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GPBAR1 promotes proliferation and is related to poor prognosis of high-grade glioma via inducing MAFB expression

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Summary. 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.

Materials and 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.

Key words: Glioma, GPBAR1, Prognosis, MAFB, Proliferation

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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 (Ostrom et al., 2015). Glioma accounts for over 70% of malignant brain tumors (Gusyatiner and Hegi, 2018). In the WHO staging system, glioma has 4 histological grades (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 (approximately 45% of all gliomas) (Alexander and Cloughesy, 2017). The median survival of GBM is less than 2 years, even if patients receive the standard treatment including maximal safe resection and post-operative radio-chemotherapy with the alkylating agent Temozolomide (Stupp et al., 2009).

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 (Keitel and Haussinger, 2018). GPBAR1 is ubiquitously expressed in human tissues, not only in liver and biliary system. GPBAR1 is widely involved in either physiological or pathological processes, including cell proliferation, migration, immune response, secretion and anti-apoptosis (Deutschmann et al., 2018). Many downstream signaling pathways are influenced by GPBAR1 activation, such as cAMP-PKA and MAPK-ERK signaling (Donepudi et al., 2017; Deutschmann et al., 2018). In cancers, overexpression of GPBAR1 has been reported in several types of cancers including gastric and breast cancer, cholangiocarcinoma, etc (Cao et al., 2013; Rodrigues and Moshage, 2016; Zhao et al., 2018; Chen et al., 2021b). 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 (Park et al., 2016). In the seven MAF members, MAFB is considered to be substantially oncogenic (Pouponnot et al., 2006). Aberrant expression of MAFB increases the risk of many diseases including diabetes, atherosclerotic diseases and tumors (Hamada et al., 2014; Pettersson et al., 2015; Chen et al., 2020). 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 (1) enough specimens for IHC, (2) gross total resection (>95%) was performed. The excluding criteria included (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-parraffinized and rehydrated with graded ethanol and xylene, and then incubated in 0.3% H_2O_2 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) were 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 (Chen et al., 2021a; Liu et al., 2021). 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 (Li et al., 2021).

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) following 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 $2^{-\Delta\Delta Ct}$ method normalization. The qRTPCR primers were as follows:

GPBAR1: forward: 5'-CCCAGGCTATCTTCCC AGC-3'; reverse: 5'- GCCAGGACTGAGAGGA

GCA-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 a 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 from the 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 (Fig. 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 (Fig. 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 (Fig. 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.

