoxon, and Chi-square tests were conducted. Human beings turn were preconceived to put to use the Kaplan–Meier come near, and fix it comparisons were obligated functioning the logarithmic autocratic annex confirmation. Cox sinking was occupied to accomplish both univariate and multivariate inquiry utilizing clinical variables and AQP1 transport equalize, down a relation rest of p<0.05. Omen models were constructed profit by WHO classification, IP/19q co-deletion, IDH novelty caste, and time e as predictors for 1-year, 5-year, and 10-year ordinary human beings. The Wilcoxon sure enlarge impede was conducted in the settle nearby high–low AQP1 articulation to assess the aspect between aggression of epitomized cells and immunological checkpoints. A p-value in the matter of than 0.05 was thorough to be statistically monstrous. To criticize the development of AQP1 mRNA emancipation in gliomas and brain-damaged relations substantiate, a t-test was conducted.

Results

AQP1 exhibits varying expression in both pan-carcinoma and glioma

AQP1 is highly expressed in 27 different types of cancer, such as CHOL, DLBC, GBM, LGG, LICH, PAAD, PCPG, TGCT, and THYM (as shown in Figure 1A and B). Notably, glioma exhibits particularly high levels of AQP1 expression (as depicted in Figure 1C and D). Additionally, immunohistochemical findings from the HPA database further validate the significant overexpression of AQP1 protein in glioma (as demonstrated in Figure 1E). Furthermore, we confirmed the mRNA and protein expression of AQP1 in tissue samples using RT-qPCR and Western Blot techniques. The findings indicated that the AQP1 expression level in glioma tissue was elevated compared to normal brain tissue, as depicted in Figure 1F and G.

Assessment of the predictive significance of AQP1 and clinical variables in glioma

In order to assess the predictive impact of AQP1, individuals with glioma were divided into categories with either low or high AQP1 levels, determined by the median TPM value (Table 1). According to the ROC curve, AQP1’s area under the curve (AUC) was 0.788 with a 95 % confidence interval (CI) of 0.526–1.000. Moreover, the presence
Figure 1: AQP1 expression in tumors differs. (A) AQP1 mRNA expression in pancarcinoma was demonstrated in TCGA and GTEX. (B) AQP1 mRNA expression in pancarcinoma was shown in GEPIA. (C, D) The mRNA expression of AQP1 in the TCGA and GEO in glioma. (E) Immunohistochemistry was used to detect AQP1 expression at the protein level in glioma via the HPA database. (F) The expression level of AQP1mRNA in tissue samples was detected by RT-qPCR. (G) The expression level of AQP1 protein in tissue samples was detected by Western Blot. *p<0.05, **p<0.01, ***p<0.001.

Table 1: Logistic regression was used to analyze the relationship between AQP1 expression and clinical features (n=699).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low expression of AQP1 n=349</th>
<th>High expression of AQP1 n=350</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO grade, n, %</td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>G2</td>
<td>154 (42.4 %)</td>
<td>70 (11 %)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>122 (19.2 %)</td>
<td>123 (19.3 %)</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>31 (4.9 %)</td>
<td>137 (21.5 %)</td>
<td></td>
</tr>
<tr>
<td>IDH status, n, %</td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>WT</td>
<td>52 (7.5 %)</td>
<td>194 (28.2 %)</td>
<td></td>
</tr>
<tr>
<td>Mut</td>
<td>291 (42.2 %)</td>
<td>152 (22.1 %)</td>
<td></td>
</tr>
<tr>
<td>1p/19q codeletion, n, %</td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Non-codel</td>
<td>209 (30.2 %)</td>
<td>311 (44.9 %)</td>
<td></td>
</tr>
<tr>
<td>Codel</td>
<td>140 (20.2 %)</td>
<td>32 (4.6 %)</td>
<td></td>
</tr>
<tr>
<td>Age, n, %</td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>≤60</td>
<td>310 (44.3 %)</td>
<td>246 (35.2 %)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>39 (5.6 %)</td>
<td>104 (14.9 %)</td>
<td></td>
</tr>
<tr>
<td>Histological type, n, %</td>
<td></td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>82 (11.7 %)</td>
<td>114 (16.3 %)</td>
<td></td>
</tr>
<tr>
<td>Oligodendrogloma</td>
<td>155 (22.2 %)</td>
<td>45 (6.4 %)</td>
<td></td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>31 (4.4 %)</td>
<td>137 (19.6 %)</td>
<td></td>
</tr>
</tbody>
</table>
of AQP1 was significantly correlated with the grade of pathology, patient age, type of tumor, mutation in IDH, and deletion of the 1p/19q chromosome (p<0.001; Figure 2B–G). The results suggest that AQP1 can serve as a clinical diagnostic marker for pathological grade, age, tumor type, IDH mutation, and 1p/19q chromosomal deletion, providing a new method for the early diagnosis of glioma.

