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Maybe a New Target for Gliomas: AQP1

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Abstract: Gliomas are the most common and aggressive tumors of the central nervous system, with poor prognosis. Studies on their diagnosis and treatment are critical. This study investigates the roles of Aquaporin family members, specifically AQP1 and AQP4, in gliomas using in silico methods. Expression levels of AQPs in Low-Grade Glioma (LGG) and Glioblastoma Multiforme (GBM) glioma subtypes were analyzed using GEPIA, UCSC Xena, Gliovis, cBioPortal, and Ivy GAP tools. Findings revealed that AQP1 and AQP4 expressions were significantly higher in tumor tissues compared to normal tissues in LGG and GBM datasets. Survival and prognosis analyses showed AQP1 levels were lower in the Oligodendrogram subtype of LGG, whereas both AQP1 and AQP4 levels were elevated in other subtypes. These results highlight AQP1 and AQP4 as key contributors to glioma pathogenesis and patient survival. While AQP4 is already known, AQP1 emerges as a potential novel biomarker or drug target for aggressive gliomas. Future studies should further explore its therapeutic potential.

Gliomlar için Belki Yeni Bir Hedef: AQP1

Anahtar Kelimeler
AQP4,
AQP1,
LGG,
GBM,
In silico

Öz: Gliomlar, merkezi sinir sisteminin en yaygın ve agresif tümörleridir ve prognozları genellikle kötüdür. Bu nedenle, gliomların tanı ve tedavisine yönelik çalışmalar büyük önem taşımaktadır. Bu çalışmada, in silico yöntemlerle Aquaporin ailesi üyeleri, özellikle AQP1 ve AQP4'ün gliomlardaki rolleri incelenmiştir. LGG ve GBM gliom alt tiplerinde AQP ekspresyon seviyeleri GEPIA, UCSC Xena, Gliovis, cBioPortal ve Ivy GAP analiz araçlarıyla değerlendirilmiştir. Bulgular, AQP1 ve AQP4 gen ekspresyonlarının LGG ve GBM veri setlerinde tümör dokularında normal dokulara kıyasla daha yüksek olduğunu göstermiştir. Sağkalım ve prognoz analizleri, AQP1 seviyelerinin LGG'nin Oligodendrogram alt tipinde düşük, diğer tüm alt tiplerde ise AQP1 ve AQP4 seviyelerinin yüksek olduğunu ortaya koymuştur. Bu sonuçlar, AQP1 ve AQP4'ün gliom patogenezi ve hasta sağkalımında önemli roller oynadığını göstermektedir. AQP4'ün yanı sıra AQP1 de agresif gliomlara karşı yeni bir biyobelirteç veya ilaç hedefi olarak değerlendirilebilir. Bu genlerin terapötik potansiyeli, gelecekteki çalışmalarda araştırılmalıdır.

1. INTRODUCTION

Gliomas, the most common tumors of the central nervous system, also make up 80% of the most deadly malignant brain tumors [1, 2]. It has been observed that the age, gender, ethnicity and other factors affecting the incidence vary between about 2-10/100,000 [3, 4]. When classified histopathologically, they can be divided up to 4 degrees, although they can generally be classified into low-grade gliomas (LGGs) (astrocytomas, oligodendrogliomas and oligoastrocytomas) and glioblastoma multiforme (GBMs) [5, 6]. LGG has a 5-year overall survival rate of approximately 60%, while GBM has a median survival of 14.6 months. Therefore, GBM can be described as the most aggressive brain tumor [5, 7]. Examining the survival based on grades, it has been shown that grade 4 has the worst rate among others and that approximately 7% of patients can survive in the next 5 years [8, 9]. Another bad news is that patients with a diagnosis of LGG may evolve to GBM within 5-10 years. During this process, many gene expression changes occur, and it is important to understand the course of the disease by monitoring their changes [10]. The most known glioblastoma markers have been studied as O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, Epidermal growth factor receptor

(EGFR) changes, and isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) mutations [11, 12], but new genes need to be investigated and applied as biomarkers to accelerate the diagnosis and treatment of gliomas of varying degrees.

With the widespread use of RNA sequencing studies, it has become increasingly easy to monitor and compare changes in gene expression. When examined in terms of clinical oncology, the advantages of this situation are known in terms of discovering new genes effective in tumors, monitoring the effects of applied therapeutic agents and making discoveries related to tumor prognosis [13-15]. Differentially expressed genes (DEGs) are obtained after many bioinformatic analyses and database usage used to carry out these studies [16, 17]. It is obtained by examining the signal pathways and interaction networks of their effects on the tumor, after comparative examination of these obtained genes. New gene discoveries create new targets for tumors and thus new hopes for improved patient survival [18, 19].

Although it is known that aquaporins (AQP) can play different roles in various tissues, they are most commonly involved in water balance, as well as in fat metabolism, cell proliferation, migration and adhesion [20-22]. AQPs, which are water-selective transmembrane transport channel proteins, are a family of 13 proteins with high conservativity. Of these, only AQP1, AQP4 and AQP9 are found in the brain in mammals [23]. The AQP1 and AQP4 as classical aquaporins are selective for water, urea, gases, H2O2, ammonia, and charged particles. The AQP9 is one of the groups of listed as aquaglyceroporins and permeable for water, glycerol, and, in some cases, urea, lactate, or H2O2 [22].

Given the therapy-resistant nature of GBM and the potential for LGG to progress into GBM, it becomes imperative to emphasize research concerning these diseases and gain insight into potential drug target proteins and genes. One notable aspect of studies on differentially expressed genes is their capacity to pinpoint genes exhibiting distinct expression patterns between normal and tumor tissues. This capability facilitates comparative analysis and the identification of genes and proteins that exhibit significant variations, making them potential candidates for drug targeting.

Moreover, it's of utmost importance not only to spot individual genes with significant differences but also to identify those that are interconnected and collectively influential. Biological studies have established that proteins can compensate for each other's functions. Therefore, rather than concentrating solely on a single protein or gene, investigating pathways and interconnected genes that function in tandem is another crucial aspect of drug targeting.

The primary objective of this study is to assess the expression status of transmembrane transporter proteins like AQP1 and AQP4 in GBM and LGG, in conjunction with both individual and correlated known genes associated with these diseases. This comprehensive approach aims to provide a deeper understanding of potential drug targets.

