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Original Article

Fucosyltransferase 8 modulates receptor tyrosine kinase activation and temozolomide resistance in glioblastoma cells

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Abstract: Alteration of extracellular glycosylation is a hallmark of malignant characteristics. In this study, we revealed that fucosyltransferase 8 (FUT8), an enzyme that mediates the core fucosylation of N-linked glycosylation, is an important regulator of malignant characteristics in human glioma that acts by modifying the activities of both the HGF receptor (MET) and epidermal growth factor receptor (EGFR). mRNA and protein expression levels of FUT8 were frequently upregulated in gliomas, and these events were showed positive correlations with advanced tumor grade, recurrence, and decreased overall survival. Silencing FUT8 expression in glioma cells suppressed cell growth, migration, and invasion, whereas overexpression of FUT8 was sufficient to enhance these phenotypes. Mechanistic investigations revealed that FUT8 was involved in the alteration of fucosylation status that was attached to MET and EGFR, changing MET responses after HGF stimulation, as well as in the transactivation of EGFR. Importantly, altering FUT8 expression or using the fucosylation inhibitor 2F-peracetyl-fucose sensitized the efficacy of of temozolomide (TMZ) therapy. Collectively, these results suggested that FUT8 dysregulation contributed to the malignant behaviors of glioma cells and provide novel insights into the significance of fucosylation in receptor tyrosine kinase activity and TMZ resistance.

Keywords: Fucosyltransferase 8, glioma, HGF, MET, EGFR

Introduction

Gliomas are solid tumors that arise from glial cells in the brain or spine, and account for around 80 percent of all malignant brain tumors. High-grade gliomas, particularly grade IV glioblastoma multiforme (GBM), are almost incurable; patients with GBM have an the estimated five-year survival rate of only 5.5 percent, and the median survival time is only 5 to 7 months after recurrence [1]. Currently, glioma therapies still mainly rely on surgical resection and radiation combined with TMZ treatment [2]. The outcomes for GBM patients after the stander treatments are still poor, and the cancer cells often develop resistance to TMZ therapy. TMZ resistance is mainly caused by an upregulation of the DNA damage repair proteins [3]. The coactivation of receptor tyrosine kinases (RTKs) such as MET and ErbB family receptors often enhances cell survival by triggering DNA damage repair signals [4-7]. Therefore, strategies that simultaneously suppress RTK activation possess the potential to overcome TMZ resistance in glioma.

Glycosylation is a common post-translational modification in cells. This process is an enzymatic reaction that catalyzes linear and/or branched glycan chains on lipids, peptides, or other organic molecules [8]. Abnormal protein glycosylation is frequently observed in human cancers and is associated with malignant transformation and tumor progression [9, 10].

The alteration of glycans on RTKs can modulate their biological properties, including protein folding, protein turnover rate, and receptor dimerization [11-13]. Therefore, modification or inhibition of glycans on RTK provides possible strategies to combat RTK-driven tumor cells [14, 15]. Additionally, the altered expression levels and distinct glycan structures in cancer cells compared to their normal counterparts are often considered as tumor-associated antigens, and have been used in cancer diagnosis or relapse tracking. For example, carbohydrate epitopes such as carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9), are often highly expressed in the serum of cholangiocarcinoma, pancreatic, and colorectal cancer patients, and elevated expression levels of these glycan structures are a sign of tumor recurrence [16]. Increases in fucosylation and sialylation are also well-established signatures of malignant cell transformation, which lead to tumor progression and metastasis [17, 18].

Fucosylation refers to the transfer of a fucose residue to oligosaccharides on glycoproteins or glycolipids [19]. This process is catalyzed by at least 11 types of fucosyltransferases (FUTs), which transfer a fucose residue from GDP-fucose to oligosaccharides at different positions. The addition of fucose to carbohydrates through α1,2-linkage is catalyzed by FUT1 and FUT2. FUT3-FUT7 and FUT9-FUT11 catalyze the addition of α1,3/4-linked fucose to oligosaccharides. FUT8 is the only enzyme responsible for the attachment of α1,6-linked fucose to N-glycan [19].

Dysregulated fucosylation has been reported in many types of cancers. The aberrant expression of FUTs and their respective synthesized carbohydrate structures is associated with tumor progression and malignant behaviors, including enhanced cancer cell proliferation, tissue invasion and metastasis, increased cell survival, and multidrug resistance. Nevertheless, abnormal FUTs may also involve in cancer stemness, epithelial to mesenchymal transition, endothelial and immune cell interaction, and angiogenesis [17, 20-23]. These results suggest that fucosylation is a key event in the regulation of cancer development and progression. Although protein fucosylation has been studied extensively in cancers, and its role in regulating cell physiology, cell-cell interaction, and tumor progression has been established, its functions and clinical relevance in human glioma have not been reported.

Materials and methods

Reagents and antibodies

Recombinant HGF protein was purchased from PeproTech (100-39H). Full length FUT8 cDNA clone was purchased from Sino Biological. Mouse monoclonal antibody against FUT8 (2F7H2) was purchased from Proteintech Group, Inc. Antibodies against p-MET (#3077), MET (#3127), p-EGFR (#3777), EGFR (#2232), p-AK (#4060), AKT (#4691), p-ERK (#9107), and ERK (#9102) were purchased from Cell Signaling Technology, Inc. Biotinylated-LCA (B-1045-5) and AAL (B-1395-1) were purchased from Vector Laboratories. Antibodies against ACTB were purchased from GeneTex, Inc. 2F-peracetyl-fucose and Temozolomide (TMZ) was purchased from Cayman Chemical and CCK8 reagent were purchased from Sigma-Aldrich.

