GPBAR1 Promotes Proliferation and Is Related to Poor Prognosis of High-Grade Glioma via Inducing MAFB Expression

Suohui Sun  
Shandong First Medical University

Hui Guo  
Taian City Central Hospital

Nan Liang  
Shandong First Medical University

Tao Wu  
Shandong First Medical University

Chunpu Zhang  
Shandong First Medical University

Huaqing Li (✉ lxicjn315dcj@163.com)  
Shandong First Medical University

Research

Keywords: Glioma, GPBAR1, prognosis, MAFB, proliferation.

DOI: https://doi.org/10.21203/rs.3.rs-419714/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Glioma is the most prevalent brain tumors with extremely poor prognosis, but the prognostic biomarkers of high-grade (grade III and IV) gliomas (HGG) are still insufficient.

Methods

In our study, we investigated the expression of GPBAR1 in HGG by qRT-PCR and immunohistochemistry (IHC), and evaluated the clinical significance of GPBAR1 with univariate and multivariate analyses. By retrieving the data from TCGA, we screened the genes significantly associated with GPBAR1, and identified the correlation between GPBAR1 and MAFB. By experiments in vitro, we showed the pivotal role of MAFB in GPBAR1-induced proliferation of HGG.

Results

GPBAR1 expression in HGGs was significantly higher than that in normal brain tissues. GPBAR1 was an independent prognostic biomarker of HGG. GPBAR1 promoted the proliferation of HGG by inducing MAFB expression. MAFB was also a prognostic biomarker of HGG, and patients with co-expression of MAFB and GPBAR1 had worse prognosis.

Conclusions

GPBAR1 promoted the proliferation of HGG by inducing MAFB expression. Both GPBAR1 and MAFB were prognostic biomarkers of HGG, and patients with co-expression of MAFB and GPBAR1 had worse prognosis than those with only GPBAR1 or MAFB expression.

Introduction

Gliomas are the most common malignant primary brain tumors in adults, with an estimated annual incidence of 6.6 per 100,000 individuals in the USA\(^\text{18}\). Glioma accounts for over 70\% of malignant brain tumors\(^\text{11}\). In the WHO staging system, glioma has 4 histological grade (grade I-IV). Low-grade gliomas (LGG) (grade I and II) are less common and affect younger patients. Patients with LGG usually have a more favorable prognosis and better response to adjuvant therapy. In the high-grade gliomas (HGG) (grade III and IV), glioblastoma (GBM) is the most malignant glioma (grade IV) with the highest prevalence (approximate 45\% of all gliomas)\(^1\). The median survival of GBM is less than 2 years, even if patients receive the standard treatment including maximal safe resection and post-operative radiochemotherapy with the alkylating agent Temozolomide\(^\text{25}\).
G protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5) is a G protein-coupled receptor which can be activated by primary and secondary bile acids\textsuperscript{14}. GPBAR1 was ubiquitously expressed in human tissues, not only in liver and biliary system. GPBAR1 was widely involved in either physiological or pathological processes, including cell proliferation, migration, immune response, secretion and anti-apoptosis\textsuperscript{7}. Many downstream signaling pathways are influenced by GPBAR1 activation, such as cAMP-PKA and MAPK-ERK signaling\textsuperscript{7; 8}. In cancers, overexpression of GPBAR1 has been reported in several types of cancers including gastric and breast cancer, cholangiocarcinoma, etc\textsuperscript{2; 24; 30}. In glioma, the expression and role in tumor progression of GPBAR1 has not been elucidated.

V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) is a transcription factor which can activate or suppress transcription of downstream genes\textsuperscript{19}. In the seven MAF members, MAFB is considered to be substantially oncogenic\textsuperscript{21}. Aberrant expression of MAFB increases the risk of many diseases including diabetes, atherosclerotic diseases and tumors\textsuperscript{6; 12; 20}. However, the expression and clinical significance of MAFB in glioma, and the correlation between MAFB and GPBAR1, are not understood.

In our study, we investigated the expression of GPBAR1 in HGG by qRT-PCR and immunohistochemistry (IHC), and evaluated the clinical significance of GPBAR1 with univariate and multivariate analyses. By retrieving the data from TCGA, we screened the genes significantly associated with GPBAR1, and identified the correlation between GPBAR1 and MAFB. By experiments in vitro, we showed the pivotal role of MAFB in GPBAR1-induced proliferation of HGG.