2. MATERIAL AND METHOD

2.1. Gene Expression Analysis of AQP Family Genes in Glioma and Normal Tissues

The gene expression profiles of human aquaporins in LGG and GBM datasets were analyzed with the Gene Expression Profiling Interactive Analysis (GEPIA) platform (http://gepia.cance r- pku.cn/). Normal and tumor samples were compared to determine the differences [24]. Expression levels in tumors were assessed using The Cancer Genome Atlas (TCGA) dataset whereas transcript levels in healthy tissue samples were obtained from the Genotype Tissue Expression (GTEx) project. The cut-off of p value and fold change were defined as 0.05 and 1.5, respectively.

2.2. AQP1 and AQP4 Gene Expressions Based on Histology

Samples from the TCGA-LGG dataset were analyzed using the GlioVis (http://gliov is.bioin fo.cnio.es/) web application.18 AQP1 and AQP4 expressions in patients having different histologies were assessed and plotted as log2(norm_count+1). In the current analysis, 188 patients with Oligodendroglioma, 129 patients with Oligoastrocytoma, 193 patients with Astrocytoma and in total 510 patients were included in the expression query. Samples from the TCGA-GBM dataset (RNA-seq platform) were analyzed using the GlioVis (http://gliov is.bioin fo.cnio.es/) web application [25]. AQP1 and AQP4 expressions in patients having different histologies were assessed and plotted as log2 (norm_count+1). In the current analysis, 4 patients with non-tumor, 156 patients with GBM and in total 160 patients were included in the query.

2.3. AQP1 and AQP4 Transcript Level Analyses Based on Molecular Subtype

For glioma subtype analysis TCGA-LGG and TCGA-GBM datasets were used. TCGA- LGG dataset includes 85 patients with IDHmut-codel, 141 patients with IDHmut-non-codel, 55 patients with IDHwt subtypes, and in total 281 patients were included for this analysis. TCGA- GBM dataset includes 56 patients with Classical, 51 patients with Mesenchymal, 46 patients with Proneural subtypes and in total 156 patients were included for this analysis. Samples were analyzed using the GlioVis (http://gliov is.bioinfo.cnio.es/) web application [25].

2.4. Overall Survival Analyses of AQP1 and AQP4

Patients' overall survival Kaplan-Meier plots were drawn to the UCSC Xena browser using records of primary tumor samples obtained from the TCGA-LGG, TCGA-GBM and TCGA-GBMLGG cohorts. The median for each gene in the selected cohort was used for groups [26].

2.5. Correlation Analysis of AQP1 and AQP4 Gene Transcript Levels

Correlation between AQP1 and AQP4 transcript levels were determined by retrieving gene expression RNAseq illuminaHiseq data on primary tumors in the TCGA- LGG and TCGA- GBM cohorts from GlioVis. A log2(X + 1) transformed RSEM normalized count was depicted on both axes. Pearson tests were used for correlation analyses [25].

2.6. AQP4 Correlated Genes and Protein Interaction Analyses

AQP1 and 4 and their correlated genes (filtered by r>0.5 for AQP1 and r>0.6 for AQP4) were obtained from The Ivy Glioblastoma Atlas Project (IVY GAP) [27]. STRING v11.5 (Search Tool for the Retrieval of Interacting Genes) was used to examine protein–protein interaction networks [28]. Functional annotation analysis was performed using DAVID v6.7 (Database for Annotation, Visualization, and Integrated Discovery), which provides a comprehensive set of functional annotation tools to understand the biological significance associated with large lists of genes or proteins [29].

2.7. Statistical Analysis

GraphPad Prism 9 was used for statistical analysis in the study. Kruskal Wallis test was applied for comparisons between groups, and Dunn's post hoc test was applied for multiple comparisons. Comparison between two groups was made using Student's t-test. All values are represented as mean ± SD.

Kaplan–Meier curves were plotted using the UCSC Xena database. The difference between the curves was determined by the Log-rank test. Correlation analysis was performed by calculating Pearson correlation coefficients. $p < 0.05$ indicates statistical significance.

3. RESULTS

3.1. The Expressions of AQP1 & AQP4 in Different Human Cancers

The expression levels of AQP1 were observed as increased in tumor tissues of Cholangio carcinoma (CHOL), Glioblastoma multiforme (GBM), Brain Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Pancreatic adenocarcinoma (PAAD), Pheochromocytoma and Paraganglioma (PCPG), Stomach adenocarcinoma (STAD), Testicular Germ Cell Tumors (TGCT) and Thymoma (THYM) (Figure 1A). AQP4 was found to be higher in tumor tissue in CHOL, GBM, LGG, PCPG, THYM and Uterine Carcinosarcoma (UCS) human cancer types (Figure 1B).

As a result of the comparison of the expressions of the AQP family genes in the brain parts, although the cerebellar hemisphere and cerebellum are slightly different from the other parts, in general, it has been observed that AQP1 and AQP4 show very high activity in almost all brain regions (Figure 1C). The most striking difference in the cerebellar hemisphere and cerebellum is the higher expression of AQP7 compared to other brain tissues.