Human tissue samples

Post-surgery paraffin-embedded glioma tissues for immunohistochemistry were obtained from the Chang-Gung Memorial Hospital (Taoyuan, Taiwan). The usage of human tissues for this study was approved by the Chang Gung Medical Foundation Institutional Review Board, and all patients gave informed consent to have their tissues before collection (Approve Number: 201802150B0). Adult normal human brain tissue lysates were purchased from Novus Biologicals. Primary human astrocytes (Cat. #1800) was purchased from ScienCell Research Laboratories.

Immunohistochemistry

Paraffin-embedded glioma tissues were incubated with anti-FUT8 antibody (1:200) in 5% bovine serum albumin/PBS and 0.1% Triton X-100 (Sigma) for 16 h at 4°C. UltraVision Quanto Detection System (Thermo Fisher Scientific Inc.) was used to amplified the signal. The specific immunostaining was visualized with 3,3-diaminobenzidine and counterstained with hematoxylin (Sigma). Images were scanned by TissueFAX Plus Cytometer. The FUT8

positive cells were counted by two scorers blinded to the clinical parameters.

Cell culture and transfection

The source and the species of glioma cell lines GBM8401, U251-MG, Ln18, U118, A172, and GL261 were listed in the Table S1. Cells were cultured in DMEM containing 4.0 mM L-glutamine and 10% FBS in 5% CO_2 at 37°C.

For overexpression experiments, empty pCMV6 and FUT8-pCMV6 plasmids were transfected to Ln18 cells using Lipofectamine 3000 (Invitrogen). The transfected cells were selected with 150 μg/mL of Hygromycin B for 14 days. For knockdown of FUT8, ON-TARGETplus SMARTpool siRNA against FUT8 and control siRNA were purchased from Dharmacon. Cells were transfected with 20 nmol of siRNA using Lipofectamine RNAiMAX (Invitrogen) for 48 to 72 h.

Cell proliferation, cell invasion, and cell migration assay

Cells (2×10^3) were seeded into 96-well plates with culture medium. Living cells were analyzed by CCK8 at 0, 24, 48, and 72 h. Four wells per group for each time point were measured following manufacturer's protocol. The experiments were repeated for three times, and relative fold changes of OD 450 nm were shown.

Transwell inserts for 24-well plate (Corning) with uncoated porous filters (pore size 8 μm) were used to evaluate cell migration, and Matrigel (BD Biosciences) coated porous filters were used to examine cell invasion. Ln18 (2×10^4 cells) and U251-MG (2×10^4 cells) in 0.1 ml serum-free DMEM were seeded into inserts, and 0.6 ml DMEM containing 10% FBS was added in lower part of the well. Cells were incubated for 24 h, and cells moving to the other side of the filters were stained by crystal violet and counted manually. Values were shown as the average number of cells per microscopic field over four fields of each filter. Independent experiments were repeated for three times.

Western blotting and lectin pulldown assay

The protein samples (30 μg) were separated by 8% SDS-PAGE and transferred to PVDF membrane. The total protein was measured by stain-free technology (Bio-Rad). To analyze HGF-triggered signaling, cells were incubated in DMEM medium with 2% FBS for 16 h, and then stimulated with 20 ng/ml of HGF for 10 min or 30 min. Streptavidin-HRP (Vector Laboratories) was used for lectin bloting.

For lectin pulldown assay, total cell lysates (600 μg) were incubated with biotinylated-lectins at 4°C for 16 hours. Streptavidin-conjugated agarose beads (Vector Laboratories) were then added and lysates were further incubated for additional 6 hours. The precipitated proteins were then subjected to western blotting. Intensity of signals on western blotting was quantified by ImageJ software (Wayne Rasband).

Phospho-RTK array assay

For phospho-RTK array assay, cells were incubated in DMEM medium with 2% FBS for 16 h, and then stimulated with additional FBS (10% in final) for 15 min. Three hundred micrograms of protein lysate were applied to human phospho-RTK array Kit (ARY001B, R&D SystemsTM) according to the manufacturer's protocol.

Durg sensitivity assay

Cells (4×10^3) were seeded into 96-well plates with culture medium for 16 h, and the serial diluted TMZ (200 μM to 6.25 μM) was added to medium for 24 h. For 2PF treatment, U251-MG cells were pre-treated with 2PF (200 μM) or solvent control for 6 days, and then seeding to 96-well plates for TMZ treatments. Four wells per group for each TMZ concentration were measured by CCK8 assay. The experiments were repeated for three times, and relative fold changes of OD 450 nm were shown.

Statistical analysis

All data analysis was performed using GraphPad Prism 6. One-way analysis of variance multiple comparisons (ANOVA) were used for analyzing FUT8 expressing cells of tissue sections in different glioma subtypes. Student t-test was used for statistical analyses. Two-sided Fisher exact test was used for comparisons between FUT8 expression and clinicopathologic features. Kaplan-Meier analysis and the log-rank test were used to estimate overall survival. $P < 0.05$ was considered statistically significant.