Moreover, the examination of AQP1 mRNA expression through univariate and multivariate Cox regression analysis demonstrated that it served as a significant autonomous risk factor [hazard ratio (HR)=1.629; 95 % CI: 1.175–2.258, p<0.01]. Furthermore, the WHO classification also encompasses G3 (HR=1.870; 95 % CI: 1.222–2.861, p<0.01) and G4 (HR=4.227; 95 % CI: 2.514–7.107, p<0.001). Additionally, age (HR=1.455; 95 % CI: 1.069–1.981, p<0.05) emerged as a separate prognostic risk factor for OS, while IDH status (HR=0.293; 95 % CI: 0.195–0.439, p<0.001) could potentially serve as an independent prognostic protective factor for OS (Figure 3).

Figure 2: A correlation study of AQP1 expression and clinical characteristics. (A) Glioma AQP1 Receiver Operating Characteristic Analysis (ROC). (B) An examination of AQP1 expression in TCGA subgroups according to glioma grade. (C) Relationship between AQP1 expression and age. (D) Correlation between AQP1 expression and histological type. (E) Correlation between AQP1 expression and IDH status. (F) AQP1 expression in the codel and non-codel of the 1p/19q co-deletion. (G) Analysis of association between clinical features and AQP1 expression. *p<0.05, **p<0.01, ***p<0.001.
Glioma prognosis is negatively impacted by elevated AQP1 expression

According to the Kaplan–Meier survival analysis of the TCGA-GBMLGG dataset, individuals exhibiting elevated levels of AQP1 expression experienced a more unfavorable prognosis compared to those with lower AQP1 expression (HR=3.52; 95% CI: 2.7–4.6, p<0.001, as shown in Figure 4A). Likewise, we analyzed Kaplan–Meier plots for disease-specific survival (DSS) and platinum-free interval (PFI) in the TCGA-GBMLGG dataset. Decreased AQP1 expression was associated with shorter DSS (HR=3.66; 95% CI: 2.76–4.85, p<0.001, Figure 4B) and PFI (HR=2.38; 95% CI: 1.91–2.96, p<0.001, Figure 4C).

Generation and evaluation of prognostic models in glioma patients

In both univariate and multivariate regression analyses, AQP1 was discovered to be a separate prognostic element for glioma. In order to accomplish this, we developed a predictive map for the operating system by utilizing TCGA AQP1 mRNA expression data and incorporating additional clinicopathological features. Using AQP1 expression data and clinical prognostic factors like tumor grade, IDH status, 1p/19q co-deletion, age, and gender, a nomogram was developed (Figure 4D). The prognosis improves as the total score increases, which is determined by summing up the scores of all elements in the chart. To evaluate the nomogram’s performance, both the calibration curve and the ROC curve were utilized. AQP1’s nomograms predicted AUC values of 0.723, 0.762, and 0.821 for 1-year, 5-year, and 10-year, respectively (Figure 4E). The viability of the method was confirmed by calibration curves (Figure 4F). To sum up, the presence of AQP1 is closely linked to OS in individuals with glioma, suggesting that it could serve as a potential indicator for predicting the survival of glioma patients.

The level of immune cell infiltration showed a strong correlation with the expression of AQP1

To better examine the function of AQP1 in early gliomas, we employed ssGSEA to analyze the correlation between AQP1
mRNA expression and the level of immune cell infiltration (Table 2). The relationship between the infiltration of immune cells and the expression of AQP1 is illustrated in Figure 5A. Moreover, we examined the correlation between elevated and reduced AQP1 levels and the infiltration of macrophages, neutrophils, eosinophils, aDCs, T cells, and pDCs. Macrophages (R=0.514, p<0.001, Figure 5B), Neutrophils (R=0.406, p<0.001, Figure 5C), Eosinophils (R=0.473, p<0.001, Figure 5D), aDC (R=0.305, p<0.001, Figure 5F), T cells (R=0.305, p<0.001, Figure 5E), but exhibited a negative correlation with infiltration of pDC cells (R=-0.370, p<0.001, Figure 5G).