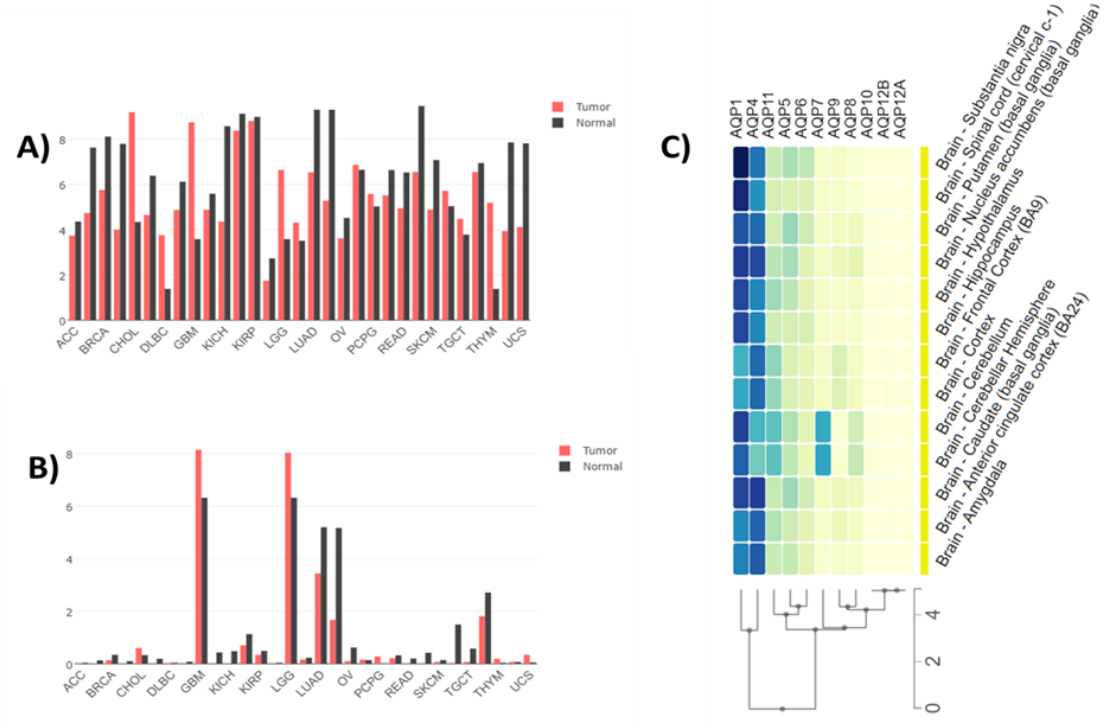

Figure 1. (A) AQP1 and **(B)** AQP4 expression levels in Human Cancers by GEPIA. **(C)** Comparison of the expressions of AQP family genes in brain regions using GTEx portal.

3.2. AQP Family Genes in LGG and GBM Platforms Have Different Expression Results in Glioblastoma and Normal Tissue Samples

It is known that intercellular and intracellular traffic is very rapid in cancer. One of the protein families responsible for these traffics is aquaporins. Depending on the increase in cancer cell activities, the increase of these proteins, which are responsible for transport, may be specific to some types of cancer or cancer tissues. Considering this situation, it aims to establish a relationship by looking at the expression rates of AQP family genes in glioblastoma cancers and their significance levels.

In the expression profiles comparison, it is observed that the gene expression levels of AQP1 and AQP4 are highly expressed in both LGG and GBM datasets compared to the others (Figure 2). It is observed that the amount of AQP1 in tumor tissue is quite significant compared to normal tissue. According to Figure 2, AQP11 is another highly expressed aquaporin after AQP1 and AQP4.

Figure 2. The expression profiles of human AQP1-12 (12A and 12B) in LGG and GBM platforms. The transcript levels of AQP family members in LGG and GBM tumors and normal tissues were analyzed using the GEPIA database.

Considering the significance of expression levels of AQP genes in tumor and normal tissues in LGG and GBM datasets, AQP1 and AQP4 seem to be remarkable in both datasets. Figure 3 (A-M) shows the differential expression levels of all AQP genes in LGG and GBM datasets. Figure 5A (AQP1) and Figure 3D (AQP4) were statistically significantly higher in tumor tissue than in normal tissue. Although AQP5 and AQP6 were higher in tumor tissue in both datasets, the results were not statistically significant. AQP7,8,11,12A and 12B appear to be higher in normal tissue, but the results are also not statistically significant in these groups. Overall, these data highlight the possible roles of AQP1 and AQP4 in glioma progression.

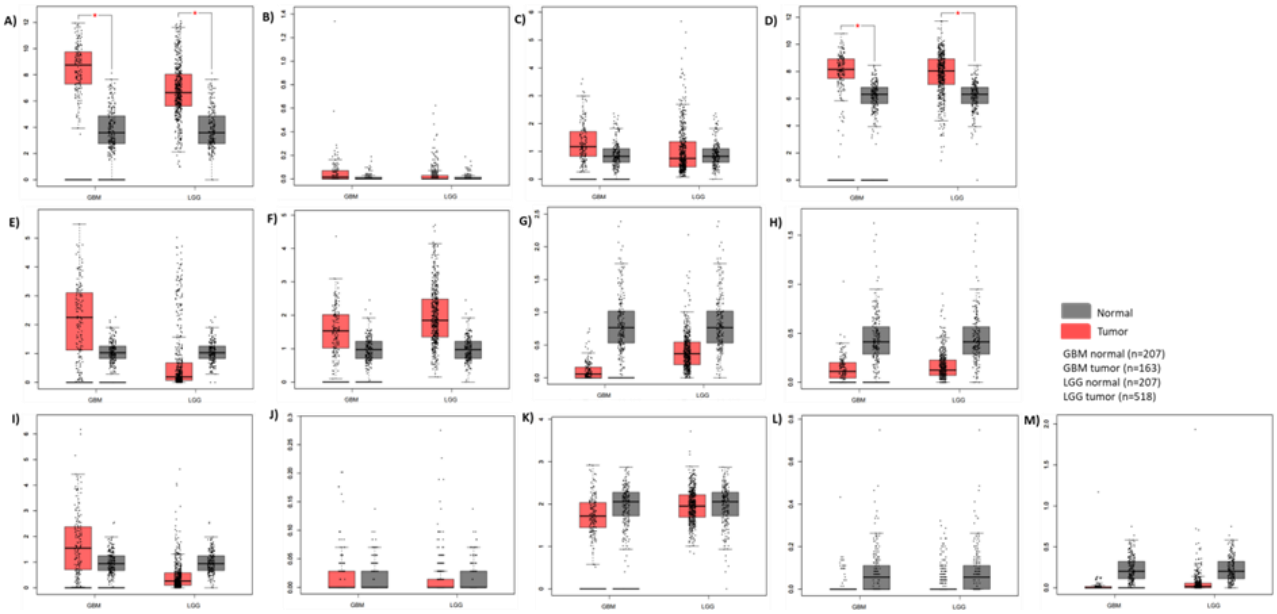

Figure 3. The differential expressions of AQP family members in LGM and GBM tumor tissues compared to normal counterparts. The expression levels of AQPs in LGM and GBM were analyzed using the GEPIA database. Box plots showing the expression profile of (A) AQP1, (B) AQP2, (C) AQP3, (D) AQP4, (E) AQP5, (F) AQP6, (G) AQP7, (H) AQP8, (I) AQP9, (J) AQP10, (K) AQP11, (L) AQP12A, (M) AQP12B in tumor and healthy tissues. The asterisk (*) shows statistical significance ($p<0.05$).