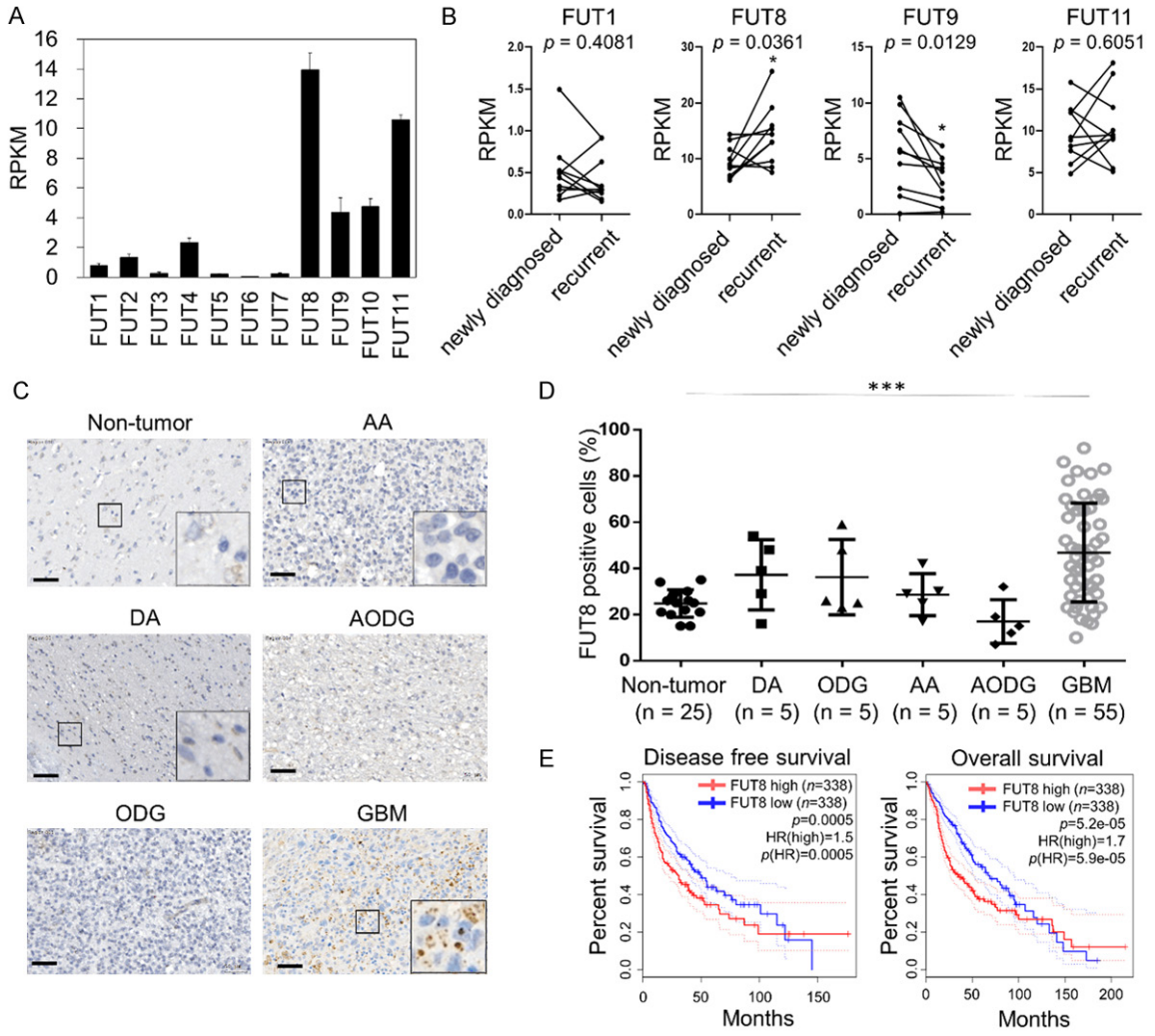

Figure 1. FUT8 is frequently upregulated in human GBM. A. The expression of fucosyltransferase family in 8 glioma tissues using RNA-Seq. The data are presented as the means ± SEM. B. The expression of *FUT1, FUT8, FUT9*, and *FUT11* in 10 paired newly diagnosed and corresponding recurrent tumor tissues using RNA-Seq. N = 10. The statistic significance of the differential gene expression between newly diagnosed and recurrent tumors is analyzed using paired *t*-test. $^{*}P < 0.05$ is considered statistically significant. C. Representative images of FUT8 immunohistochemistry in different subtypes of glioma tissue (AA: anaplastic astrocytomas; DA: diffuse astrocytomas; AODG: anaplastic oligodendrogliomas; ODG: oligodendrogliomas; GBM: glioblastomas). Increased dot-like precipitations of FUT8 were observed in cytoplasm of GBM tissues. The scale bar indicates 50 μm. D. Presentage of FUT8 positve cells in each glioma case. Noted that significantly increase of FUT8 cells was found in GBM tissue. $^{***}P < 0.001$ (One-way ANOVA). E. High expression of *FUT8* was associated with decreased disease free survival and overall survival in glioma patients. The high and low expression groups were divided by median expression level of *FUT8* in 676 glioma cases (HR: hazard ratio). These data were from the GEPIA2 database (http://gepia2.cancer-pku.cn/#index).

Results

Expression of fucosyltransferases in human GBM

We first analyze gene expression of fucosyltransferases (FUTs) using 8 newly diagnosed GBM patients, and differential gene expression among *FUT* family members was observed (**Figure 1A**). Among all fucosylatransferases, the α1,6-fucosyltransferase, *FUT8,* has the highest expression level in tumor tissue. α1,3-fucosyltransferase, *FUT9*, *FUT10*, and *FUT11* have medium expression levels. Other FUTs are expressed at relatively low levels in glioma tissues. We further compared the expression of FUTs in 10 paired newly diagnosed specimen and corresponding recurrent speci-

Table 1. Correlation of FUT8 expression with clinicopathological features of glioma tissue array

Factor		FUT8 positive cells		*P* value (Two-sided Fisher's exact test)
		Low (≤ 40%)	High (> 40%)	
Tissue types	Non-tumor	25	0	< 0.0001
	Tumor	36	39	
Age	< 55 years	16	15	0.6443
	≥ 55 years	20	24	
Tumor number	Single	33	31	0.1948
	Multiple	3	8	
CSF dissemination	Yes	2	1	0.6049
	No	34	38	
Tumor stage	Grade I-III	15	5	0.0081
	Grade IV (GBM)	21	34	

men. Results indicated that only FUT8 is significantly upregulated in recurrent glioma tissue, while FUT9 is downregulated in recurrent tissue (**Figure 1B**). To examine the protein expression of FUT8 in glioma subtypes, immunohistochemistry was performed on diffuse astrocytomas, oligodendrogliomas, anaplastic oligodendrogliomas, anaplastic astrocytomas, glioblastomas, and non-tumor adjacent tissues. The immunohistochemistry showed paranuclear dot-like precipitates of FUT8 in the cytoplasm (**Figure 1C**), which is similar to the intracellular localization of the Golgi apparatus. The frequency of FUT8 positive cells in GBM tissue (grade IV) is significantly higher than other subtypes (grade I-III) and non-tumor tissue (**Figure 1D** and **Table 1**). Analyzing the expression of *FUT8* in glioma patients by GEPIA2 websites, we found that high expression of *FUT8* was significantly associated with decreased disease free survival and overall survival (**Figure 1E**, n = 676). Overall, these results suggested that FUT8 frequently upregulated in GBM, and its expression is associated with poor prognosis of glioma patients.

