Pre-Clinical Research Report

GPCR genes as a predictor of glioma severity and clinical outcome

Eun-A Ko¹ and Tong Zhou²

Abstract

Objective: To undertake a comprehensive analysis of the differential expression of the G protein-coupled receptor (GPCR) genes in order to construct a GPCR gene signature for human glioma prognosis.

Methods: This current study investigated several glioma transcriptomic datasets and identified the GPCR genes potentially associated with glioma severity.

Results: A gene signature comprising 13 GPCR genes (nine upregulated and four downregulated genes in high-grade glioma) was developed. The predictive power of the 13-gene signature was tested in two validation cohorts and a strong positive correlation (Spearman’s rank correlation test: \( \rho = 0.649 \) for the Validation1 cohort; \( \rho = 0.693 \) for the Validation2 cohort) was observed between the glioma grade and 13-gene based severity score in both cohorts. The 13-gene signature was also predictive of glioma prognosis based on Kaplan–Meier survival curve analyses and Cox proportional hazard regression analysis in four cohorts of patients with glioma.

Conclusions: Knowledge of GPCR gene expression in glioma may help researchers gain a better understanding of the pathogenesis of high-grade glioma. Further studies are needed to validate the association between these GPCR genes and glioma pathogenesis.

Keywords
Gene signature, transcriptome, glioma, GPCR genes

Date received: 16 January 2022; accepted: 30 May 2022

Introduction

Glioma is the most prevalent brain tumour and its diagnosis is based on clinical characteristics, histopathological analysis and radiographic features.¹⁻⁴ Conventional tumour grading has not been suitable for outcome prediction in the case of glioma,
particularly between grades III and IV; in fact, grade IV brain tumours are the most malignant with poor clinical outcomes. The Cancer Genome Atlas provides information on genome alterations in various types of cancers present in large cohorts of humans. Gene expression-based molecular classification has been established with four glioma subtypes: classical, mesenchymal, proneural and neural. To understand the biology of glioma and its optimal treatment, multidisciplinary approaches, including the use of morphological features for imaging-based phenotypic characterization using magnetic resonance images, distant metastasis prediction and molecular characteristic classification, are being used to predict the survival outcome. The gene expression analyses based on high-throughput platforms and microarrays have improved the diagnostic ability by incorporating genetic signatures of glioma pathology. For example, genes related to inflammation, energy metabolism, autophagy and DNA methylation have previously been reported as prognostic markers of glioma.

G-protein-coupled receptors (GPCRs), a family of membrane receptors, play an important role in cellular signalling involving ligands, hormones, neurotransmitters and proteases. Indeed, GPCRs relevant to tumour growth and metastasis have been observed in many forms of cancer. Numerous GPCRs bearing ligand-binding sites are located on the extracellular domain with agonist, thereby, these sites are easily accessible at the cell surface membrane. Therefore, GPCRs have become the focus of research interest as novel drug targets for several diseases.

This current study undertook a comprehensive analysis of the differential expression of the GPCR genes in order to construct a GPCR gene signature for human glioma prognosis.