3.3. High AQP1 and Low AQP1 in GBM & Low AQP1 and AQP4 in LGG are related with better prognosis

To assess the prognostic values of the AQP1 and AQP4 genes in two different datasets (GBM and LGG), Kaplan-Meier plots were generated to visualize the overall survival (OS) patterns (Figure 4). It was observed that increased AQP1 expression in the GBM dataset (Figure 4A) had a positive impact on the overall survival curve. Similarly, the effect of AQP4 on overall survival in the GBM dataset was found to contribute (Figure 4B). In the overall survival plots of the LGG dataset, lower gene expression levels for both AQP1 and AQP4 were associated with longer OS (Figure 4C-D).

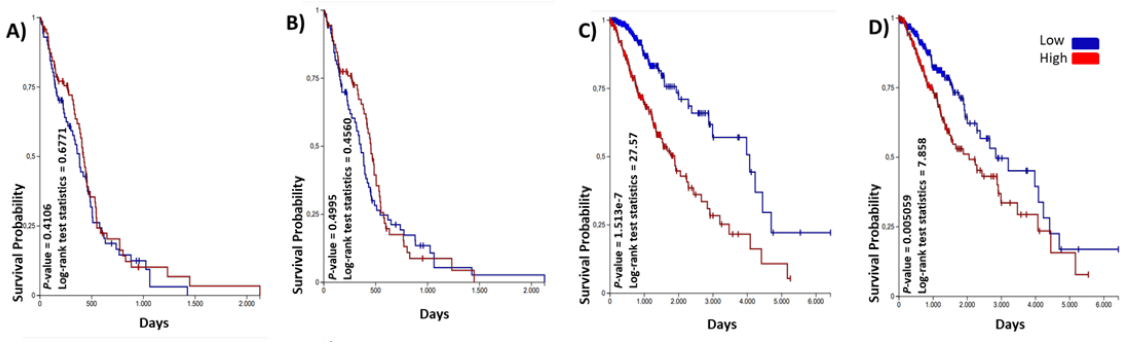

Figure 4. AQP1 and AQP4 effect on overall survival in TCGA-GBM and TCGA-LGG datasets. A) AQP1 GBM overall survival, B) AQP4 GBM overall survival, C) AQP1 LGG overall survival, D) AQP4 LGG overall survival

3.4. The Expression Patterns of AQP1 and AQP4 in Datasets Based on Histology, Subtypes and Grade of Glioma

The GBM dataset was analyzed histologically and subtype-based in terms of AQP1 and AQP4 gene expressions. The histology-based analysis of AQP1 expression showed a statistically significant difference between the non-tumor and tumor groups (Figure 5A). However, there was no significant change in AQP4 mRNA expression between the non-tumor and tumor groups (Figure 5C).

Subtype-based analyses were also conducted, yielding controversial results. According to Figure 5B, there was no statistically significant difference in the expression ratios of AQP1 among the subtypes. However, AQP4 displayed significant differences between the subtype groups. Notably, the Classical and Mesenchymal subtypes exhibited a significant difference in AQP4 expression ($p<0.01$) (Figure 5D). The most significant difference was observed between the Classical and Preneural groups ($p<0.01$).

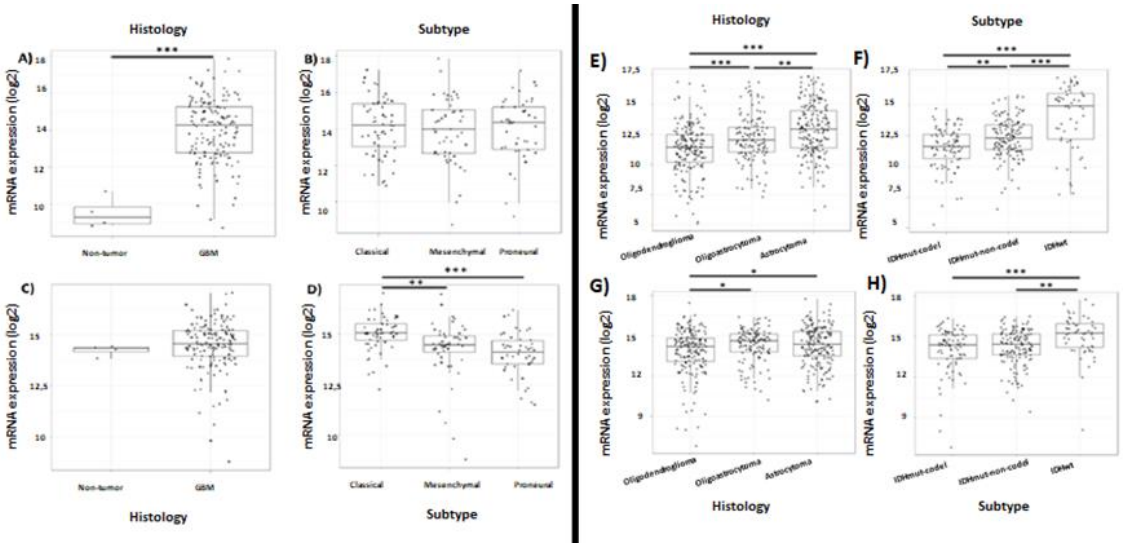

Figure 5. The expression results of AQP1 and AQP4 genes in the TCGA-GBM dataset by using the GlioVis platform. A) AQP1 gene expressions result in histology-based, B) AQP1 gene expressions result subtype-based, C) AQP4 gene expressions result in histology-based, D) AQP4 gene expressions result subtype-based. The expression results of AQP1 and AQP4 genes in the TCGA-LGG dataset by using the GlioVis platform. E) AQP1 gene expressions result in histology-based, F) AQP1 gene expressions result subtype-based, G) AQP4 gene expressions result in histology based, H) AQP4 gene expressions results subtype based. The table shows the difference between pairs, the 95% confidence interval and the p-value of the pairwise comparisons. ***p<0.001; **p<0.01. Not significant results were not shown on graphics. No significant results were not shown on graphics.

The analysis of the LGG dataset yielded highly satisfactory results. At least two subgroups in the graphs displayed significant values, particularly in the case of AQP1 gene expression. Significant values were observed in every comparison, both in terms of histology and subtype (Figure 5E and F). Although AQP4 showed lower statistics in histology-based analyses, two comparisons yielded significant results, except for the Oligoastrocytoma and Astrocytoma comparison (Figure 5G). In the subtype-based analysis, comparing the

presence of wild-type and mutated status for the AQP4 gene expression level revealed significant results in both cases (Figure 5H).