FUT8 mediates protein fucosylation on glioma cells

To investigate the role of FUT8 in protein fucosylation in glioma cells, we first analyzed its expression in normal human brain tissue, primary cultured human astrocyte, and GBM cell lines by western blotting. The expression level of FUT8 was relatively high in U251-MG cells, while low in Ln18 cells, brain tissues, and astrocytes (**Figure 2A**). Thus, we used Ln18 cell line for the overexpression experiments, and silenced FUT8 expression in U251-MG cells by small interfering RNA (siRNA) transfection (**Figure 2B**). Mock and stable FUT8 transfectants were obtained from the pooled colonies of Ln18 cells transfected with pCMV6 or FUT8/pCMV6 plasmids, respectively. The FUT8-modulated fucosylation was quantified by flow cytometry using a fucose-recognizing lectin, LCA, staining. We found that LCA intensity was decreased 60% in FUT8-silenced U251-MG cells, while increased 22% in FUT8-overexpressing L18 cells (**Figure 3C**). Furthermore, we showed that silence of FUT8 decreased the LCA and AAL (another fucose-recognizing lectin) binding to several glycoproteins, including the 150 kDa bands (**Figure 2D**), whereas overexpression of FUT8 enhanced binding of LCA and AAL to 150 kDa glycoproteins (**Figure 2E**). These results indicate that FUT8 modulates the protein fucosylation on glioma cells.

FUT8 regulates malignant phenotypes of glioma cell

Next, we investigated the effects of FUT8 on malignant phenotypes of glioma cells, including cell viability, cell migration, and invasion. The CCK8 assay showed that knockdown of FUT8 significantly suppressed cell viability in U251-MG cells, whereas overexpression of FUT8 promoted cell viability (**Figure 3A**). Furthermore, transwell assays were used to study the effects of FUT8 on migration and invasion. We found that knockdown of FUT8 significantly decreased FBS-induced migration and invasion of U251-MG cells, while overexpression of FUT8 significantly enhanced migration and invasion of Ln18 cells (**Figure 3B** and **3C**).