Materials and methods

GPCR genes

The definition of GPCR genes was obtained from the IUPHAR database (https://www.guidetopharmacology.org/). Only the well-annotated human GPCR genes were considered. A total of 407 GPCR genes were collected in this study (see supplementary materials, Table S1), including 5-hydroxytryptamine receptors, acetylcholine receptors, adenosine receptors, adhesion class GPCRs, adrenoceptors, angiotensin receptors, apelin receptor, bile acid receptor, bombesin receptors, bradykinin receptors, calcitonin receptors, calcium-sensing receptor, cannabinoid receptors, chemein receptors, chemokine receptors, cholecystokinin receptors, class A orphans, class C orphans, class frizzled GPCRs, complement peptide receptors, corticotropin-releasing factor receptors, dopamine receptors, endothelin receptors, formylpeptide receptors, free fatty acid receptors, G protein-coupled oestrogen receptor, gamma-aminobutyric acid (GABA) B receptors, galanin receptors, ghrelin receptor, glucagon receptor family, glycoprotein hormone receptors, gonadotrophin-releasing hormone receptors, histamine receptors, hydroxycarboxylic acid receptors, kisspeptin receptor, leukotriene receptors, lysophospholipid receptors, melanin-concentrating hormone receptors, melanocortin receptors, melatonin receptors, metabotropic glutamate receptors, motilin receptor, neuropeptide Y receptors, neuropeptide W/neuropeptide Y receptors, neuropeptide FF/neuropeptide AF receptors, neuropeptide S receptor, neuropeptide W/neuropeptide B receptors, neuropeptide Y receptors,
neurotensin receptors, opioid receptors, opsin receptors, orexin receptors, oxoglutarate receptor, purinergic receptors, parathyroid hormone receptors, platelet-activating factor receptor, prokineticin receptors, prolactin-releasing peptide receptor, prostaglandin receptors, proteinase-activated receptors, pyroglutamylated RFamide peptide receptor, relaxin family peptide receptors, somatostatin receptors, succinate receptor, tachykinin receptors, taste 1 receptors, taste 2 receptors, thyrotropin-releasing hormone receptors, trace amine receptor, urotensin receptor, vasopressin and oxytocin receptors, vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors.

Glioma transcriptomic data

Seven glioma transcriptomic datasets were investigated in this study, which were generated using the following arrays: Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix Human Genome U133A/B Array, Affymetrix Human Genome U95Av2 Array or Affymetrix Human Exon 1.0 ST Array. The Robust Multi-array Average (RMA) tool was used to quantify the genome-wide gene expression profile for the Affymetrix Human Genome U133 Plus 2.0 Array, U133A/B Array and U95Av2 Array, while the Affymetrix Human Exon 1.0 ST Array data were summarized using Affymetrix Power Tools. One of the transcriptomic datasets was used as a discovery cohort and all the other datasets were used for validation purposes. In the discovery cohort, only the probesets with unique annotations that were present in at least two-thirds of the samples were retained for further analysis.

Severity score based on GPCR gene expression

A signature based on multiple GPCR genes was developed, which can be used to predict severity and survival for glioma patients. A severity score for each glioma patient was computed based on the gene expression information of the signature, which was a combination of the expression values of the GPCR genes within the signature:

\[ S = \sum_{i=1}^{n} \frac{W_i(e_i - \mu_i)}{\tau_i} \]

Here, \( S \) was the severity score; \( n \) was the number of genes in the signature; \( W_i \) denoted the weight of gene \( i \) in the signature (+1 was assigned to the genes that were upregulated in high-grade glioma while –1 was assigned to the genes that were downregulated in high-grade glioma); \( e_i \) was the expression level of gene \( i \); \( \mu_i \) was the mean of the expression for gene \( i \) across the samples in each cohort; and \( \tau_i \) was the standard deviation of the expression for gene \( i \) across the samples in each cohort.

Statistical analyses

All statistical analyses were undertaken using the R statistical package (R version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria). Spearman’s rank correlation test was performed using the ‘cor.test’ function. The ‘pROC’ package was used to generate the receiver operating characteristic (ROC) curve and compute the area under the ROC curve (AUC). The ‘survival’ package was used to perform the log-rank test using the ‘survdiff’ function and Cox proportional hazard regression using the ‘coxph’ function. A \( P \)-value <0.05 was considered statistically significant.