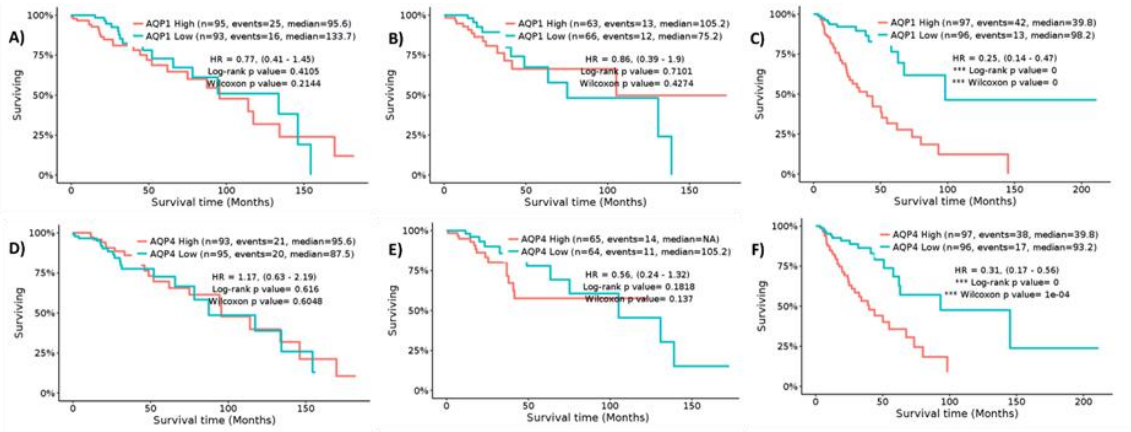

Figure 6. The survival graphics of histology-based LGG dataset A) Oligodendroglioma-AQP1 B) Oligoastrocytoma-AQP1 C) Astrocytoma-AQP1 D) Oligodendroglioma-AQP4 E) Oligoastrocytoma-AQP4 F) Astrocytoma-AQP4.

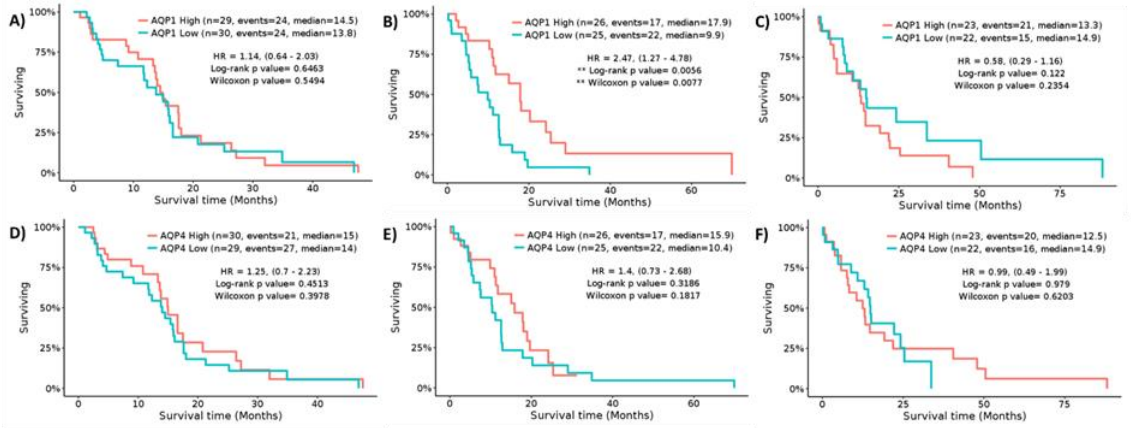

Figure 7. The survival graphics of histology GBM subtype based GBM dataset A) Classical-AQP1 B) Mesenchymal-AQP1 C) Proneural-AQP1 D) Classical-AQP4 E) Mesenchymal-AQP4 F) Proneural-AQP4.

3.5. Correlation of the AQP1 and AQP4 gene expression levels in TCGA-GBM and TCGA-LGG datasets

The correlation of the expression levels of the AQP1 and AQP4 genes was investigated in the TGCA-GBM and TCGA-LGG datasets. In the TCGA-GBM dataset, a lower gene expression correlation value was obtained with r=0.46 (Figure 8A). The correlation between AQP1 and AQP4 was relatively higher in the TCGA-LGG dataset (Figure 8B).

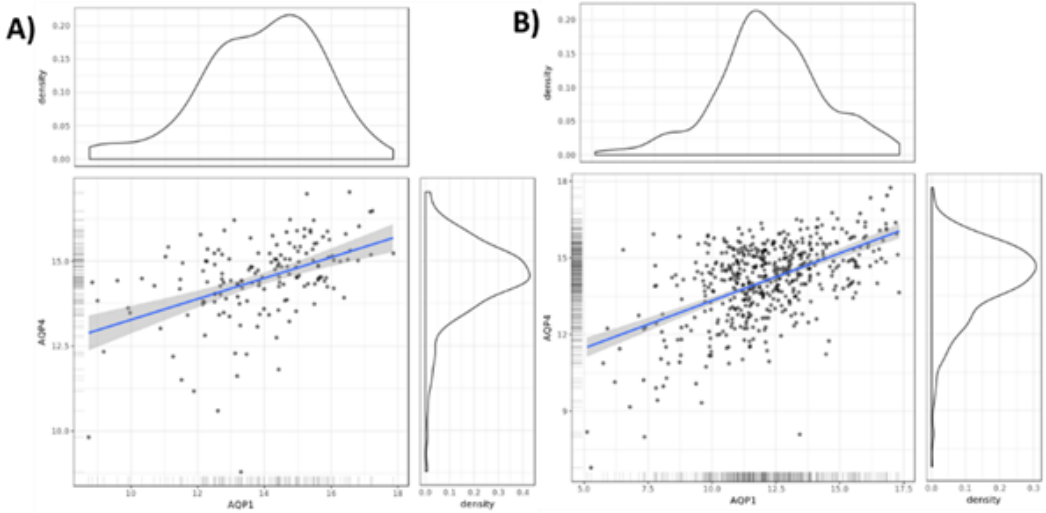

Figure 8. Correlation of AQP1 and AQP4 levels in A) TCGA_ GBM (r= 0.46) and B) TCGA_ LGG (r= 0.53) datasets. Pearson's test was used for correlation analyses.