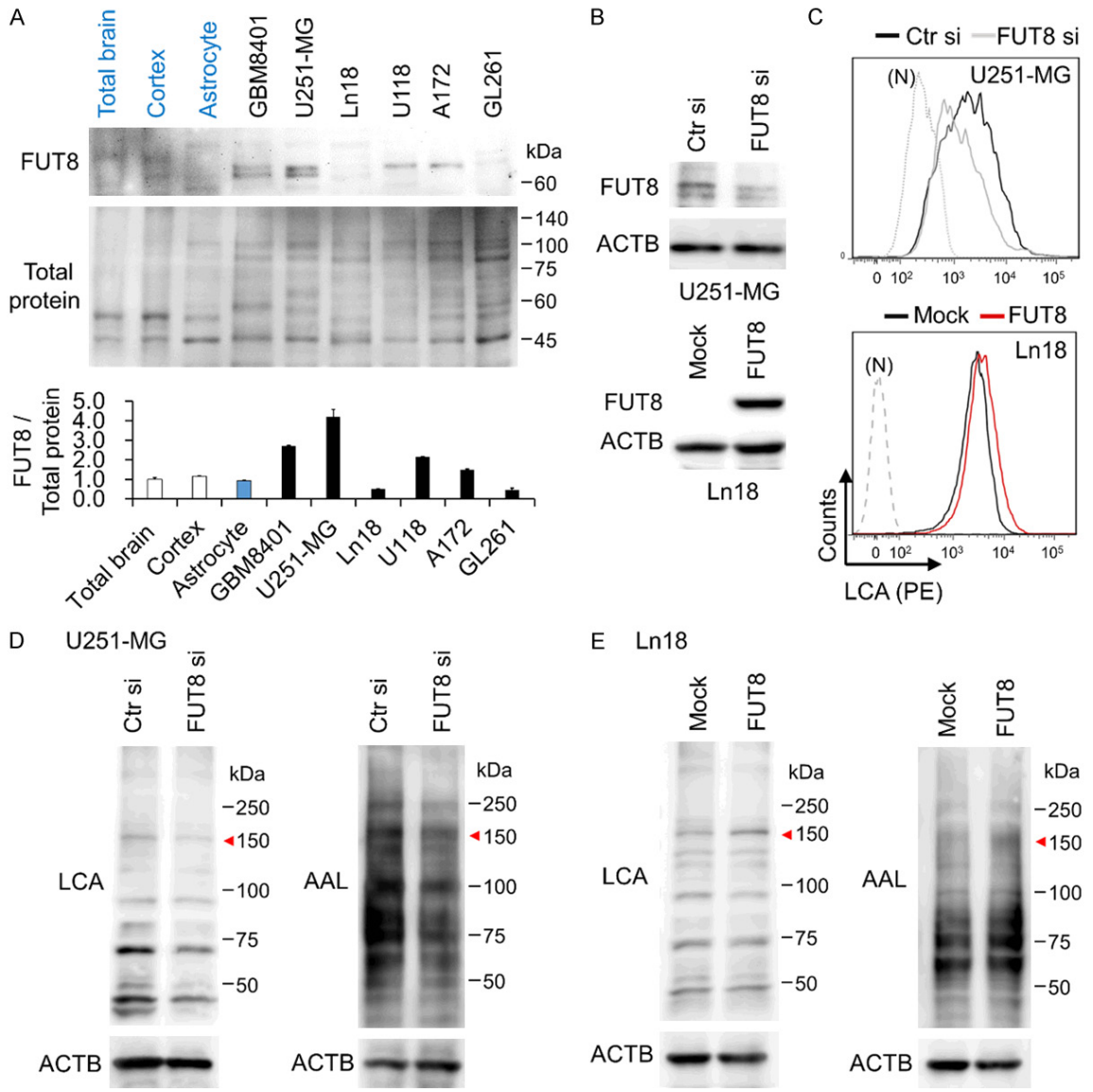

Figure 2. FUT8 modulates protein fucosylation on glioma cells. (A) Expression of FUT8 in glioma cell lines, human astrocyte, and normal brain tissue. The protein expression was analyzed by western blotting. Total loading protein is shown at bottom. Relative expression levels to total brain tissue form three independent blots are shown. (B) siRNA knockdown of FUT8 in U251-MG cells (upper), and stable overexpression of FUT8 in Ln18 cells (lower). The expression of FTU8 was analyzed by western blots. ACTB was used as loading control. Control-siRNA (Ctr si); FUT8-specific siRNA (FUT8 si). (C) Effects of FUT8 on cell surfaces fucosylation. Surface protein fucosylation were analyzed by flow cytometry with PE-LCA. Negative (N) refers to cells without addition of PE-LCA. The changes in fucosylation on glycoproteins were revealed by western blotting with biotinylated LCA and AAL on U251-MG cell lysate (D) and Ln18 cell lysate (E). Proteins with evident changes at 150 kDa of all tested cell lines are indicated by red arrowheads.

FUT8 mediates activity of multiple receptor tyrosine kinases in glioma cells

Activation of receptor tyrosine kinase (RTK) signaling is the most common alteration in human glioma, and several studies demonstrated that core fucosylation modulates RTK activities [24, 25]. Thus, we used phospho-RTK antibody arrays after 10 minutes FBS stimulation to examine which RTKs were regulated by FUT8 in cultured GBM cells. Interestingly, our data indicated that the phosphorylation of MET, EPHA1, and RYK was decreased in FUT8-silenced U251-MG cells. In contrast, overexpression of FUT8 in Ln18 cells enhanced phosphorylation of several RTKs, such as

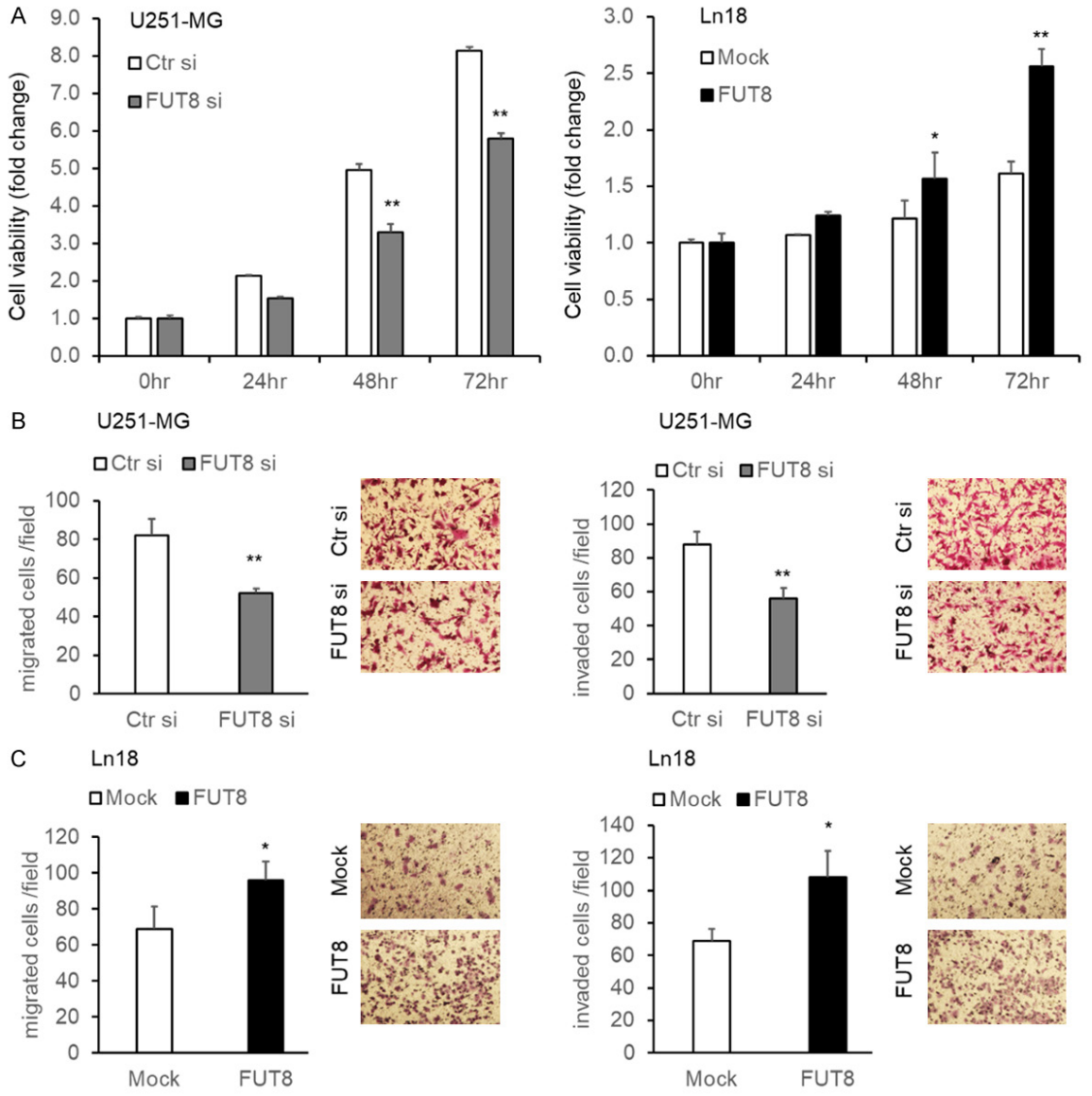

Figure 3. FUT8 regulates malignant phenotypes in glioma cells. A. FUT8 modulated cell viability *in vitro*. The cell viability of U251-MG and Ln18 cells was determined using a CCK-8 assay at the indicted time-points. Data represent means ± SD from three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. B and C. Effects of FUT8 on transwell cell migration (left), and matrigel invasion (right). Representative images are shown. All results are represented as means ± SD from three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$.