Table 2: Correlation analysis between AQP1 expression and infiltration levels of 24 kinds of immune cells.

<table>
<thead>
<tr>
<th>AQP1</th>
<th>Immune cells</th>
<th>Correlation (Pearson)</th>
<th>p-Value (Pearson)</th>
<th>Correlation (Spearman)</th>
<th>p-Value (Spearman)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>aDC</td>
<td>0.287391884</td>
<td>p&lt;0.001</td>
<td>0.304635509</td>
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</tr>
<tr>
<td></td>
<td>B Cells</td>
<td>0.111581248</td>
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<td>0.087210968</td>
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<td>CD8 T cells</td>
<td>−0.200318573</td>
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<td>−0.218114364</td>
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<td>Cytotoxic cells</td>
<td>0.226491159</td>
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<td>0.261422756</td>
<td>p&lt;0.001</td>
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<tr>
<td></td>
<td>DC</td>
<td>−0.000110617</td>
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<td>0.007530177</td>
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<td></td>
<td>Eosinophils</td>
<td>0.449628123</td>
<td>p&lt;0.001</td>
<td>0.473056838</td>
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<tr>
<td></td>
<td>iDC</td>
<td>0.204421691</td>
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<td>0.239598713</td>
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<td>Macrophages</td>
<td>0.477589872</td>
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<td>0.51438146</td>
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<td>Mast cells</td>
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<td>Neutrophils</td>
<td>0.344145161</td>
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<td>0.406060479</td>
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<td>NK CD56 bright cells</td>
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<td>NK cells</td>
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<td></td>
<td>pDC</td>
<td>−0.350995402</td>
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Table 2: (continued)

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<tr>
<th>AQP1</th>
<th>Immune cells</th>
<th>Correlation (Pearson)</th>
<th>p-Value (Pearson)</th>
<th>Correlation (Spearman)</th>
<th>p-Value (Spearman)</th>
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<tr>
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<td>T Cells</td>
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<td>0.304815405</td>
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<tr>
<td></td>
<td>T Helper cells</td>
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<td>-0.054102832</td>
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<tr>
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<td>Tcm</td>
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<td>-0.243309517</td>
<td>p&lt;0.001</td>
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<td></td>
<td>Tem</td>
<td>-0.179821419</td>
<td>p&lt;0.001</td>
<td>-0.180001335</td>
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<td></td>
<td>Tfh</td>
<td>-0.004079478</td>
<td>p&gt;0.05</td>
<td>-0.022810693</td>
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<td>Tgd</td>
<td>-0.058688159</td>
<td>p&gt;0.05</td>
<td>-0.134673368</td>
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<td>Th1 cells</td>
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<td>p&lt;0.01</td>
<td>0.118331433</td>
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<td>Th17 cells</td>
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<td>0.156320994</td>
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<td>0.234174112</td>
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<td>TReg</td>
<td>-0.077742478</td>
<td>p&lt;0.05</td>
<td>-0.090985101</td>
<td>p&lt;0.05</td>
</tr>
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</table>

p<0.05, p<0.01 and p<0.001 all indicated statistical significance.

Figure 5: ssGSEA analysis of AQP1 in glioma and its correlation with immune infiltration level. (A) Correlation analysis between AQP1 expression and 24 kinds of immune cell infiltration. (B–G) AQP1 expression was positively correlated with the infiltration levels of macrophages, neutrophils, eosinophils, aDC and T cells, and negatively correlated with the infiltration levels of pDC cells.
Examining the relationship between the expression of AQP1 and the immune microenvironment of tumors through correlation analysis

To gain a deeper comprehension of how AQP1 affects the immune environment of gliomas, we investigated its simultaneous expression with key MHC molecules, immune activators, immune inhibitors, chemokine receptors, and chemokines. AQP1 receptors co-express nearly all MHC marker genes (Figure 6A) and chemokine receptors (Figure 6B). Figure 6D shows that AQP1 has a positive correlation with CXCL10, which plays a role in regulating the immune response of T cells. Additionally, AQP1 is significantly correlated with TNFRSF14, a protein linked to immune activation. Based on the co-expression heat map, it is evident that, apart from LAG3, the expression of AQP1 shows both positive and negative correlation with immunosuppression (Figure 6E). Moreover, the tumor mutation load (TML) and microsatellite instability (MSI) both have significant impacts on tumor immune response. AQP1 expression showed a positive correlation with TMB score (Figure 6F) and a negative correlation with MSI score (Figure 6G). Furthermore, glioma patients exhibiting elevated AQP1 expression displayed higher TIDE scores, indicating a potential favorable response to ICB treatment (Figure 6H). Additionally, we examined the correlation between AQP1 and temozolomide, a commonly prescribed medication for glioma. Figure 6I demonstrated a negative correlation between the expression of AQP1 and temozolomide.