3.6. AQP1, AQP4 and their correlated genes and their protein interaction analyses

Genes correlated with AQP1 (r=0.5) and AQP4 (r=0.6) were listed separately by The Ivy Glioblastoma Atlas Project. The 86 genes were listed for AQP1, while 100 genes were listed for AQP4. The DAVID tool was used to search for the functional annotations of these lists. Thus, the roles of genes that are separately correlated with AQP1 and AQP4 in cellular components, molecular function and biological processes have been annotated.

As expected in the cellular component analysis, cell membrane and membrane-related parts were more enriched for both AQP1 and AQP4 (Table 1 and Table 2). After analyzing the results of the Cellular component analysis of AQP1 and its correlated genes, several prominent locations were identified. The Plasma membrane, Endosome, Integral component of the plasma membrane, Extracellular region, and Extracellular space were found to be the major cellular components, each constituting more than 10 percent of the total (Table 1). The Membrane and Cell membrane were listed as the major cellular components of AQP4 and correlate (Table 2). Although p-value<0.05 for both aquaporins in Molecular Function and Biological Process results, enrichment scores were listed as quite high. Of these, only AQP1's Cell Adhesion, listed in Biological Process, was able to exceed the 10 percent limit (Table 1).

Table 1. Functional annotation of AQP1 and its correlated proteins functional annotation results with DAVID (2023q2). (Red marked ones are significant in terms of p-value and/or enrichment score values are less than 0.05.)

Category	Term Name	Count (%)	PValue	Enrichment Score
CELLULAR COMPONENT	Plasma membrane	36 (46,8)	5,70E-05	9,00E-03
	Endosome	7 (9,1)	1,00E-03	8,10E-02
	An integral component of plasma membrane	14 (18,2)	1,70E-03	8,90E-02
	Extracellular region	17 (22,1)	4,10E-03	1,60E-02
	Extracellular space	15 (19,5)	9,60E-03	2,70E-02
	Neuronal cell body	6 (7,8)	1,00E-02	2,70E-02
	Basolateral plasma membrane	5 (6,5)	1,30E-02	2,80E-02
	Extracellular matrix	5 (6,5)	1,40E-02	2,80E-02
	Astrocyte end-foot	2 (2,6)	1,80E-02	3,10E-02
	Caveola	3 (3,9)	3,00E-02	4,40E-02
	Membrane	21 (27,3)	3,00E-02	4,40E-02
	Perineuronal net	2 (2,6)	3,50E-02	4,70E-02
	Neuron projection	5 (6,6)	4,30E-02	5,10E-02
	Perinuclear region of cytoplasm	7 (9,1)	4,80E-02	5,10E-02
	Lysosomal lumen	3 (3,9)	4,80E-02	5,10E-02
	Integral component of membrane	27 (35,1)	5,50E-02	5,30E-02
	Golgi lumen	3 (3,9)	5,70E-02	5,30E-02
	Filopodium membrane	2 (2,6)	6,30E-02	5,50E-02
	Filopodium tip	2 (2,6)	7,30E-02	6,10E-02
	Extracellular exosome	13 (16,9)	9,60E-02	7,70E-02
MOLECULAR FUNCTION	Glyceraldehyde-3-phosphate dehydrogenase (NAD+) (non-phosphorylating) activity	2 (2,6)	2,60E-02	1,00E+00
	Water transmembrane transporter activity	2 (2,6)	2,60E-02	1,00E+00
	Beta-amyloid binding	2 (2,6)	4,20E-02	1,00E+00
	GTPase activating protein binding	2 (2,6)	5,60E-02	1,00E+00
	Aldehyde dehydrogenase (NAD) activity	2 (2,6)	5,60E-02	1,00E+00
	Water channel activity	2 (2,6)	6,30E-02	1,00E+00
	Peptidase inhibitor activity	2 (2,6)	7,30E-02	1,00E+00
	Semaphorin receptor binding	2 (2,6)	8,40E-02	1,00E+00
	Hyaluronic acid binding	2 (2,6)	9,10E-02	1,00E+00
	Phosphatidylinositol phospholipase C activity	2 (2,6)	9,40E-02	1,00E+00
	Channel activity	2 (2,6)	9,40E-02	1,00E+00
	Inward rectifier potassium channel activity	2 (2,6)	9,40E-02	1,00E+00
BIOLOGICAL PROCESS	Cell adhesion	9 (11,7)	1,20E-03	7,80E-01
	Skeletal system development	4 (5,2)	1,40E-02	1,00E+00
	Cell migration	5 (6,5)	2,00E-02	1,00E+00
	Retina development in camera-type eye	3 (3,9)	2,10E-02	1,00E+00
	Cellular water homeostasis	2 (2,6)	2,60E-02	1,00E+00
	Hippocampus development	3 (3,9)	2,80E-02	1,00E+00
	Negative regulation of neuron projection development	3 (3,9)	2,80E-02	1,00E+00
	Intracellular signal transduction	6 (7,8)	2,90E-02	1,00E+00
	Potassium ion transport	3 (3,9)	3,30E-02	1,00E+00
	Alpha-linolenic acid metabolic process	2 (2,6)	3,70E-02	1,00E+00
	Bergmann glial cell differentiation	2 (2,6)	4,10E-02	1,00E+00
	Multicellular organismal water homeostasis	2 (2,6)	4,10E-02	1,00E+00
	Cell cycle	5 (6,5)	4,70E-02	1,00E+00
	Adaptive thermogenesis	2 (2,6)	4,80E-02	1,00E+00
	Carbon dioxide transport	2 (2,6)	5,10E-02	1,00E+00
	Synapse maturation	2 (2,6)	5,90E-02	1,00E+00
	Sodium ion transmembrane transport	3 (3,9)	6,80E-02	1,00E+00
	Renal water homeostasis	2 (2,6)	6,90E-02	1,00E+00
	Positive regulation of neuron projection development	3 (3,9)	7,20E-02	1,00E+00
	Water transport	2 (2,6)	8,30E-02	1,00E+00
	Glial cell differentiation	2 (2,6)	8,60E-02	1,00E+00
	Potassium ion transmembrane transport	3 (3,9)	8,90E-02	1,00E+00
	Ion transport	3 (3,9)	9,80E-02	1,00E+00

*Red lines are statistically significant groups (p<0.05).