ErbB2, FGFR1, FGFR2, AXL, and MET (**Figure 4A**). To investigate FUT8-mediated fucosylation on RTKs, we examined LCA pulldown protein with MET and EGFR immunoblotting in FUT8 knockdowned or overexpressed glioma cells. We found that knockdown of FUT8 decreased LCA binding to MET and EGFR in U251-MG cells; whereas, overexpression of FUT8 increased LCA binding to MET and EGFR in Ln18 cells (**Figure 4C**). Although fucosylation of MET and EGFR were both modified by FUT8, the phosphor-RTK array assay indicated that the alternation of MET is common in U251-MG and Ln18 cell lines. To analyze MET-induced signaling, FUT8 silenced or overexpressed glioma cells were stimulated with HGF. Our data revealed that knockdown of FUT8 inhibited HGF-induced phosphorylation of MET at Y1234/5 and suppressed phosphorylation of ERK in U251-MG cells (**Figure 4C**, left). In

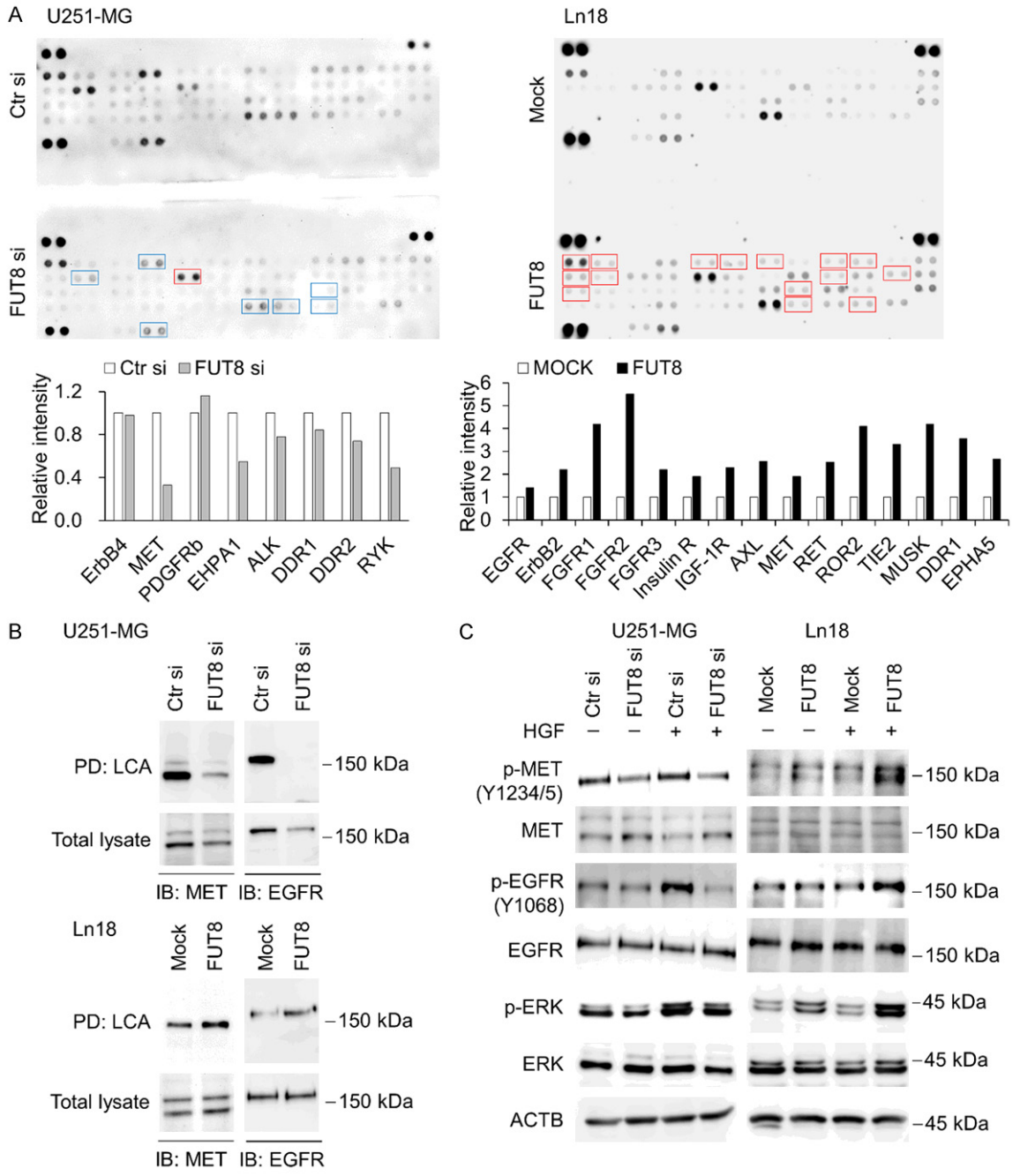

Figure 4. FUT8 regulates activation and fucosylation of RTKs in glioma cells. A. Human phospho-RTK (p-RTK) array showing the tyrosine phosphorylation of RTKs in glioma cells. Cells were starved for 3 h and then stimulated with FBS (10%) for 15 min. Cell lysates of control siRNA (Ctr si) and FUT8-silenced (FUT8 si) U251-MG cells (left), and mock and FUT8-overexpressed Ln18 cells (right) were applied to p-RTK arrays including 39 human RTKs. B. Fucosylaton of MET and EGFR were modified by FUT8 in glioma cells. Cell lysates were pulled down by LCA agarose beads. The pulled down glycoproteins were immunoblotted (IB) with anti-MET antibody or anti-EGFR antibody. C. FUT8 modulated HGF-induced signaling in glioma cells. U251-MG and Ln18 transfectants were starved for 3 h and then treated with (+)/without (-) HGF (20 ng/mL) for 10 min. Cell lysates (30 μg) were analyzed by western blotting with various antibodies, as indicated.