**Materials And Methods**

**Patients and follow-ups**

A total of 189 patients with HGG underwent surgical resection in the Second Hospital affiliated to Shandong First Medical University from 2009 to 2017. The enrolling criteria included that (1) enough specimens for IHC, (2) gross total resection (>95%) was performed. The excluding criteria included that (1) post-operational survival less than 3 months, (2) patients suffer other malignancies. The final cohort was comprised of 149 patients with HGG, including 115 male and 34 female patients. Moreover, we collected 10 fresh HGGs and corresponding tissues for mRNA detection. All the specimens were obtained with the consent of patients. The study was approved by the Ethics Committee of Second Hospital affiliated to Shandong First Medical University. The data of GBM patients were retrieved from the Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) for in silico analysis of clinical databases.

**Cells and transfection**

Human GBM cell lines U251, U118, U87 and A172 were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM containing 10% fetal bovine serum (FBS) supplemented with streptomycin (100 μg/ml) and penicillin (100 U/ml). Forskolin, 6R-ethyl-23(S)-
methylcholic acid (S-EMCA, INT777), NF449 were all purchased from Sigma-Aldrich. The applied antibodies were as follows: GPBAR1 (Novus Biologicals, NBP2-23669), MAFB (Santa Cruz Biotechnology, sc-376387), Phospho-p44/42 Erk1/2 (9101), GAPDH (Cell signaling technology, 5174). siRNAs and shRNAs were purchased from GenePharma (Shanghai, China). GPBAR1 open reading frame was translocated into pFLAG-CMV with double enzyme digestion reaction. The shRNA, siRNA and pFLAG-GPBAR1 vector were all transfected into GBM cell lines with Lipofectamine 2000 according to the manual.

**Immunohistochemistry and evaluation**

IHC with the streptavidin-biotin immunoperoxidase was used to evaluate and semi-quantify the expression of GPBAR1 and MAFB in GBM. In brief, the specimens were de-paraffinized and rehydrated with graded ethanol and xylene, and then incubated in 0.3% H2O2 for endogenous hydrogen peroxidase inactivation. The optimal antigen retrieval was accomplished by citrate buffer. Unspecific antigen binding was blocked by 5% fetal bovine serum. After that, primary antibodies of GPBAR1 (1:100) or MAFB (1:100) were used to incubate the specimens at 4°C overnight. The slides were rinsed 3 times with phosphate buffer saline, and the corresponding secondary antibodies (Sangon, Shanghai, China) was used at room temperature for 1 hour. The antigens were finally displayed with a DAB kit.

The results of IHC were blindly evaluated by two senior pathologists unaware of the clinical data. IHC results were semi-quantified by calculating the final score, which was the product of the score of staining intensity and the score of positive cell percentage according to previous reports. The scores of staining intensity were as follows: 0 for negative staining; 1 for weak staining; 2 for moderate staining and 3 for strong staining. The scores of positive cell percentage had 4 grades: score 1 for <25% positive cells; 2 for 25%-50% positive cells; 3 for 50%-75% positive cells; 4 for 75%-100% positive cells. The final score was the product of two aspects multiplication, and the cut-off of the final score was determined by Receiver operating characteristic (ROC) curve, which was the point with the highest sum of sensitivity and specialty referring to the previous study.

**RNA extraction and quantified real-time PCR**

mRNA levels of GPBAR1 or MAFB in glioma cell lines and tissues were detected with qRT-PCR. mRNAs of cells or tissues were extracted with TRizol reagent (Thermo Fisher) and RNeasy protect mini kit (Qiagen, Hilden, Germany) with the manual. Reverse transcription PCR and quantification was accomplished by the Primescript RT reagent kit (Takara BIO INC.) with Thermo Fisher 7500 PCR System. The mRNA level of GAPDH was used as the internal control for 2^-ΔΔCt method normalization. The qRT-PCR primers were as follows:

GPBAR1: forward: 5’-CCCAGGCTATCTTCCCAGC-3’;
reverse: 5’- GCCAGGACTGAGGAGGA-3’.
Proliferation assay

The proliferation of GBM cell lines was evaluated by CCK8 kit (Beyotime, Beijing, China). Cells were transfected with scrambled siRNA, siRNA or pFLAG-GPBAR1. 48 hours after the transfection, cells were seeded into 96-well plates and cultured for another 48 hours in the stimulation of Int777, Forskolin, NF449 or Ulixertinib. At the end of stimulation, CCK8 was added and the optical density (OD) at 450nm was measured with a spectrophotometer (Molecular Devices Company, USA). The proliferation ratio was calculated with the control group as a baseline.