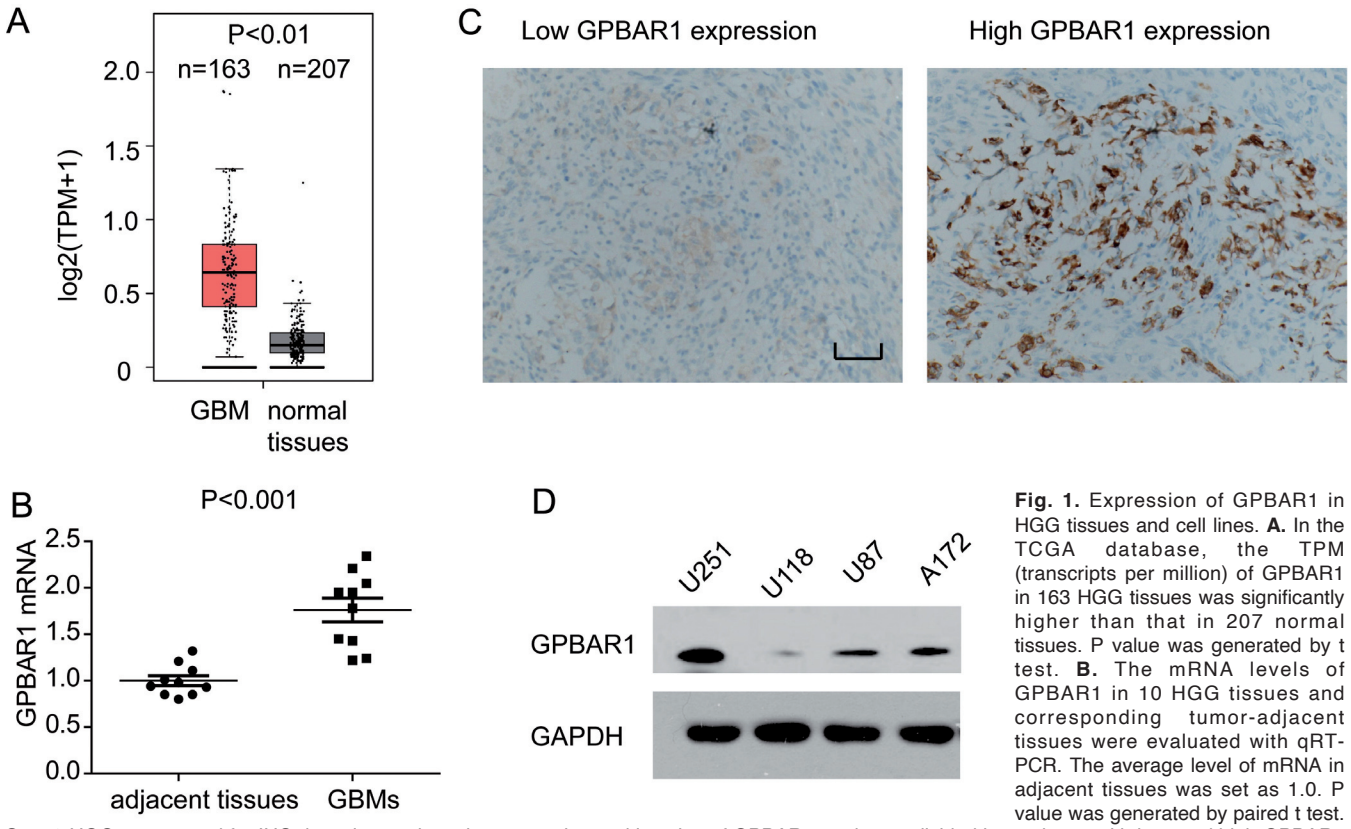

Fig. 1. Expression of GPBAR1 in HGG tissues and cell lines. **A.** In the TCGA database, the TPM (transcripts per million) of GPBAR1 in 163 HGG tissues was significantly higher than that in 207 normal tissues. P value was generated by t test. **B.** The mRNA levels of GPBAR1 in 10 HGG tissues and corresponding 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. **D.** The expressions of GPBAR1 in GBM cell lines U251, U118, U87 and A172 were detected with western blot. Scale bar: 100 μm.

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 (Fig. 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 (Fig. 2B,C).

Table 1. Correlation between GPBAR1 and clinicopathological variables.

Parameters	number	GPBAR1		P*
		Low	High	
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.

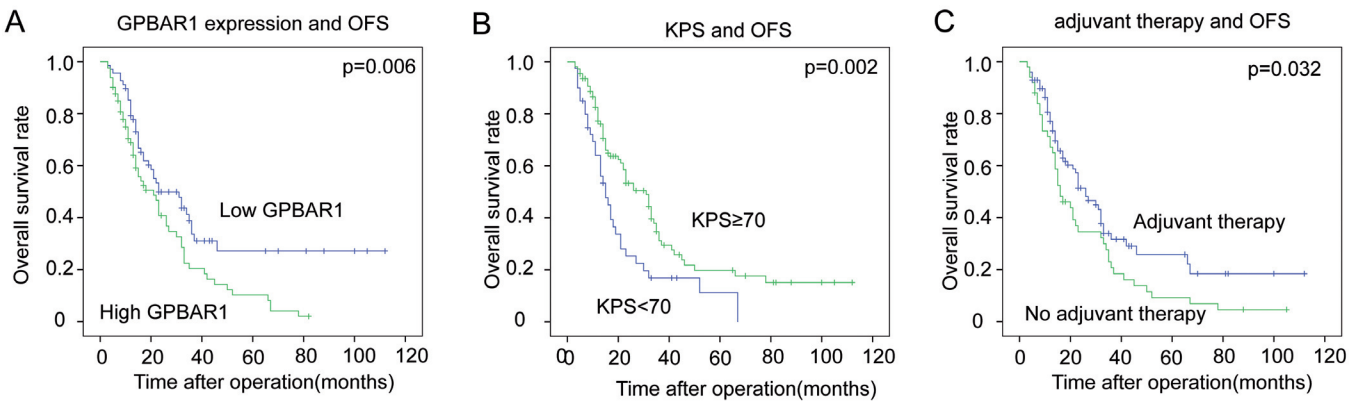

Fig. 2. The survival rates of patients with low- and high-GPBAR1 expression. **A.** The total of 149 HGG patients were divided into subsets with low- and high-GPBAR1 expression, accounting for 68 and 81 patients, respectively. **B, C.** The correlation between KPS, adjuvant therapy and OS rate. The statistical significance was analyzed with the log-rank test.