Table 2. Functional annotation of AQP4 and its correlated proteins functional annotation results with DAVID (2023q2). (Red marked ones are significant in terms of p-value and/or enrichment score values are less than 0.05.)

Category	Term Name	Count (%)	PValue	Enrichment Score
CELLULAR COMPONENT	Membrane	63 (68,5)	3,20E-07	7,30E-06
	Cell membrane	40 (43,5)	7,00E-07	8,10E-06
	Microsome	4 (4,3)	1,80E-02	1,40E-01
	Cytoskeleton	12 (13)	5,90E-02	3,40E-01
	Endoplasmic reticulum	11 (12)	1,00E-01	4,60E-01
MOLECULAR FUNCTION	Developmental protein	12 (13)	9,20E-03	1,50E-01
	Developmental protein	12 (13)	9,20E-03	1,50E-01
	Actin-binding	6 (6,5)	1,30E-02	1,50E-01
	Ion channel	6 (6,5)	3,40E-02	1,00E-02
BIOLOGICAL PROCESS	Cell adhesion	9 (9,8)	2,80E-03	6,30E-02
	Symport	5 (5,4)	2,90E-03	6,30E-02
	One-carbon metabolism	3 (3,3)	4,40E-03	6,40E-02
	Transport	19 (20,7)	1,10E-02	1,10E-01
	Neurogenesis	6 (6,5)	1,40E-02	1,10E-01
	Ion transport	9 (9,8)	1,50E-02	1,10E-01
	Differentiation	8 (8,7)	8,40E-02	5,30E-01

*Red lines are statistically significant groups (p<0.05).

The common 31 genes in both AQP1 and AQP4-related gene lists were examined for protein-protein interactions using the STRING database. The most interacted genes with AQP1 and AQP4 were ADCYAP1R1, KCNJ16, FAM107A, ALDH1L1, S100B in Figure 9A. In addition, GPR56 and TSAPN3 were also associated with each other.

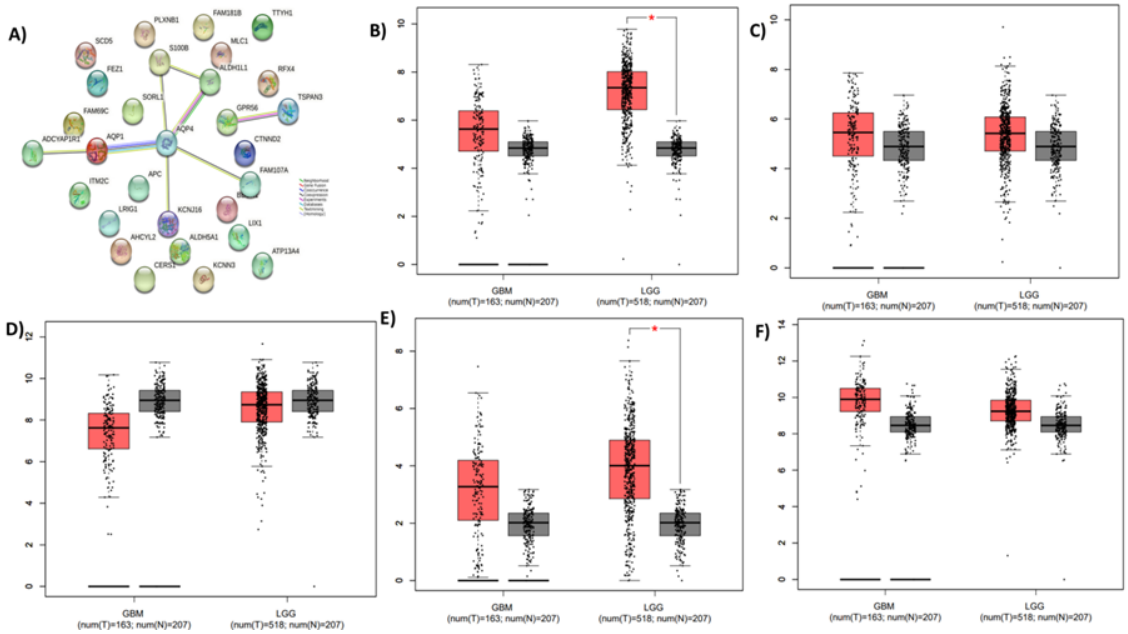

Figure 9. Common AQP1 and AQP4 related genes and their interaction and expression values. A) Protein-protein interactions of common genes in the list of AQP1 and AQP4 correlated genes using STRING v11.5. Most interacted genes with AQP1&4 were selected for further analyses. The expression pattern of (B) ADCYAP1R1, (C) ALDH1L1, (D) FAM107A, (E) KJN16 and (F) S100B in GBM and LGG datasets using the GEPIA database. The asterisk (*) shows statistical significance (p<0.05).

4. DISCUSSION AND CONCLUSION

Discussion

Among the central nervous system tumors in humans, diffuse gliomas are the most common. These include GBM (associated with poor prognosis) and LGGs (associated with better prognosis) that are at risk of developing into GBM in the future. Changes in many genes and molecular pathways are required for this differentiation [10]. In this study, the changes of AQP1 and AQP4 between LGG and GBM were compared and whether they could be used as biomarkers and/or therapeutic targets was investigated. Individuals with GBM have much shorter overall survival periods than those with LGG because of factors such as fast development, high invasiveness, and resistance to treatment [30]. LGG also leads to the secondary subtype of GBM, which contains primary and secondary subtypes. Previous researches have also revealed that IDH mutation, TP53 mutation, and 19q deletion are some of the most prevalent modifications in the creation of the secondary type [12, 14, 31]. Although there may be similarities between LGG and GBM in terms of gene expression, there are also very significant differences, as shown above. Therefore, it is important to recognize these differences and investigate biomarkers and potential therapeutic targets.

Although ion channel pharmacology has been used in the clinic for a long time, the idea of aquaporins being used in the clinic is still a developing idea [32-34]. In this study, which focused on the classical AQPs, AQP1 and AQP4, the expression levels of both genes in different cancer types were examined (Figure 1). Accordingly, it shows that both AQP1 and AQP4 are highly expressed in tumor tissue in GBM and LGG types (Figure 1A). On the other hand, since both cancers are glioma types, the expression status of the members of the aquaporin family in the brain was compared (Figure 1B). The results obtained show that the expression of AQP1 and AQP4 are aquaporins that are actively used in almost all tissues of the brain. AQP11 is right next to it. However, it has been observed that two regions of the brain (brain-cerebellum and brain-cerebellar hemisphere) can be targeted by AQP7, which is highly expressed in these regions. Therefore, while AQP1 and AQP4 can be used in studies that can target almost the entire brain, it is thought that AQP7 can be used more specifically in targeting these two regions of the brain.