contrast, overexpression of FUT8 enhanced HGF-induced activation of MET and increased p-ERK levels in Ln18 cells (**Figure 4C**, right). In addition, we found that the trans-activation of

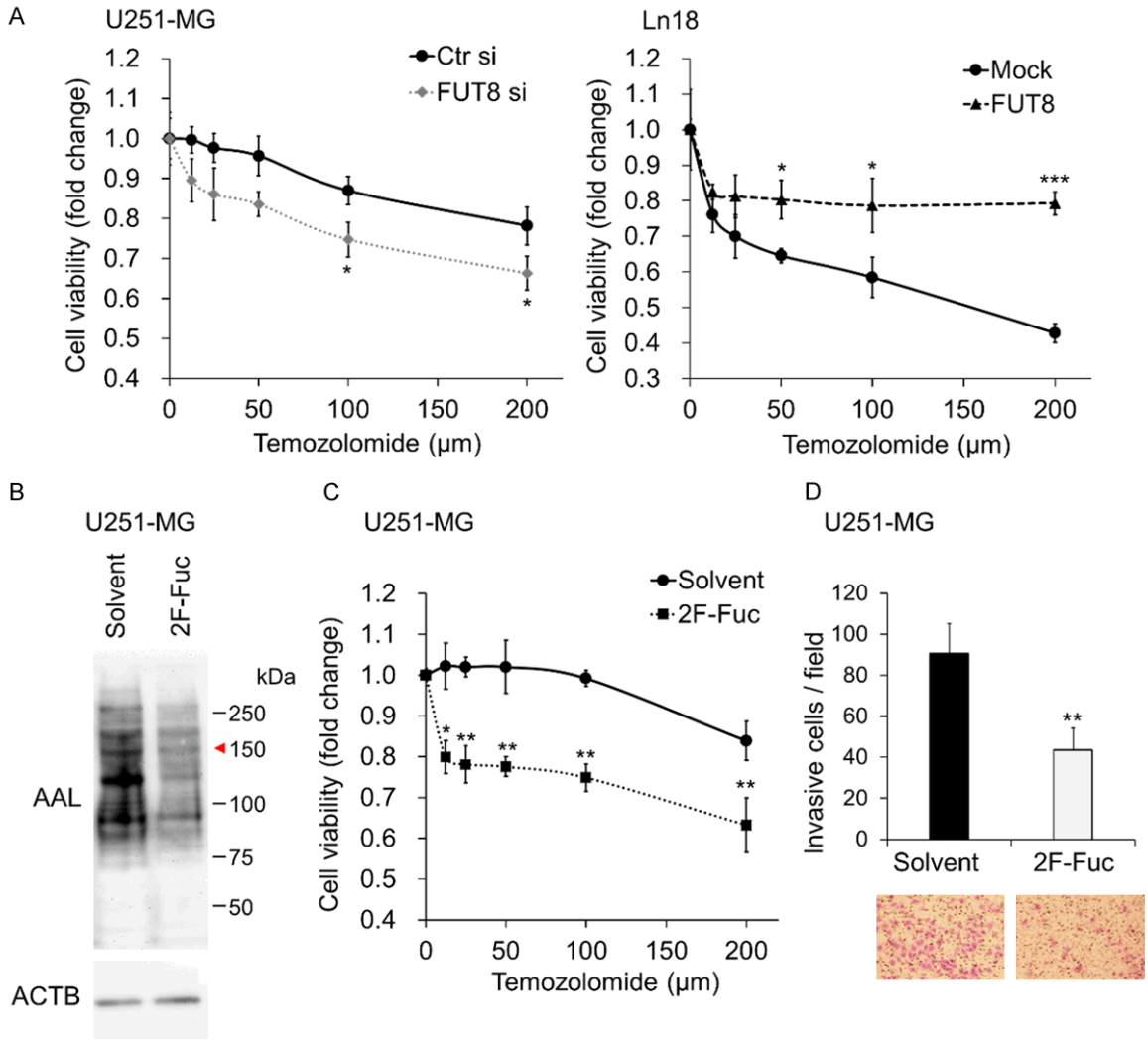

Figure 5. Fucosylation of glioma cell regulates TMZ sensitivity and cell invasiveness. A. U251-MG and Ln18 transfectants were treated with TMZ at indicated concentrations for 24 h. Cell viability was measured using a CCK8 assay. B. LCA blotting of U251-MG cells treated with solvent (methanol) or 2F-Fuc (200 μM) for 5 days. ACTB was taken as loading control. C. U251-MG cells were pretreated with solvent (methanol) or 2F-Fuc (200 μM) for 5 days and applied to TMZ sensitivity test. Cell viability was measured using a CCK8 assay. D. The solvent or 2F-Fuc treated cells were applied to cell invasion assay. All results are represented as means ± SD from three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

EGFR also be affected by FUT8 expression. These results suggest that FUT8 significantly activates the HGF/MET signaling pathway.