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 independently indicated a 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 tumor proliferation, so we further screened the potential target proteins of GPBAR1 which may participate in the GPBAR1-invovled proliferation. MAFB was previously reported to promote cancer progression such as tumorigenesis, proliferation and stemness in several cancer types including osteosarcoma and colon cancer (Yang et al., 2016; Chen et al., 2020). In the TCGA database, MAFB expression was substantially correlated with GPBAR1 expression (Fig. 3A). In our study, we also investigated the mRNA correlation between GPBAR1 and MAFB, and demonstrated that GPABR1 was also positively associated with MAFB (Fig. 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 (Fig. 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 (Fig. 3D,E).

GPBAR1 activation promoted the expression of MAFB

GPBAR1 is a GPCR receptor which can 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 (Fig. 4A). On the contrary, NF449 was able to inhibit GPBAR1-induced MAFB expression. These results suggested that GPBAR1 regulated MAFB expression in

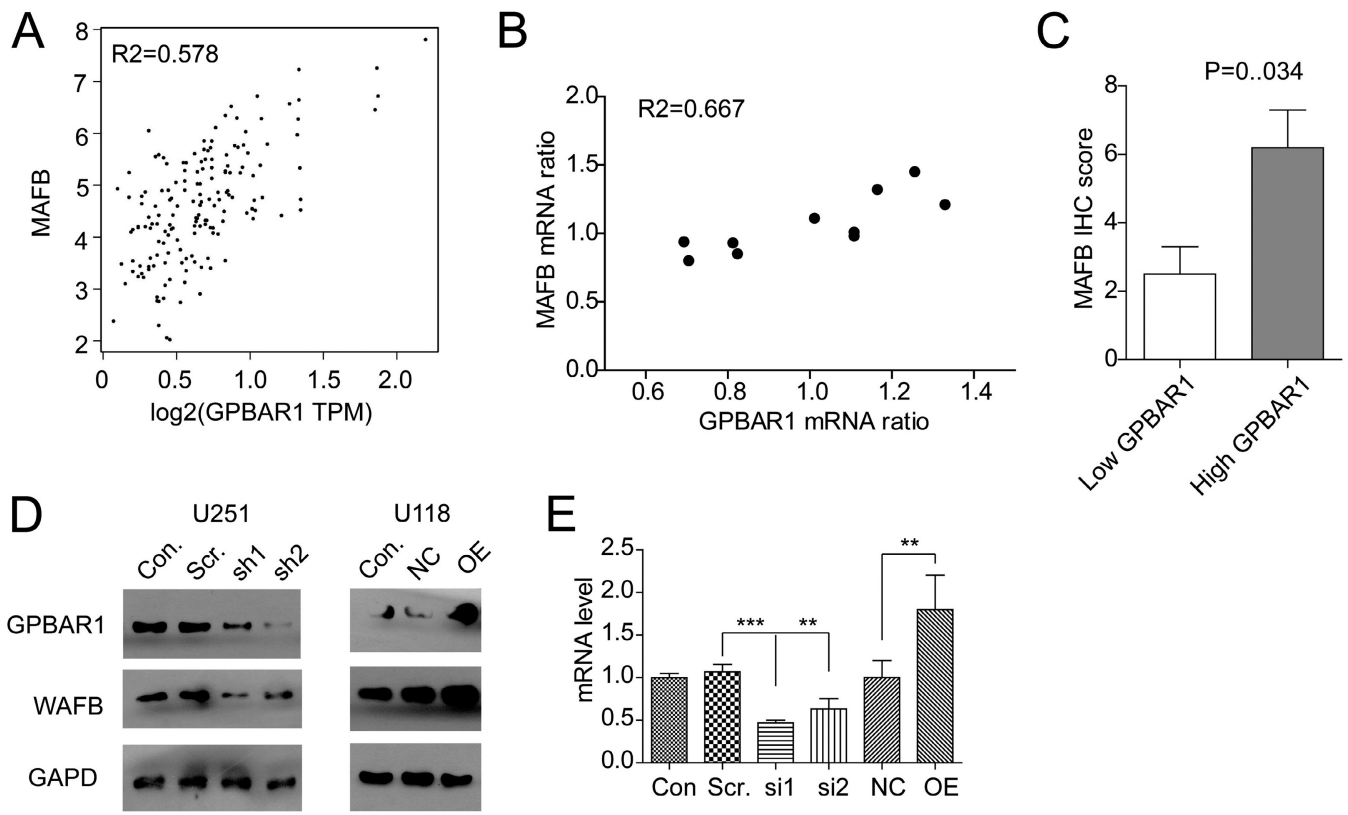

Fig. 3. GPBAR1 expression was associated with the expression of MAFB. **A.** In the TCGA database, MAFB expression was significantly associated with GPBAR1. **B.** In the 10 GBMs, MAFB mRNA level was significantly associated with GPBAR1 mRNA. **C.** In the 250 HGG patients, patients with high GPBAR1 had higher MAFB IHC scores compared with patients with low GPBAR1. **D, 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 consistently 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.