Results

First, the GPCR genes that were correlated with glioma World Health Organization grade were identified. A glioma
transcriptomic dataset was obtained from the Gene Expression Omnibus (GEO) database with an accession of GSE43289, which was used as a discovery cohort to prioritize the GPCR genes that are associated with glioma severity. In total, the GPCR genes in three grade I patients, three grade II patients, six grade III patients and 28 grade IV patients were investigated. The correlation between GPCR gene expression and glioma grade was computed by Spearman’s rank correlation test. Figure 1 presents the GPCR genes with a correlation coefficient $\rho > 0.2$ or $< -0.2$, which represents a list of GPCR genes that are potentially differentially expressed in glioma: a positive $P$ suggests upregulation while a negative $P$ suggests downregulation in high-grade glioma. Among these genes, the genes with an adjusted $P$-value $< 0.05$ were selected. This step yielded a list of 13 GPCR genes (Figures 1 and 2), which included nine genes upregulated in high-grade glioma (CELSR1, F2RL2, FZD1, FZD2, FZD5, FZD7, GPR107, HRH1 and P2RY1) and four genes downregulated in high-grade glioma (GABBR1, GPRC5B, HTR2A and P2RY12). These 13 GPCR genes were assigned as a 13-gene signature. To test whether the correlation between GPCR gene expression and glioma grade was robust, two independent validation cohorts were investigated: Validation1 and Validation2. The Validation1 cohort (GEO accession number: GSE4290) consists of 23 non-glioma subjects and 45 grade II, 31 grade III, and 77 grade IV glioma patients; while the Validation2 cohort (GEO accession number: GSE19728) consists of four non-glioma subjects, and two grade I, five grade II, five grade III and five grade IV glioma patients. Spearman’s rank correlation test between expression and grade was applied to the GPCR genes listed in Figure 1. The $\rho$ of the discovery cohort was significantly correlated with the $\rho$ of the two validation cohorts (Spearman’s rank correlation test: $\rho = 0.859$ and $P < 10^{-10}$ for the Validation1 cohort; $\rho = 0.640$ and $P = 2.2 \times 10^{-6}$ for the Validation2 cohort) (Figure 3), which suggests that the GPCR gene expression profile in the discovery cohort can be robustly

![Figure 1](http://imr.sagepub.com)
mirrored by the pattern in the two validation cohorts.

A 13-gene signature based on 13 GPCR genes with nine upregulated and four downregulated in high-grade glioma was developed. It was assumed that the expression of the 13-gene signature could be used to predict glioma severity, i.e. tumour grade. A weight was assigned to each gene among the 13-gene signature according to the direction of differential expression, i.e. +1 for the upregulated genes and −1 for the downregulated genes in high-grade glioma. In the discovery cohort, a severity score was assigned to each subject based on the expression pattern of the 13-gene signature.

Figure 2. The 13-gene signature. The G-protein-coupled receptor (GPCR) gene expression data were derived from the discovery cohort. For the GPCR genes with multiple probesets, only the probeset with the most significant P-value was demonstrated. The X-axis denotes glioma grade while the Y-axis represents log2-transformed gene expression. The colour version of this figure is available at: http://imr.sagepub.com.

Figure 3. The ρ between G-protein-coupled receptor (GPCR) gene expression and glioma grade. Each dot denotes a GPCR gene. Only the genes listed in Figure 1 were included. The pink and red dots stand for the genes with positive ρ in the discovery cohort, while the dark and light blue dots represent the gene with negative ρ in the discovery cohort. The left and right panels are for the Validation1 and Validation2 cohorts, respectively. The ρ in the discovery cohort is significantly correlated with that of the two validation cohorts. The colour version of this figure is available at: http://imr.sagepub.com.
as detailed in the ‘Materials and methods’ section. A greater severity score suggests more severe glioma. A strong positive correlation (Spearman’s rank correlation test: \( \rho = 0.739 \) and \( P = 5.2 \times 10^{-8} \)) was observed between the tumour grade and 13-gene based severity score (Figure 4a). The predictive power of the 13-gene signature was tested in the two validation cohorts. In both validation cohorts, a strong positive correlation (Spearman’s rank correlation test: \( \rho = 0.649 \) and \( P < 10^{-10} \) for the Validation1 cohort; \( \rho = 0.693 \) and \( 2.2 \times 10^{-4} \) for the Validation2 cohort) was observed between the glioma grade and 13-gene based severity score (Figure 4a), which suggests a promising predictive power of the 13-gene signature in predicting glioma severity. In addition, the ROC curve was plotted to quantify the classification power of the 13-gene based severity score in differentiating between the non-glioma and glioma subjects (Figure 4b). The AUC was 0.844 between the glioma and non-glioma subjects in the Validation1 cohort. All these results suggest that the 13-gene signature can be used not only for glioma severity prediction but also for glioma screening.