Statistical analysis

The Chi-square test was applied to calculate the correlation between GPBAR1 expression and the clinicopathological factors. The Kaplan-Meier method was used to show the survival curves and the log-rank test was used to analyze the statistical difference of different subsets. The independent prognostic factors were identified by the Cox-regression proportional hazards model. One-way ANOVA was used to compare the statistical difference between different groups. The software SPSS 22.0 (IBM Corporation, Chicago, USA) was used to analyze all the data, and the \( P \) less than 0.05 was considered as statistically significant.

Results

GPBAR1 expression in HGG tissues and cell lines.

First of all, data were retrieved in TCGA database to show GPBAR1 expression in high-throughput assay. The TPM (transcripts per million) of GPBAR1 in 163 HGGs was significantly higher than in 207 normal brain tissues (Figure 1A). In our collection of 10 fresh HGGs and their corresponding normal tissues, GPBAR1 mRNA was detected with qRT-PCR. The results also showed the up-regulation of GPBAR1 in HGGs (Figure 1B). In our cohort with 149 HGG patients, GPBAR1 expression was detected with IHC, and evaluated with IHC score which divided the cohort into subsets with low and high GPBAR1 expression (Figure 1C). The percentages of patients with low and high GPBAR1 account for 45.6% (68/149) and 54.4% (81/149), respectively. In addition, GPBAR1 expression in GBM cell lines U251, U118, U87 and A172 cells were detected with Western blot.

The clinical significance of GPBAR1 expression

The clinical significance of GPBAR1 was first evaluated by analyzing its correlation between clinicopathological factors with chi-square test (Table 1). The clinical information including the sex and age of patients, the tumor size, Karnofsky Performance Scale (KPS) score, and adjuvant therapy. Intriguingly, high expression of GPBAR1 was significantly associated with large tumor size \( (P=0.004) \). The correlation between GPBAR1 and other factors had no obvious statistical significance.

Furthermore, the prognostic value of GPBAR1 and other clinicopathological factors were analyzed with univariate and multivariate analyses (Table 2). The Kaplan-Meier method was applied to analyze the
correlations between these factors and the overall survival rates. In our study, GPBAR1 expression was substantially associated with the overall survival (OS) rate (Figure 2A). High GPBAR1 predicted the poor outcome of patients with high-grade glioma. Moreover, high KPS and adjuvant therapy were also associated with the OS rate of patients (Figure 2B and 2C).

In addition, the independent prognostic factors were identified with multivariate analysis (Table 2). In the Cox-regression Hazard model, GPBAR1 was an independent prognostic factor of high-grade glioma ($P=0.016$), with a hazard ratio of high GPBAR1 as 1.64. In addition to GPBAR1, higher KPS could independently indicated the favorable prognosis ($P=0.008$).

**GPBAR1 expression was correlated with MAFB**

In the clinical analysis, we found that GPBAR1 was associated with larger tumor size, indicating that GPBAR1 may be involved in the tumor proliferation, so we further screened the potential target proteins of GPBAR1 which may participate in the GPBAR1-involved proliferation. MAFB was previously reported to promote the cancer progression such as tumorigenesis, proliferation and stemness in several cancer types including osteosarcoma and colon cancer$^{6,29}$. In TCGA database, MAFB expression was substantially correlated with GPBAR1 expression (Figure 3A). In our study, we also investigated the mRNA correlation between GPBAR1 and MAFB, and demonstrated that GPABR1 was also positively associated with MAFB (Figure 3B). In the cohort with 149 patients, the average IHC score of MAFB in patients with low GPBAR1 was significantly lower than that in patients with high GPBAR1 (Figure 3C). Chi-square test also validated the significant correlation between GPBAR1 and MAFB (Table 1). In U118 and U251 cells, we silenced or overexpressed GPBAR1, and found that MAFB expression was correspondingly changed (Figure 3D and 3E).