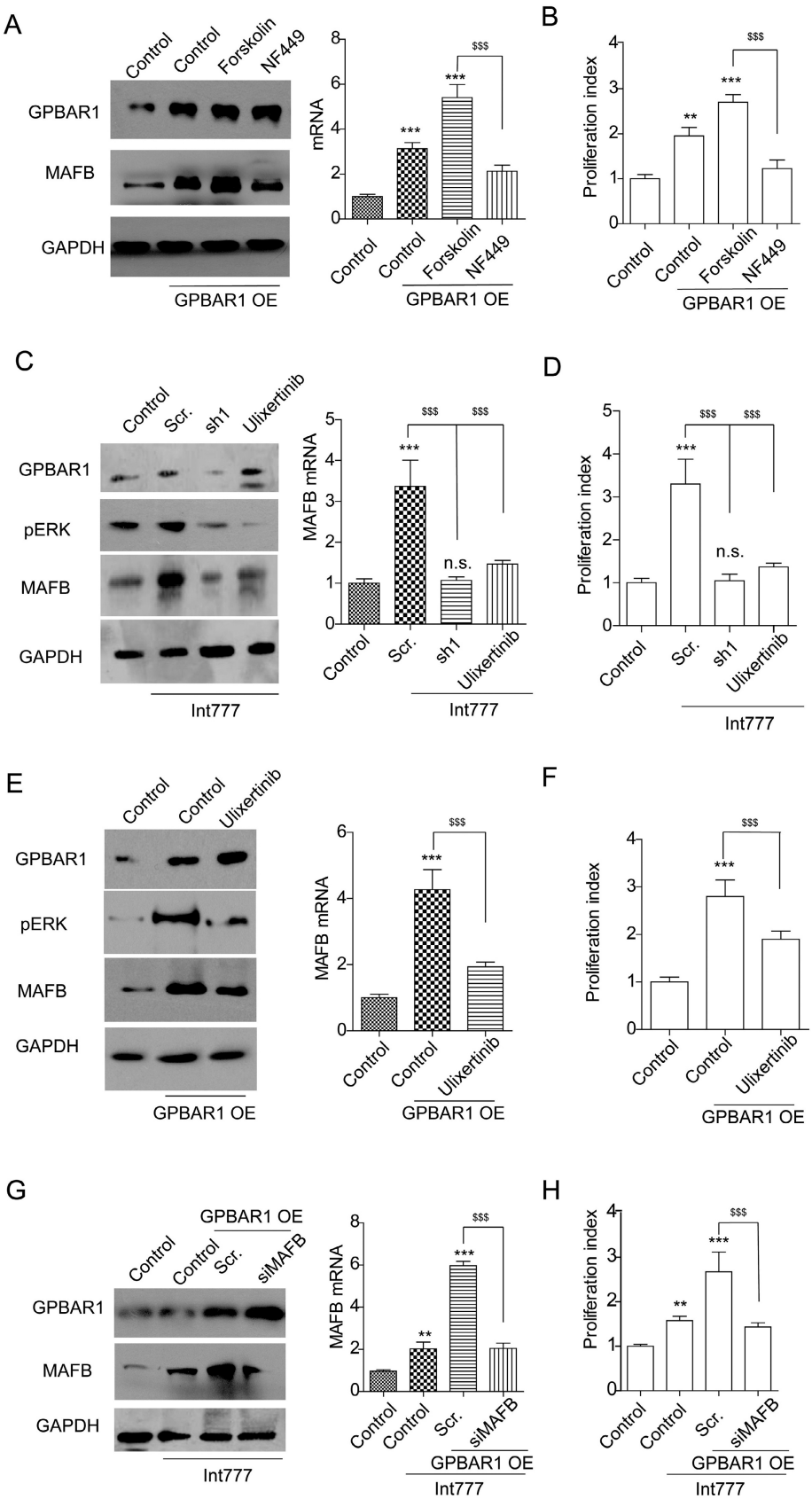

Fig. 4. GPBAR1 activation promoted the expression of MAFB. **A.** In U118 cells, GPBAR1 was overexpressed, and the cAMP stimulator forskollin (10μM) or inhibitor NF449 (1μM) was used for 24 hours. The expression of MAFB was detected with WB (left) or qRT-PCR (right). **B.** After GPBAR1 overexpression, 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.** GPBAR1-overexpressed U118 cell was stimulated\with or without ulixertinib (1 μM) treatment for 30 minutes. ERK phosphorylation and MAFB expression were detected with WB (left) or qRT-PCR (right). **F.** Control U251 or GPBAR1-overexpressed U118 cells were incubated in ulixertinib (1 μM) for 48 hours, and cell proliferation was detected with CCK8 assay. **G.** In the stimulation of Int777 (1 μM) stimlation 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). **H.** After GPBAR1 over-expression and/or MAFB silencing, U118 cells were stimulated with 1 μM Int777 for 48 hours, and cell proliferation was detected with CCK8 assay. ** and *** represent P<0.01 and <0.001 compared with control group, with one-way ANOVA. $$$ represents P<0.001 between indicated groups. All data were shown as mean±SEM, and analyzed with one-way ANOVA.

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 (Fig. 4B). GPBAR1 can stimulate and activate the MAPK-ERK pathway by phosphorylating ERK (Reich et al., 2016), so we further verified that in GBM cells. Int777 is a well-accepted specific stimulator of GPBAR1 (Zuo et al., 2019), 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 ERK inhibitor Ulixertinib decreased ERK activation and MAFB expression (Fig. 4C), suggesting that ERK activation was essential in GPBAR1-induced MAFB expression. U251 cell proliferation had a similar tendency with MAFB expression (Fig. 4D). Moreover, we overexpressed GPBAR1 and inhibited ERK with Ulixertinib to further validate the involvement of ERK in the activation of MAFB via GPBAR1. As expected, GPBAR1 overexpression increased ERK phosphorylation and MAFB expression, while Ulixertinib inhibited MAFB expression by suppressing ERK phosphorylation (Fig. 4E). U251 proliferation was also promoted by GPBAR1 overexpression and suppressed