The study then determined whether the 13-gene based severity score could be used to predict clinical outcome in glioma by investigating four independent glioma transcriptomic datasets: (i) the European Organisation for Research and Treatment of Cancer (EORTC) cohort including 95 glioma patients with a GEO accession of GSE43107;35 (ii) the University of California at Los Angeles (UCLA) cohort including 85 glioma patients with a GEO accession of GSE4412;36 (iii) the Massachusetts General Hospital (MGH) cohort composed of 50 glioma patients;37 and (iv) the MD Anderson Cancer Center (MDACC) cohort including 77 glioma patients with a GEO accession of GSE4271.38 The severity score was computed for all the subjects within the four validation cohorts based on the GPCR gene expression of the 13-gene signature. Kaplan–Meier survival curves demonstrated a significant difference in survival between the glioma patients with positive and negative severity scores in the four cohorts (log-rank test: \( P = 2.0 \times 10^{-3} \) for the EORTC cohort; \( P = 3.0 \times 10^{-3} \) for the UCLA cohort; \( P = 2.0 \times 10^{-4} \) for the MGH cohort).

![Figure 4](http://imr.sagepub.com). The performance of the 13-gene signature in predicting glioma severity: (a) comparison of the 13-gene based severity score in different categories. The heavy central black horizontal lines for each grade are the median; the extremities of the boxes are the 25th and 75th percentiles; and the whiskers represent 1.5 interquartile ranges (IQRs) (in case there is no data point exceeding 1.5 IQR, the lower/upper whiskers denote the minimum/maximum values respectively). NT, non-tumour samples and (b) The receiver operating characteristic curve of the 13-gene based severity score in distinguishing between the glioma and non-glioma subjects in the Validation1 cohort. The colour version of this figure is available at: http://imr.sagepub.com.
cohort; \( P = 3.0 \times 10^{-4} \) for the MDACC cohort) (Figure 5). The relationship between the severity score and survival was further confirmed by Cox proportional hazard regression analysis. Compared with the patients with a negative severity score, the patients with a positive severity score had a 2.84-fold increased risk of death in the EORTC cohort (\( P = 2.8 \times 10^{-3} \)), 2.51-fold in the UCLA cohort (\( P = 3.4 \times 10^{-3} \)), 2.51-fold in the MGH cohort (\( P = 4.7 \times 10^{-4} \)) and 3.95-fold in the MDACC cohort (\( P = 4.2 \times 10^{-4} \)). All these results suggest that the 13-gene signature can be used for glioma prognosis.

**Discussion**

To date, various anatomical and histological features and molecular alterations related to glioma have been identified, enabling the development of various novel approaches for the identification of glioma progression.\(^9\)\(^{39–42}\) Although several molecular signatures have been used to predict glioma, they are yet to be adopted clinically for the prognostic evaluation of glioma patients.\(^43\) Diverse and precise molecular profiling resources will be needed to provide diagnosis and prognosis predictions of glioma. GPCRs are critically involved in cell proliferation and survival and are abnormally expressed in tumour cells.\(^44\) With increasing evidence that demonstrates the crucial role of GPCRs in carcinogenesis, this current study aimed to develop prognostic molecular signatures based on the expression of aberrant \( GPCR \) genes to predict the survival of patients with glioma. In this current study, the \( GPCR \) genes that were differentially expressed in glioma patients were identified and a 13-gene signature was established to improve the prognostic evaluation of glioma. The grade-dependent \( GPCR \) genes were significantly associated with survivability in glioma cohorts.