**GPBAR1 activation promoted the expression of MAFB.**

GPBAR1 was a GPCR receptor which could be stimulated by bile acid, so we further investigated the molecular signaling between GPBAR1 and MAFB. The cAMP stimulator forskolin and inhibitor NF449 were used to incubate U118 cells for 24 hours, and we showed that MAFB expression was enhanced when GPBAR1 was overexpressed, or when Forskolin was used (Figure 4A). On the contrary, NF449 could inhibit GPBAR1-induced MAFB expression. These results suggested that GPBAR1 regulated MAFB expression in a cAMP-independent pathway. U118 cell proliferation was also detected after GPBAR1 overexpression, in the presence of forskolin or NF449 with CCK8 assay. GPBAR1 overexpression and forskolin stimulation accelerated U118 proliferation, while NF449 attenuated the proliferation (Figure 4B). GPBAR1 can stimulate and activate MAPK-ERK pathway by phosphorylating ERK$^{23}$, so we further verified that in GBM cells. Int777 is a well-accepted specific stimulator of GPBAR1$^{31}$, which was used to activate GPBAR1 signaling in U251. In our study, Int777 promoted the phosphorylation of ERK and the expression of MAFB. However, GPBAR1 knockdown and Ulixertinib decreased ERK activation and MAFB expression (Figure 4C), suggesting that ERK activation was essential in GPBAR1-induced MAFB expression. U251 cell proliferation had the similar tendency with MAFB expression (Figure 4D). To show the role of MAFB in
GPBAR1-induced proliferation, we silenced MAFB when overexpressing GPBAR1 and/or stimulating U118 with Int777 (Figure 4E), and we detected the influence of these factors to U118 proliferation. MAFB knockdown significantly impaired cell proliferation, which was increased by Int777 or GPBAR1. These results showed an essential role of MAFB in GPBAR1-induced proliferation (Figure 4F).

Co-expression of GPBAR1 and MAFB was a more sensitive prognostic biomarker

The clinical significance of MAFB has never been elucidated in glioma, so we further evaluated the prognostic significance of MAFB. The cohort was divided into low and high MAFB according to MAFB IHC score (Figure 5A). The number of patients with low and high MAFB was 87 and 62, respectively, accounting for 58.39% and 41.61%. With univariate analysis, we showed that patients with low MAFB expression had more favorable prognosis than those with high MAFB ($P=0.002$), with the 3-year OS rate as 32.7% and 19.1% respectively (Figure 5B). Moreover, we divided the patients into subsets with those with co-expression of GPBAR1 and MAFB, and those with other expression patterns, which accounted for 42 and 107 patients respectively. Co-expression of GPBAR1 and MAFB can predict the poor prognosis of high-grade glioma more effectively and sensitively ($P<0.001$), showing that detecting GPBAR1 and MAFB may be a possible method for individual treatment.

Discussion

Identifying potential biomarker is important in patient stratification, definition of risk groups and predicting adjuvant therapy response. Compared with many other tumors, the genetic or immunohistochemical biomarkers derived from the resected tumor or biopsy are far away from sufficiency. HGG is one of the most aggressive tumor types among all the solid tumors, requiring more effective treatments. However, encouraging outcomes are not observed, though many large-cohort comprehensive genome analyses were made and the molecular landscape of glioma was depicted. Two reasons may attributed to the slow progresses: (1) glioma is highly heterogenous, (2) the genetic detection is not sufficient to describe the overall variation of glioma, and protein detection is also needed. Here in our study, we demonstrated that GPBAR1 and MAFB were prognostic biomarkers of HGG, and showed that co-expression was a more sensitive indicator of HGG. This result provided more detailed evidence to stratify the high-risk patients with glioma.

The expressions of GPBAR1 in different cancer types, and GPBAR1 functions in cancer progression and prognosis are not in consensus. GPBAR1 expression was decreased in renal neoplasms but up-regulated in gastric cancer. Moreover, GPBAR1 led to poor prognosis of gastric cancer and pancreatic cancer, but was reported to be associated with good prognosis of ampullary adenocarcinoma. There are many conflicting evidence of GPBAR1 function even in the same cancer type. Previous studies indicated that GPBAR1 suppressed proliferation and migration of gastric cancer cell, but another line showed that GPBAR1 promoted epithelial mesenchymal transition in gastric cancer cell lines. Moreover, many interesting phenomena of GPBAR1 need to be solved. When GPBAR1 is expressed in the primary cilium of cholangiocytes, it couples to Goi and inhibits cell proliferation. However, when located in the apical
plasma membrane, it interacts with Gαs and promotes cell proliferation\textsuperscript{17}. Here we demonstrated that GPBAR1 could induce MAFB expression dependent on ERK phosphorylation and cAMP activation, but the underlying mechanism of how ERK and cAMP activation induced MAFB expression is still unknown. More experiments should be performed to reveal the exact mechanism of GPBAR1-induced MAFB expression, and MAFB-involved proliferation of glioma.