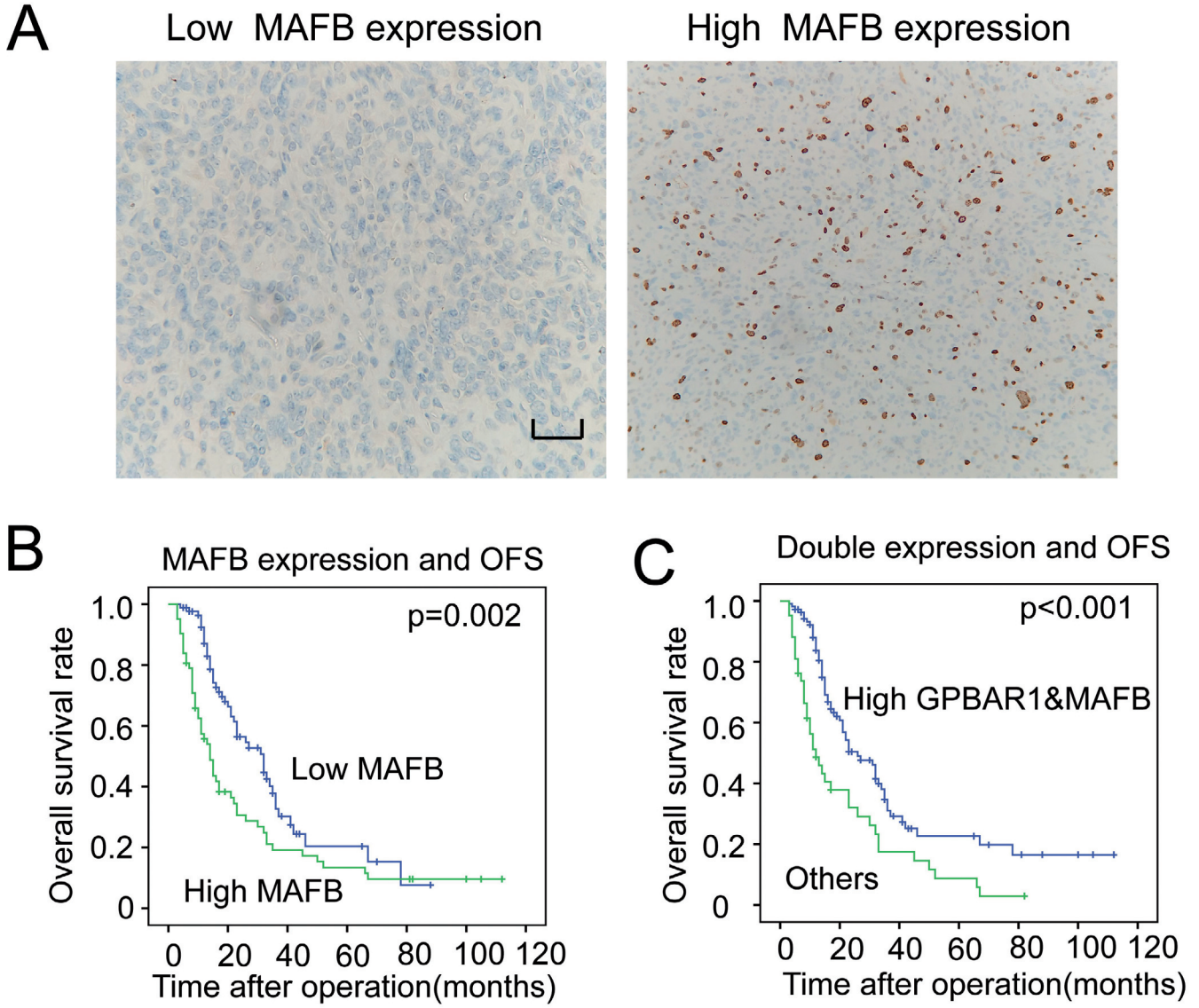

Fig. 5. Co-expression of GPBAR1 and MAFB was a more sensitive biomarker of HGG. **A.** MAFB expression in the 149 HGG patients was detected with IHC, and divided into subsets with low and high MAFB expression. **B.** The correlation between MAFB expression and overall survival rates were analyzed with log-rank test. **C.** The patients were divided into a subset with co-expression of GPBAR1 and MAFB, and the other subset with other expression profiles. The survival rates between GPBAR1 and MAFB co-expression and other patterns were compared. Scale bar: 100 μm.

by Ulixertinib incubation (Fig. 4F).

To show the role of MAFB in GPBAR1-induced proliferation, we silenced MAFB when overexpressing GPBAR1 and/or stimulating U118 with Int777 (Fig. 4G), 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 (Fig. 4H).

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 (Fig. 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 (Fig. 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 biomarkers 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 from sufficiency (Westphal and Lamszus, 2015). 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 have been done and the molecular landscape of glioma has been depicted. Two reasons may account for the slow progress: (1) glioma is highly heterogenous, (2) 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 provids 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 (Carino et al., 2016). Moreover, GPBAR1 led to poor prognosis of gastric cancer and pancreatic cancer, but was reported to be associated with good prognosis of ampullary adenocarcinoma (Chen et al., 2016). There is much conflicting evidence of GPBAR1 function even in the same cancer type. Previous studies