Figure 6: Correlation analysis between AQP1 expression and tumor immune microenvironment. Co-expression analysis of AQP1 with MHC genes (A), chemokine receptors (B), chemokine genes (C), Immunostimulator (D), immunosuppression (E). (F, G) The correlation between AQP1 expression and TMB score and MSI score. (H) In glioma patients, the AQP1 group responds differently to immune checkpoint inhibition. (I) The relationship between AQP1 and the temozolomide IC50. *p<0.05, **p<0.01, ***p<0.001.
Performing functional enrichment analysis on glioma samples exhibiting high and low levels of AQP1 expression

In order to explore the likely process through which AQP1 promotes the malignant progression of gliomas, TCGA data were utilized to analyze glioma samples categorized into high- and low-expression groups of AQP1. This analysis revealed a total of 943 differentially expressed genes (DEGs). Figure 7A displayed a total of 836 differentially expressed genes (DEGs) exhibiting high expression and 107 DEGs with low expression, meeting the criteria of $|\log_2 \text{(Fold Change)}| > 1.5$ and p-adj 0.05. Coexpression heat maps (Figure 7B and C) demonstrated the correlation between AQP1 expression and the top 50 genes co-expressed with AQP1. To assess the role of coexpressed genes, enrichment analysis was utilized, and enrichment items were ordered based on their p-values. The enrichment items in the BP, MF, CC, and KEGG groups include anterior/posterior pattern specification, pattern specification, regionalization, high-density lipoprotein particle, protein–lipid complex, glycosaminoglycan binding, DNA-binding transcription repressor activity, RNA polymerase II-specific, DNA-binding transcription repressor activity, Nitrogen metabolism, alpha-linolenic acid metabolism, and Fat digestion and absorption (Figure 8).

Figure 7: (A) Volcano map of differentially expressed genes in glioma. (B, C) Heat map of co-expression of the top 50 genes adjacent to AQP1. *p<0.05, **p<0.01, ***p<0.001.
Discussion

Glioma ranks among the frequently occurring primary tumors in the brain [18, 19]. Despite long-standing dedication to enhancing glioma patient treatment, neuro tumor research continues to face numerous challenges in clinical and patient prognosis [20]. Hence, the quest for biomarkers that can enhance prognosis is of utmost significance [21, 22]. In our investigation, we found that gliomas exhibited significant overexpression of AQP1, which was associated with an unfavorable prognosis. Functional enrichment analysis revealed that AQP1 expression was linked to the specification of anterior/posterior pattern, pattern specification, process, regionalization, high-density lipoprotein particle, protein–lipid complex, glycosaminoglycan binding, DNA-binding transcription repressor activity, RNA polymerase II-specific, and DNA-binding repressor activity. Consequently, this study emphasizes the possible importance of AQP1 in the development of tumors and its utility as a biomarker for glioma.

Analysis of data from the public databases TCGA and GEO indicated that AQP1 exhibited higher expression levels in gliomas compared to normal brain tissue (p<0.05). These findings were confirmed by clinical tissue samples that included six samples of traumatic brain tissue and 10 samples of glioma tissue, consisting of 4 grade 2 gliomas and 6 grade 4 gliomas. The TCGA dataset confirmed this finding, indicating that AQP1 could potentially serve as a diagnostic indicator for various types of cancers [23–25]. AQP1 expression proved to be a successful diagnostic indicator for glioblastoma, as evidenced by its AUC of 0.788. Furthermore, AQP1 is associated with the pathological stage of glioma, the age of the patient, the presence of IDH mutation, and the co-deletion of 1p/19q. The clinical observations provide evidence that the expression of AQP1 is associated with the malignancy of glioma.