The frizzled (FZD) family of proteins consists of 10 isoforms (FZD1–10) and many aspects of FZD signal transduction are critical for cell cycle regulation, embryonic development, cell proliferation and the development of the central nervous system.\(^45–49\) Accumulating evidence shows that aberrant FZD signalling is involved in many different types of cancers and a critical role of FZD has been reported in the later stages of tumour development.\(^50–52\) For example, the expression of FZD2 and FZD7 was upregulated in later-stage tumours in pancreatic adenocarcinoma; and FZE10 was highly expressed in the later stages of tumour progression in colon cancer.\(^53,54\) In particular, FZD7

**Figure 5.** Kaplan-Meier curves for the EORTC, UCLA, MGH and MDACC cohorts. The 13-gene based severity score predicts clinical outcome in all the cohorts. \( P \)-values were calculated using log-rank test. EORTC, European Organisation for Research and Treatment of Cancer; UCLA, University of California at Los Angeles; MGH cohort, Massachusetts General Hospital; MDACC, MD Anderson Cancer Center. The colour version of this figure is available at: http://imr.sagepub.com.
upregulation in glioma patients was associated with a poor prognosis.\textsuperscript{55} In fact, aberrant FZD signalling and complex formation between FZDs and Wnt have been associated with several human cancers.\textsuperscript{56} Blocking FZDs using antibodies or inhibitors has been explored as an anticancer therapy and is known to disrupt Wnt signalling and inhibit their downstream genes.\textsuperscript{51,57} In this current study, higher expression levels of FZD1, FZD2, FZD5 and FZD7 were identified in glioma tissues than in normal tissues. In addition to the FZD family, prominent expression of purinergic receptors P2RY1 was identified in high-grade glioma. Previous studies on prostate and gastric cancers have demonstrated that extracellular adenosine triphosphate promotes cancer progression through the activation of purinergic receptors and the resulting induction of apoptosis.\textsuperscript{58–60} This current study also identified increased expression of \textit{HRH1} in higher grades of glioma.

In this current study, a low expression of \textit{GPCR} genes, such as \textit{GABBR1}, \textit{GPRC5B}, \textit{HTR2A} and \textit{P2RY12} was observed in high-grade glioma. GABA is an inhibitory neurotransmitter in the nervous system, which functions via both GABA\textsubscript{A} and GABA\textsubscript{B} receptors. Considering the importance of GABA\textsubscript{B} receptors in maintaining neuronal excitability, the lack of the GABA\textsubscript{B1} subunit causes epileptic seizures, pain disorders and an impaired memory.\textsuperscript{61} GPRC5B is an orphan receptor belonging to the GPCR family and contributes to neurogenesis.\textsuperscript{62} The 5-hydroxytryptamine receptor \textit{HTR2A} gene is a prognostic marker for low-grade glioma\textsuperscript{63} and downregulation of \textit{HTR5A} has been found in high-grade glioma.\textsuperscript{64} This current study identified 13 \textit{GPCR} genes that can be used to predict the severity and clinical outcomes of glioma. As this gene signature was derived from grade-specific \textit{GPCR} genes and strongly associated with glioma severity, it is reasonable to anticipate that this gene signature can be used to monitor glioma treatment. A change in the 13-gene based severity score potentially reflects the treatment response in glioma patients, which may be applicable for optimizing the treatment plan for individual patients. Further studies are needed to validate the association between these \textit{GPCR} genes and glioma pathogenesis for a better clinical application.

\textbf{Author contributions}  
E.K. and T.Z. conceived the idea. T.Z. performed the statistical analysis. E.K. interpreted and discussed the results. E.K. and T.Z. wrote the manuscript. All authors read and approved the final manuscript.

\textbf{Declaration of conflicting interest}  
The authors declare that there are no conflicts of interest.

\textbf{Funding}  
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: E.K. was supported by a research grant from the Jeju National University Hospital Research Fund of Jeju National University School of Medicine in 2021.

\textbf{ORCID iD}  
Eun-A Ko https://orcid.org/0000-0002-1585-6886

\textbf{References}  
1. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. \textit{Acta Neuropathol} 2007; 114: 97–109.
2. Perry A and Wesseling P. Histologic classification of gliomas. \textit{Handb Clin Neurol} 2016; 134: 71–95.
3. Bakas S, Akbari H, Sotiras A, et al. Advancing The Cancer Genome Atlas glioma MRI collections with expert
segmentation labels and radiomic features. *Sci Data* 2017; 4: 170117.

4. Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, et al. Clinical, Radiologic, Pathologic, and Molecular Characteristics of Long-Term Survivors of Diffuse Intrinsic Pontine Glioma (DIPG): A Collaborative Report From the International and European Society for Pediatric Oncology DIPG Registries. *J Clin Oncol* 2018; 36: 1963–1972.

5. Hartmann C, Hentschel B, Wick W, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol* 2010; 120: 707–718.

6. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008; 455: 1061–1068.

7. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 2010; 17: 98–110.

8. Chaddad A, Desrosiers C, Hassan L, et al. A quantitative study of shape descriptors from glioblastoma multiforme phenotypes for predicting survival outcome. *Br J Radiol* 2016; 89: 20160575.

9. Zhou L, Tang H, Wang F, et al. Bioinformatics analyses of significant genes, related pathways and candidate prognostic biomarkers in glioblastoma. *Mol Med Rep* 2018; 18: 4185–4196.

10. Han T, Zuo Z, Qu M, et al. Comprehensive Analysis of Inflammatory Response-Related Genes, and Prognosis and Immune Infiltration in Patients With Low-Grade Glioma. *Front Pharmacol* 2021; 12: 748993.

11. Chen J, Li Y, Han X, et al. An autophagic gene-based signature to predict the survival of patients with low-grade gliomas. *Cancer Med* 2021; 10: 1848–1859.

12. Butler M, Pongor L, Su YT, et al. MGMT Status as a Clinical Biomarker in Glioblastoma. *Trends Cancer* 2020; 6: 380–391.

13. Bingxiang X, Panxing W, Lu F, et al. A Prognostic Model for Brain Glioma Patients Based on 9 Signature Glycolytic Genes. *Biomed Res Int* 2021; 2021: 6680066.

14. Alivand MR, Najafi S, Esmaeili S, et al. Integrative analysis of DNA methylation and gene expression profiles to identify biomarkers of glioblastoma. *Cancer Genet* 2021; 258–259: 135–150.

15. Cho U, Yang SH and Yoo C. Estimation of the occurrence rates of IDH1 and IDH2 mutations in gliomas and the reconsideration of IDH-wildtype anaplastic astrocytomas: an institutional experience. *J Int Med Res* 2021; 49: 300605211019258.

16. Kadioglu O, Saeed MEM, Mahmoud N, et al. Identification of novel drug resistance mechanisms by genomic and transcriptomic profiling of glioblastoma cells with mutation-activated EGFR. *Life Sci* 2021; 284: 119601.

17. Brandner S, McAleenan A, Kelly C, et al. MGMT promoter methylation testing to predict overall survival in people with glioblastoma treated with temozolomide: a comprehensive meta-analysis based on a Cochrane Systematic Review. *Neuro Oncol* 2021; 23: 1457–1469.

18. Guo CF, Zhuang Y, Chen Y, et al. Significance of tumor protein p53 mutation in cellular process and drug selection in brain lower grade (WHO grades II and III) glioma. *Biomark Med* 2020; 14: 1139–1150.

19. Shelly D, Epari S, Arora I, et al. Epidermal growth factor receptor (EGFR) gene amplification in high-grade gliomas: Western Indian tertiary cancer center experience. *Neuro India* 2016; 64: 115–120.

20. Rosenbaum DM, Rasmussen SG and Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature* 2009; 459: 356–363.

21. Feve M, Saliou JM, Zeniou M, et al. Comparative expression study of the endo-G protein coupled receptor (GPCR) repertoire in human glioblastoma cancer stem-like cells, U87-MG cells and non malignant cells of neural origin unveils new
potential therapeutic targets. *PLoS One* 2014; 9: e91519.