indicated that GPBAR1 suppressed proliferation and migration of gastric cancer cells (Guo et al., 2015), 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 Gαi and inhibits cell proliferation. However, when located in the apical plasma membrane, it interacts with Gαs and promotes cell proliferation (Masyuk et al., 2013). 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-invovled proliferation of glioma.

Up-regulation of MAFB is reported in acute leukemia, myeloma, hepatocellular carcinoma, and colorectal carcinoma (Pettersson et al., 2015; Yang et al., 2015; Qiang et al., 2018). MAFB was able to promote tumor-involved progress such as stemness, proliferation, and drug resistance of a variety of cancers including nasopharyngeal carcinoma and osteosarcoma (Li et al., 2017; Chen et al., 2020). MAFB was able to be either an oncogene or a tumor suppressor, depending on the cell context (Eychene et al., 2008). For the first time, we showed that MAFB was also a prognostic biomarker of HGG, indicating poor prognosis. Moreover, we showed that MAFB was required in the GPBAR1-induced proliferation of HGG. A number of proliferation-involved target genes downstream of MAFB have been identified, such as Notch and CCND2 (Hurt et al., 2004; van Stralen et al., 2009). The genes or proteins responsible for MAFB-induced proliferation should be identified to better depict the profound mechanism of the MAFB role in HGG progression.

In conclusion, 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 in vitro experiments, we showed that MAFB expression was associated with GPBAR1, and that GPBAR1 can induced 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.

Conflicts of interest. There is no conflict of interest.

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