Up-regulation of MAFB is reported in acute leukemia, myeloma, hepatocellular carcinoma, and colorectal carcinoma\textsuperscript{20, 22, 28}. MAFB could promote the tumor-involved progress such as stemness, proliferation, drug resistance of a variety of cancers including nasopharyngeal carcinoma and osteosarcoma\textsuperscript{6, 15}. MAFB could be either an oncogene or as a tumor suppressor, depending on the cell context\textsuperscript{9}. For the first time, we showed that MAFB was also a prognostic biomarker of HGG, indicating the poor prognosis. Moreover, we showed that MAFB was required in the GPBAR1-induced proliferation of HGG. A number of proliferation-involved target genes downstream MAFB have been identified, such as Notch and CCND2\textsuperscript{13, 26}. The genes or proteins for responsible MAFB-induced proliferation should be identified to better depict the profound mechanism of MAFB role in HGG progression.

**Conclusions**

We investigated the expression of GPBAR1 in HGG by qRTPCR and IHC, and showed an up-regulation of GPBAR1 in HGG compared with normal tissues. With univariate and multivariate analyses, we demonstrated that GPBAR1 was an independent prognostic biomarker of HGG. By retrieving the data from TCGA and \textit{in vitro} experiments, we showed that MAFB expression was associated with GPBAR1, and that GPBAR1 can induce the MAFB expression. We showed that MAFB was required in GPBAR1-induced proliferation of HGG. MAFB was also a prognostic biomarker of HGG, and patients with co-expression of MAFB and GPBAR1 had worse prognosis than those with only GPBAR1 or MAFB expression. Our results identified more effective biomarkers of HGG, which could stratify the high-risk patients with HGG. Moreover, we investigated the underlying mechanism of GPBAR1-induced progression of HGG, suggesting that GPBAR1 could be a potential drug target of HGG, and providing more evidence on the precise treatment of HGG.

**Abbreviations**

HGG: high-grade gliomas, GPBAR1: G-protein coupled bile acid receptor 1, IHC: immunohistochemistry, GBM: glioblastoma, LGG: Low-grade gliomas, MAFB: V-maf musculoaponeurotic fibrosarcoma oncogene homolog B, ROC: Receiver operating characteristic, qPCR: quantified real-time PCR, TPM: transcripts per million, KPS: Karnofsky Performance Scale, OS: overall survival.

**Declarations**

\textbf{Ethical approval and consent to participate:} All procedures performed in studies involving human participants were in accordance with the ethical standards of YIDU Central Hospital, and with the 1964...
Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

**Consent for publication:** All authors agree for publication.

**Availability of supporting data:** The original datasets are available from the corresponding author on reasonable request

**Competing interests:** The authors declare no conflict of interest.

**Funding:** No funding information was applicable.

**Author contributions:** Huaqing Li and Suohui Sun conceived and designed the experiments, Hui Guo, Nan Liang and Tao Wu analyzed and interpreted the results of the experiments, Suohui Sun, Hui Guo, Nan Liang, Tao Wu, Chunpu Zhang performed the experiments. Huaqing Li drafted the manuscript. The authors read and approved the final manuscript

**Acknowledgments:** Not applicable.

**Authors information:**

1. Departments of Neurosurgery, the Second Hospital of Shandong First Medical University, Taian, Shandong, China.

2. Departments of Pediatrics, Central Hospital of Taian, Taian, Shandong, China.

3. Departments of Intensive Care Unit, the Second Hospital of Shandong First Medical University, Taian, Shandong, China.

**References**

1. Alexander BM, Cloughesy TF (2017). Adult Glioblastoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **35**(21): 2402-2409.

2. Cao W, Tian W, Hong J, Li D, Tavares R, Noble L et al (2013). Expression of bile acid receptor TGR5 in gastric adenocarcinoma. *American journal of physiology Gastrointestinal and liver physiology* **304**(4): G322-327.