22. Lecointre C, Desrues L, Joubert JE, et al. Signaling switch of the urotensin II vasoactive peptide GPCR: prototypic chemotaxic mechanism in glioma. *Oncogene* 2015; 34: 5080–5094.

23. Byun S, Affolter KE, Snow AK, et al. Differential methylation of G-protein coupled receptor signaling genes in gastrointestinal neuroendocrine tumors. *Sci Rep* 2021; 11: 12303.

24. Arakaki AKS, Pan WA, Wedegaertner H, et al. Alpha-arrestin ARRDC3 tumor suppressor function is linked to GPCR-induced TAZ activation and breast cancer metastasis. *J Cell Sci* 2021; 134: jcs254888.

25. Hauser AS, Attwood MM, Rask-Andersen M, et al. Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov* 2017; 16: 829–842.

26. Stomach J, Collin F, et al. Targeting G protein-coupled receptors for the treatment of chronic pain in the digestive system. *Gut* 2021; 70: 970–981.

27. Muthiah I, Rajendran K and Dhanaraj P. In silico molecular docking and physicochemical property studies on effective phytochemicals targeting GPR116 for breast cancer treatment. *Mol Cell Biochem* 2021; 476: 883–896.

28. Tian JY, Chi CL, Bian G, et al. A novel GPCR target in correlation with androgen deprivation therapy for prostate cancer drug discovery. *Basic Clin Pharmacol Toxicol* 2021; 128: 195–203.

29. Alexander SP, Christopoulos A, Davenport AP, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: G protein-coupled receptors. *Br J Pharmacol* 2021; 178: S27–S156.

30. Irizarry RA, Bolstad BM, Collin F, et al. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* 2003; 31: e15.

31. Wang R, Gurguis CI, Gu W, et al. Ion channel gene expression predicts survival in glioma patients. *Sci Rep* 2015; 5: 11593.

32. Xie Y, Xiao Y, Liu Y, et al. Construction of a novel ratiosensitivity-and ferroptosis-associated gene signature for prognosis cytogenetics and histopathology. *Neuro Oncol* 2010; 12: 991–1003.

33. Sun L, Hui AM, Su Q, et al. Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. *Cancer Cell* 2006; 9: 287–300.

34. Liu Z, Yao Z, Li C, et al. Gene expression profiling in human high-grade astrocytomas. *Comp Funct Genomics* 2011; 2011: 245137.

35. Erdem-Eraslan L, Gravendeel LA, de Rooi J, et al. Intrinsic molecular subtypes of glioma are prognostic and predict benefit from adjuvant procarbazine, lomustine, and vincristine chemotherapy in combination with other prognostic factors in anaplastic oligodendrogial brain tumors: a report from EORTC study 26951. *J Clin Oncol* 2013; 31: 328–336.

36. Freije WA, Castro-Vargas FE, Fang Z, et al. Gene expression profiling of gliomas strongly predicts survival. *Cancer Res* 2004; 64: 6503–6510.

37. Nutt CL, Mani DR, Betensky RA, et al. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res* 2003; 63: 1602–1607.

38. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 2006; 9: 157–173.

39. Kersch CN, Claunch CJ, Ambady P, et al. Transcriptional signatures in histologic structures within glioblastoma tumors may predict personalized drug sensitivity and survival. *Neurooncol Adv* 2020; 2: vdaa093.