While the aquaporins associated with the brain are AQP1-AQP4-AQP9 according to the literature, when the expression levels of aquaporins in LGG and GBM datasets are examined in our study, it is striking that the ones with the highest expression are AQP1-AQP4 and AQP11 (Figure 2). Unlike the others in the LGG and GBM datasets, AQP9 was observed to be higher in normal tissue in LGG and tumor tissue in GBM.

When the significance of gene expression levels of the AQP family in GBM and LGG were compared, it was found that only the expression levels of AQP1 (Figure 3A) and AQP4 (Figure 3D) differed significantly in tumor and normal tissue comparisons. It has been shown that this significant difference is found to be higher in tumor tissue than in normal ones.

In this study, in which the effects of the above-mentioned two genes on the overall survival graphs of the patients were also examined, it was found that AQP1 and AQP4 did not have significant effects in GBM-type glioma (Figure 4A-B). On the other hand, when looking at the LGG type, it was observed that there were relatively positive effects on overall survival in scenarios where the expression of both genes was low (Figure 4C-D).

It was investigated whether there was a significant difference between AQP1 and AQP4 gene levels for both GBM and LGG datasets in histology and subtype levels (Figure 5). According to the data obtained, it was observed that a very significant ($p<0.001$) difference in the GBM dataset of AQP1 was only in the histology-based set. In the same histology-based set, no significant difference was observed for AQP4. When the subtype-based set was analyzed in the GBM dataset, it was observed that the expression of AQP4 showed significant variation between Classical and Mesenchymal subtypes and between Classical and Preneural subtypes. The Classical subtype was observed to have higher expression levels than the other subtypes. As a result of the analysis of the LGG dataset, it was found that almost all groups showed significant differences for both AQP1 and AQP4 genes. It was observed that AQP1 was expressed lower in Oligodendrograms and had increased expression status in other histological groups. In the groups with IDH-wt, both genes were found to have higher expression compared to the other groups.

The amount of AQP1 and the degree of malignancy were also strongly correlated by Saadoun et al (2002)[35]. As observed in the astrocytoma group, where there was a significant difference based on histological subtype for AQP1 and AQP4 in the LGG dataset, survival was higher in the scenario with low expression of AQP1 and AQP4. Apart from these, no significant effect was found in other subtypes (Figure 6). When the same study was performed on the GBM dataset, it was observed that the high level of AQP1 could be associated with patient survival only in the Mesenchymal type. According to these findings, there is a significant difference in AQP1 levels between the relatively early stages of the disease (LGG dataset) and the late stage of the disease (GBM dataset). AQP1 water channel blockers could therefore be used as potent anti-brain tumor edema agents, according to several groups [35-37]. However, according to our study, besides being used as edema agents, it can prolong patient life by increasing survival. By using this change in gliomas due to disease progression, drug targeting or changing AQP1 expressions can affect prognosis and survival.

When the common genes associated with AQP1 and AQP4 were examined, it was observed that the expression of 31 genes changed. It was found that 5 of them were directly related to AQP1 and AQP4 proteins at the protein level. These are ADCYAP1R1, ALDH1L1, FAM107A, KJN16 and S100B. When the expression levels of these 5 genes in the LGG and GBM datasets were examined, it was observed that only the expressions of ADCYAP1R1 and KJN16 changed significantly in the LGG dataset.

Spence and his friends have found that ADCYAP1R1 has a QTL background for inheritance in their rat studies. Both AQP1 (7p15-->p14)[38] and ADCYAP1R1 (7p14.3)[39] genes are located on chromosome 7 in humans. Therefore these two genes may be inherited together and possibly have roles in similar mechanisms. ADCYAP1R1 is a G-coupled protein on the plasma membrane responsible for controlling human stress responses [40, 41] and also this protein was considered as a prognostic marker for gliomas in 2020 [42]. Considering that AQP1 may play a similar role, it can be thought that the increases that occur at the level of these two genes in tumor conditions are to protect glial cells from the stress conditions caused by the tumor.

KJN16 gene encodes a channel protein named Kir5.1. This protein is mainly responsible for potassium homeostasis and pH-electrolyte balances [43]. Possible mutations on this gene cause several disorders such as hypercapnia/hypoxia, and seizure. To explain it from the perspective of our study, statistically significant results in gliomas of this gene, which was previously thought to have therapeutic potential [43] in other studies, were observed. Its mutation status in gliomas and their

contributions to gliomas can be investigated in more detail and its use as a drug target can be examined.

In summary, it has been found that AQP1, like AQP4, has effects on gliomas, with significant differences, especially in the LGG type. Conditions with lower AQP1 and AQP4 levels are more favorable for disease progression. Therefore, it has been shown that AQP1 can be used as a therapeutic target in common glioma studies. Therefore, this study will be a starting point for further studies.

CONCLUSION

This study conducted a systematic bioinformatics analysis of DEGs between LGG and GBM. Notably, it observed differential expression of AQP1 and AQP4 aquaporin family members in diffuse gliomas for the first time. Furthermore, it revealed significant differences in the associations of expression levels according to different subtypes among aquaporins. In the LGG subtype, low expression of AQP1 and high expression of ADCYAP1R1 gene, which is correlated with AQP1, has been shown to be advantageous in terms of survival. However, in the GBM dataset, high expression of AQP1 is associated with increased survival in mesenchymal types. Based on these findings, it can be concluded that AQP1 is more promising than AQP4 in terms of being a treatment target and usability as a prognostic marker. While this study, which analyzes existing data from the literature, offers valuable insights into the potential of aquaporins as drug targets and markers, further experimental verification studies are needed.

Author contributions
SEO: Conceptualization, data analysis, and manuscript writing.

Conflict Of Interest
The authors declare that they have no competing interests.

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Consent to participate
Not applicable.

Consent for publication
Not applicable.

Data Availability Statement
The manuscript contains all data supporting the reported results. The raw data used in this study are available online in the GTEx, TCGA LGG, TCGA GBM cohorts and Rembrandt dataset. Additional questions can be directed to the relevant author(s).

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