3. Carino A, Graziosi L, D’Amore C, Cipriani S, Marchiano S, Marino E et al (2016). The bile acid receptor GPBAR1 (TGR5) is expressed in human gastric cancers and promotes epithelial-mesenchymal transition in gastric cancer cell lines. *Oncotarget* **7**(38): 61021-61035.

4. Chen MC, Chen YL, Wang TW, Hsu HP, Lai MD (2016). Membrane bile acid receptor TGR5 predicts good prognosis in ampullary adenocarcinoma patients with hyperbilirubinemia. *Oncology reports* **36**(4): 1997-2008.
5. Chen T, Li K, Liu Z, Liu J, Wang Y, Sun R et al (2021). WDR5 facilitates EMT and metastasis of CCA by increasing HIF-1alpha accumulation in Myc-dependent and independent pathways. *Molecular therapy : the journal of the American Society of Gene Therapy*.

6. Chen Y, Wang T, Huang M, Liu Q, Hu C, Wang B et al (2020). MAFB Promotes Cancer Stemness and Tumorigenesis in Osteosarcoma through a Sox9-Mediated Positive Feedback Loop. *Cancer research* 80(12): 2472-2483.

7. Deutschmann K, Reich M, Klindt C, Droge C, Spomer L, Haussinger D et al (2018). Bile acid receptors in the biliary tree: TGR5 in physiology and disease. *Biochimica et biophysica acta Molecular basis of disease* 1864(4 Pt B): 1319-1325.

8. Donepudi AC, Boehme S, Li F, Chiang JY (2017). G-protein-coupled bile acid receptor plays a key role in bile acid metabolism and fasting-induced hepatic steatosis in mice. *Hepatology* 65(3): 813-827.

9. Eychene A, Rocques N, Pouponnot C (2008). A new MAFia in cancer. *Nature reviews Cancer* 8(9): 683-693.

10. Guo C, Su J, Li Z, Xiao R, Wen J, Li Y et al (2015). The G-protein-coupled bile acid receptor Gpbar1 (TGR5) suppresses gastric cancer cell proliferation and migration through antagonizing STAT3 signaling pathway. *Oncotarget* 6(33): 34402-34413.

11. Gusyatiner O, Hegi ME (2018). Glioma epigenetics: From subclassification to novel treatment options. *Seminars in cancer biology* 51: 50-58.

12. Hamada M, Nakamura M, Tran MT, Moriguchi T, Hong C, Ohsumi T et al (2014). MafB promotes atherosclerosis by inhibiting foam-cell apoptosis. *Nature communications* 5: 3147.

13. Hurt EM, Wiestner A, Rosenwald A, Shaffer AL, Campo E, Grogan T et al (2004). Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. *Cancer cell* 5(2): 191-199.

14. Keitel V, Haussinger D (2018). Role of TGR5 (GPBAR1) in Liver Disease. *Seminars in liver disease* 38(4): 333-339.

15. Li Y, Min D, Wang K, Yin S, Zheng H, Liu L (2017). MicroRNA152 inhibits cell proliferation, migration and invasion by directly targeting MAFB in nasopharyngeal carcinoma. *Molecular medicine reports* 15(2): 948-956.

16. Li Z, Liu J, Chen T, Sun R, Liu Z, Qiu B et al (2021). HMGA1-TRIP13 axis promotes stemness and epithelial mesenchymal transition of perihilar cholangiocarcinoma in a positive feedback loop dependent on c-Myc. *Journal of experimental & clinical cancer research : CR* 40(1): 86.

17. Masyuk AI, Huang BQ, Radtke BN, Gajdos GB, Splinter PL, Masyuk TV et al (2013). Ciliary subcellular localization of TGR5 determines the cholangiocyte functional response to bile acid signaling. *American journal of physiology Gastrointestinal and liver physiology* 304(11): G1013-1024.

18. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C et al (2015). CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro-oncology* 17 Suppl 4: iv1-iv62.
19. Park JG, Tischfield MA, Nugent AA, Cheng L, Di Gioia SA, Chan WM et al (2016). Loss of MAFB Function in Humans and Mice Causes Duane Syndrome, Aberrant Extraocular Muscle Innervation, and Inner-Ear Defects. American journal of human genetics 98(6): 1220-1227.

20. Pettersson AM, Acosta JR, Bjork C, Kratzel J, Stenson B, Blomqvist L et al (2015). MAFB as a novel regulator of human adipose tissue inflammation. Diabetologia 58(9): 2115-2123.