40. Zhou H, Vallières M, Bai HX et al. MRI features predict survival and molecular markers in diffuse lower-grade gliomas. *Neuro Oncol* 2017; 19: 862–870.
prediction in gliomas. *J Cancer* 2022; 13: 2683–2693.
44. Maiga A, Lemieux S, Pabst C, et al. Transcriptome analysis of G protein-coupled receptors in distinct genetic subgroups of acute myeloid leukemia: identification of potential disease-specific targets. *Blood Cancer J* 2016; 6: e431.
45. Pascual-Vargas P and Salinas PC. A Role for Frizzled and Their Post-Translational Modifications in the Mammalian Central Nervous System. *Front Cell Dev Biol* 2021; 9: 692888.
46. Huang HC and Klein PS. The Frizzled family: receptors for multiple signal transduction pathways. *Genome Biol* 2004; 5: 234.
47. Bhanot P, Fish M, Jemison JA, et al. Frizzled and Dfrizzled-2 function as redundant receptors for Wingless during Drosophila embryonic development. *Development* 1999; 126: 4175–4186.
48. Fischer T, Guimera J, Wurst W, et al. Distinct but redundant expression of the Frizzled Wnt receptor genes at signaling centers of the developing mouse brain. *Neuroscience* 2007; 147: 693–711.
49. Zhao X, Huang H, Chen Y, et al. Dynamic expression of secreted Frizzled-related protein 3 (sFRP3) in the developing mouse spinal cord and dorsal root ganglia. *Neuroscience* 2013; 248: 594–601.
50. Scavo MP, Cigliano A, Depalo N, et al. Frizzled-10 Extracellular Vesicles Plasma Concentration Is Associated with Tumoral Progression in Patients with Colorectal and Gastric Cancer. *J Oncol* 2019; 2019: 2715968.
51. Shaw HV, Koval A and Katanaev VL. A high-throughput assay pipeline for specific targeting of frizzled GPCRs in cancer. *Methods Cell Biol* 2019; 149: 57–75.
52. Sun Y, Wang W and Zhao C. Frizzled Receptors in Tumors, Focusing on Signaling, Roles, Modulation Mechanisms, and Targeted Therapies. *Oncol Res* 2021; 28: 661–674.
53. Li Y, Liu Z and Zhang Y. Expression and prognostic impact of FZDs in pancreatic adenocarcinoma. *BMC Gastroenterol* 2021; 21: 79.
54. Scavo MP, Fucci L, Caldarola L, et al. Frizzled-10 and cancer progression: Is it a new prognostic marker? *Oncotarget* 2017; 9: 824–830.
55. Qiu X, Jiao J, Li Y et al. Overexpression of FZD7 promotes glioma cell proliferation by upregulating TAZ. *Oncotarget* 2016; 7: 85987–85999.
56. Cao TT, Xiang D, Liu BL, et al. FZD7 is a novel prognostic marker and promotes tumor metastasis via WNT and EMT signaling pathways in esophageal squamous cell carcinoma. *Oncotarget* 2017; 8: 65957–65968.
57. Zeng CM, Chen Z and Fu L. Frizzled Receptors as Potential Therapeutic Targets in Human Cancers. *Int J Mol Sci* 2018; 19: 1543.
58. Le HTT, Murugesan A, Ramesh T, et al. Molecular interaction of HIC, an agonist of P2Y1 receptor, and its role in prostate cancer apoptosis. *Int J Biol Macromol* 2021; 189: 142–150.
59. Bellefeuille SD, Molle CM and Gendron FP. Reviewing the role of P2Y receptors in specific gastrointestinal cancers. *Purinergic Signal* 2019; 15: 451–463.
60. Kuang Y, Wang Y, Cao X, et al. New prognostic factors and scoring system for patients with acute myeloid leukemia. *Oncol Lett* 2021; 22: 823.
61. Schuler V, Lüscher C, Blanchet C, et al. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA (B) responses in mice lacking GABA(B(1)). *Neuron* 2001; 31: 47–58.
62. Kurabayashi N, Nguyen MD and Sanada K. The G protein-coupled receptor GPRC5B contributes to neurogenesis in the developing mouse neocortex. *Development* 2013; 140: 4335–4346.
63. Zhang B, Wu Q, Xu R, et al. The promising novel biomarkers and candidate small molecule drugs in lower-grade glioma: Evidence from bioinformatics analysis of high-throughput data. *J Cell Biochem* 2019; 120: 15106–15118.
64. Lu Q, Ding Y, Li Y, et al. 5-HT receptor agonist Valerenic Acid enhances the innate immunity signal and suppresses glioblastoma cell growth and invasion. *Int J Biol Sci* 2020; 16: 2104–2115.