Figure 8: GO function (including BP, CC and MF) and KEGG pathway enrichment analysis of differentially expressed genes in glioma.
Gliomas exhibit an elevated expression of AQP1, leading to an unfavorable prognosis and reduced survival rate. Based on the analysis of TCGA-GBMLGG data, individuals exhibiting elevated AQP1 levels experienced unfavorable outcomes in terms of OS, disease-specific survival (DSS), and progression-free interval (PFI). In both univariate and multivariate Cox regression analyses, AQP1 was identified as a separate risk factor for glioma. Because AQP1 is a powerful predictor, we created a graph that combined AQP1 expression with clinical variables. The chart displays a superb receiver operating characteristic (ROC) curve and has the ability to precisely forecast the OS of glioma patients for durations of 1 year, 5 years, and 10 years. Regrettably, because there are several absent samples in the TCGA database, any variable with missing samples will be excluded from the single/multi-factor analysis if they are removed or if their count is zero, prior to conducting the single factor analysis. In conclusion, the use of AQP1 predictors is advantageous in identifying individuals with a high susceptibility to glioma and selecting the most suitable treatment based on their level of risk.

Next, we utilized ssGSEA to examine the involvement of AQP1 in the immune system, and the findings indicated a correlation between AQP1 and the infiltration of immune cells. AQP1 demonstrated a positive correlation with macrophage infiltration and a negative correlation with infiltration of CD8$^+$ T cells. Initial studies discovered notable amounts of macrophage infiltration and limited amounts of CD8$^+$ T cell infiltration in various cancers, which were associated with immune suppression and unfavorable prognosis [26–28]. According to the research, AQP1 might play a crucial part in facilitating immune evasion from gliomas as a result of the elevated presence of macrophages and diminished levels of CD8$^+$ T cells [29–32]. Evaluating the tumor's ability to generate an immune response and its response to immunotherapy is a crucial aspect of the TMB. Many ICBs are shown to be effective against MSI, which causes somatic mutations that can be targeted for therapeutic purposes. This study has provided a detailed explanation of the correlation between AQP1 expression and TMB and MSI, highlighting the significance of AQP1 in tumor immunity. Interestingly, AQP1 was above negatively connected around semi-inhibitory concentrations of the oft-times second-hand chemotherapy counteractant temozolomide. This suggests turning this way glioma patients hither overbearing AQP1 release may computation change for the better outsider ICB correct. The impoverished conductor AQP1 could potentially forecast ICB and chemotherapy. This information provides valuable insights into potential targets for effective pain relief in patients with glioma.

Despite revealing a connection between AQP1 and glioma and enhancing our comprehension of AQP1’s potential role in glioma, this study does have certain constraints. First, additional critical activities and processes involved in AQP1 in glioma, as well as agent mechanisms, have yet to be identified, and we intend to investigate them in future studies. Furthermore, there is little data to support the possible role of AQP1 in gliomas. Experiments in laboratory settings and on real species are required to validate the predictability of the predictions. In the recent updates of C-ImpacT-NOW, both updates three and five delve into the latest information, emphasize the significance of the molecular attributes of gliomas, and highlight the insufficiency of histological grading in describing these tumors.

In summary, gliomas exhibit an increase in AQP1 expression and this protein also has a notable impact on tumor immunity, affecting the tumor microenvironment and ultimately influencing the prognosis of glioma patients (Figure 9). These findings suggest that AQP1 could be a valuable target for future immunotherapy approaches. This research implies that AQP1 might be a glioma marker.

Figure 9: Graphical abstract of AQP1 as a biomarker of tumor immunity and prognosis of glioma.
Future prospective

Temozolomide is the current first-line chemotherapy drug for brain glioma. Combined with radiotherapy, temozolomide can partially prolong the survival time of newly diagnosed glioma patients. NCCN guidelines in the United States have taken surgery, radiotherapy and temozolomide chemotherapy as the standard treatment for newly diagnosed glioma patients. The chemotherapy effect of brain glioma is limited by the drug resistance of temozolomide chemotherapy, and tumors often relapse quickly after treatment, making the treatment of brain glioma one of the current medical problems. AQP1 plays an important role in the malignant progression of brain glioma. However, the role of AQP1 in the chemotherapy resistance of brain glioma temozolomide is still unclear, which provides a new idea for our future in-depth research.

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Research ethics: This study was approved by the Institutional Review Board (IRB) at the Jiujiang University Affiliated Hospital (approval no. jjuhmer-a-2023-0103). Patients were consented by an informed consent process that was reviewed by the IRB.

Author Contributions: Xiang Gao drafted articles for important knowledge content and made critical revisions to it and planned and monitored the study. Wengu Jiang and Qiwei Huang participated in the article structure design, manuscript drafting and main experimental research. Guofeng Zhu and Zelong Xing were responsible for cell-related experiments; Pengbo Zhu and Zunliang Ke were responsible for the article writing. All authors read and agree to the final manuscript.

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