21. Pouponnot C, Sii-Felice K, Hmitou I, Rocques N, Lecoin L, Druillennec S et al (2006). Cell context reveals a dual role for Maf in oncogenesis. Oncogene 25(9): 1299-1310.

22. Qiang YW, Ye S, Huang Y, Chen Y, Van Rhee F, Epstein J et al (2018). MAFb protein confers intrinsic resistance to proteasome inhibitors in multiple myeloma. BMC cancer 18(1): 724.

23. Reich M, Deutschmann K, Sommerfeld A, Klindt C, Kluge S, Kubitz R et al (2016). TGR5 is essential for bile acid-dependent cholangiocyte proliferation in vivo and in vitro. Gut 65(3): 487-501.

24. Rodrigues CM, Moshage H (2016). Targeting TGR5 in cholangiocyte proliferation: default topic. Gut 65(3): 369-370.

25. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC et al (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. The Lancet Oncology 10(5): 459-466.

26. van Stralen E, van de Wetering M, Agnelli L, Neri A, Clevers HC, Bast BJ (2009). Identification of primary MAFB target genes in multiple myeloma. Experimental hematology 37(1): 78-86.

27. Westphal M, Lamszus K (2015). Circulating biomarkers for gliomas. Nature reviews Neurology 11(10): 556-566.

28. Yang L, Liu Y, Zhu L, Xiao M (2015). DNMT3A R882 mutation is associated with elevated expression of MAFB and M4/M5 immunophenotype of acute myeloid leukemia blasts. Leukemia & lymphoma 56(10): 2914-2922.

29. Yang LS, Zhang XJ, Xie YY, Sun XJ, Zhao R, Huang QH (2016). SUMOylated MAFB promotes colorectal cancer tumorigenesis. Oncotarget 7(50): 83488-83501.

30. Zhao CL, Amin A, Hui Y, Yang D, Cao W (2018). TGR5 expression in normal kidney and renal neoplasms. Diagnostic pathology 13(1): 22.

31. Zuo G, Zhang T, Huang L, Araujo C, Peng J, Travis Z et al (2019). Activation of TGR5 with INT-777 attenuates oxidative stress and neuronal apoptosis via cAMP/PKCe pathway after subarachnoid hemorrhage in rats. Free radical biology & medicine 143: 441-453.

Tables

Table 1. Correlation between GPBAR1 and clinicopathological variables.
| Parameters   | number | Low | High | P*  |
|-------------|--------|-----|------|-----|
| Age         |        |     |      |     |
| ≤50         | 51     | 20  | 31   | 0.255 |
| ≥50         | 98     | 48  | 50   |      |
| Sex         |        |     |      |     |
| Male        | 115    | 53  | 62   | 0.839 |
| Female      | 34     | 15  | 19   |      |
| Tumor size  |        |     |      |     |
| ≤3cm        | 60     | 36  | 24   | 0.004 |
| ≥3cm        | 89     | 32  | 57   |      |
| KPS         |        |     |      |     |
| ≤70         | 40     | 17  | 23   | 0.712 |
| ≥70         | 109    | 51  | 58   |      |
| Adjuvant therapy |   |     |      |     |
| Yes         | 99     | 49  | 50   | 0.224 |
| No          | 50     | 19  | 31   |      |
| MAFB        |        |     |      |     |
| Low         | 87     | 48  | 39   | 0.007 |
| High        | 62     | 20  | 42   |      |

* calculated with Chi-square test.

Table 2. The univariate and multivariate analyses were performed to identify prognostic factors
| Parameters | 3-year OFS | P* | HR | CI95% | p& |
|------------|------------|----|----|-------|----|
| Age | | | | | |
| ≤50 | 29.1 | 0.34 | | | |
| ≥50 | 25.9 | | | | |
| Sex | | | | | |
| Male | 26.3 | 0.859 | | | |
| Female | 28.8 | | | | |
| Tumoe size | | | | | |
| ≤3cm | 28.6 | 0.871 | | | |
| ≥3cm | 26.3 | | | | |
| KPS | | | | | |
| ≤70 | 16.8 | 0.002 | 1 | | |
| ≥70 | 31.2 | 0.564 | 0.37-0.86 | 0.008 | |
| Adjuvant therapy | | | | | |
| Yes | 31.7 | 0.032 | 1 | | |
| No | 20.7 | 1.41 | 0.94-2.12 | 0.096 | |
| GPBAR1 | | | | | |
| Low | 33.6 | 0.006 | 1 | | |
| High | 20.4 | 1.64 | 1.10-2.45 | 0.016 | |

* calculated by log-rank test; & calculated by Cox-regression model.

**Figures**
Figure 1

Expression of GPBAR1 in HGG tissues and cell lines. A. In TCGA database, the TPM (transcripts per million) of GPBAR1 in 163 HGG tissues was significantly higher than that in 207 normal tissues. P value was generated by t test. B. The mRNA levels of GPBAR1 in 10 HGG tissues and corresponding tumor-adjacent tissues were evaluated with qRT-PCR. The average level of mRNA in adjacent tissues was set as 1.0. P value was generated by paired t test. C. 149 HGGs were used for IHC detection to show the expression and location of GPBAR1, and were divided into subsets with low- and high-GPBAR1 expression. Scale bar: 100μm. D. The expressions of GPBAR1 in GBM cell lines U251, U118, U87 and A172 were detected with western blot.
Figure 2

The survival rates of patients with low- and high-GPBAR1 expression. A. The total 149 HGG patients were divided into subsets with low- and high-GPBAR1 expression, accounting for 68 and 81 patients, respectively. B and C. The correlation between KPS, adjuvant therapy and OS rate. The statistical significance was analyzed with the log-rank test.
GPBAR1 expression was associated with the expression of MAFB. A. In TCGA database, MAFB expression was significantly associated with GPBAR1. B. In the 10 GBMs, MAFB mRNA level was significantly associated with GPBAR1 mRNA. C. In the 250 HGG patients, patients with high GPBAR1 had higher MAFB IHC scores compared with patients with low GPBAR1. D and E. GPBAR1 expression was silenced in U251 cells, and overexpressed in U118 cells. The expression of MAFB was detected with WB (D) and qRT-PCR (E). The expression of MAFB changed in consistent with the GPBAR1. In A and B, R2 was analyzed by the Pearson method. In C and E, ** and *** represent P<0.01 and <0.001, with one-way ANOVA.
GPBAR1 activation promoted the expression of MAFB. A. In U118 cells, GPBAR1 was overexpressed, and the cAMP stimulator forskollin (10μM) or inhibitor NF449 (1μM) was used for 24 hours. The expression of MAFB was detected with WB (left) or qRT-PCR (right). B. After GPBAR1 overexpression, U118 cells were incubated in forskollin or NF449 for another 48 hours, and cell proliferation was detected with CCK8 assay. C. GPBAR1 expression in U251 cells was knocked down with shRNA, with or without ulixertinib (1
μM) or Int777 (1 μM) for 30 minutes. ERK phosphorylation and MAFB expression were detected with WB (left) or qRT-PCR (right). D. 24 hours after GPBAR1 knockdown, U251 cells were incubated in ulixertinib (1 μM) or Int777 (1 μM) for 48 hours, and cell proliferation was detected with CCK8 assay. E. In the stimulation of Int777 (1 μM) stimulation for 48 hours, GPBAR1 was overexpressed in U118 cells, while MAFB was silenced with siRNA. The expression of MAFB was detected with WB (left) and qRT-PCR (right). F. After GPBAR1 overexpression and/or MAFB silencing, U118 cells were stimulated with 1 μM Int777 for 48 hours, and cell proliferation was detected with CCK8 assay. ** and *** represents P<0.01 and <0.001 compared with control group, with one-way ANOVA. $$$ represents P<0.001 between indicated groups. All data were showed as mean±SEM, and analyzed with one-way ANOVA.

![Image A](image1.png)

Low MAFB expression  
High MAFB expression

![Image B](image2.png)

MAFB expression and OFS

![Image C](image3.png)

Double expression and OFS

Figure 5
Figure 5

Co-expression of GPBAR1 and MAFB was a more sensitive biomarker of HGG. A. MAFB expression in the 149 HGG patients were detected with IHC, and divided into subsets with low and high MAFB expression. Scale bar: 100μm. B. The correlation between MAFB expression and overall survival rates were analyzed with log-rank test. C. The patients were divided into subset with co-expression of GPBAR1 and MAFB, and subset with other expression profiles. The survival rates between GPBAR1 and MAFB co-expression and other patterns were compared.