Fucosylation modulates sensitivity of glioma cells to temozolomide treatment

Temozolomide (TMZ) is the first-choice chemotherapeutic agent in GBM, while activation and RTK is demonstrated to contribute to TMZ resistance [7, 26, 27]. We treated glioma cells with TMZ to evaluate the effects of the FUT8-regulated activation of RTKs on TMZ. We found that knockdown of FUT8 in U251-MG cells enhanced cellular sensitivity to TMZ treatment. In contrast, overexpression of FUT8 in Ln18 cells significantly enhanced resistance to TMZ (**Figure 5A**). The fucose analogue 2F-peracetyl-fucose (2F-Fuc) is an inhibitor for suppressing the formation of endogenous fucosylated glycans [28]. Since the FUT8 is one of main fucosyltransferase in GBM tissue (**Figure 1A**), we examined the effects of 2F-Fuc (200 μM) on U251-MG cells, which the FUT8 and AAL blotting signal were high. Results indicated that the protein fucosylation is obviously decreased

(**Figure 5B**), while the 2F-Fuc treatment did not have significant effects on cell viability in our culture condition, comparing to that in solvent control (data not shown). Importantly, we found the 2F-Fuc treated cells were more sensitive to the TMZ administration (**Figure 5C**). Furthermore, 2F-Fuc treatment also suppressed U251-MG cells transwell invasion (**Figure 5D**).

Discussion

This is the first study demonstrated functions of FUT8 in regulating glioma malignancy. Our data showed that FUT8 is frequently upregulated in human glioma, especially in GBM tissue, and the upregulation of FUT8 in glioma tissue is associated with advance tumor grade and poor overall survival. We proposed that FUT8 is one of major fucosyltransferase which modulates protein fucosylation in glioma cells. Knockdown of FUT8 by siRNA suppresses malignant phenotypes, whereas overexpression of FUT8 enhances glioma cell malignancy. Mechanically, we found FUT8 modulates multiple RTKs activation and their downstream signaling. In addition, lectin pull-down assays revealed that fucosyltion of two crucial growth factor receptors, MET and EGFR, were regulated by FUT8 in glioma cells. Furthermore, manipulating FUT8 levels have significant impacts on cellular sensitivity to TMZ treatment. Importantly, using the fucosylation inhibitor 2F-Fuc successfully suppressed fucosylation of glioma cells and enhanced the toxicity of TMZ to GBM cells. These findings provide novel insights into the biological functions of FUT8 in the pathogenesis of glioma, and suggesting fucosylation inhibitors have potential to overcome cellular resistance to TMZ treatment.

Analyzing gene expression of *FUT8* in Oncomine Platform (https://www.oncomine.org) and GEPIA 2 website (http://gepia2.cancer-pku.cn/#index), several independent datasets indicated that *FUT8* was upregulated in glioma tissue comparing to normal brain tissue (data not shown). In consistent with these results, our IHC of FUT8 on tumor adjacent normal brain tissue revealed the FUT8 protein was observed in only 23% of cell, while higher percentage of FUT8 positive cells (43%) was observed in the glioma tissue. In addition to the percentage of FUT8 positive cells, we also observed that the staining intensity of FUT8 is obviously higher in a subset of GBM tissue. Although we need more GBM cases to analyze the correlation between FUT8 intensity and clinical outcomes, the high expression of *FUT8* gene was found in recurrent glioma tissue (**Figure 1B**) and associated with short overall survival and disease free survival (**Figure 1E**). All of these evidence indicated that upregulation of FUT8 can be a poor prognosis indicator for human glioma.

N-glycans on protein supports peptide folding and transportation to plasma membrane and secretion. FUT8 responsible for catalyzing core fucosylation (GDP-fucose linked to the innermost N-acetylglucosamine) on N-glycans. Prediction of N-glycosylation sites using NetNGlyc 1.0 indicates that all human RTKs have one to few N-glycosylation sites, suggesting all these RTKs could be modified by FUT8 [29, 30]. As expectation, our LCA and AAL blotting on Ln18 and U251 cell lysate revealed many proteins were modified by FUT8 (**Figure 2D**). The protein bands around 140 kDa to 220 kDa have the most significant changes, which were the size of ErbB family and MET. Previous studies showed that core fucosylation enhanced EGFR dimerization and activation in lung cnacer cells [31], and knockout of FUT8 impaired both MET and EGFR signaling [32, 33]. In agree with these studies, our LCA pulldown assay demonstrated both MET and EGFR were modified by FUT8, and FUT8 regulates the HGF induced MET activation and also modulates transactivation of EGFR after HGF stimulation. Thus, it is highly possible that core fucosylation is a crucial regulator for transactivation within RTKs, and it also imply manipulating FUT8 expression can modulate multiple RTK signaling in cancer cells.

Our data indicated that FUT8 significantly modulates glioma cell growth and mobility. The impact of FUT8 expression on tumor cell aggressive phenotypes has been described in many cancers, for example, in papillary carcinoma, higher FUT8 levels are significantly correlated with tumor size and lymph node metastasis [34] and in breast cancer, FUT8 promotes cell invasiveness by modification of TGF-β receptor core fucosylation and inhibition of FUT8 suppresses metastasis of the breast cancer cells to lung [35]. Core fucosylation is also involved in the immunomodulation of cancer microenvironment. T cell receptor complex

are highly fucosylated glycoprotein and loss of FUT8 expression has been confirmed to significantly reduce $CD4^+$ T cell activation [36]. Recently, expression of FUT8 have been noticed to regulate the PD-1 expression on T cells, leading to a novel thought for improving anti-tumor immunotherapy [37]. Although we found that FUT8 modulates activities of RTKs in glioma cells, we reasonably supposed that other signal pathways may also contribute to the FUT8-mediated malignant phenotypes in human glioma.

This study provides novel insights into the role of core fucosylation in modulating RTK activities in glioma cells, and consequently enhances cancer cell resistance to TMZ treatment. Importantly, we proposed for that first time that inhibits fucosylation by 2F-Fuc on glioma cell sensitivity toward TMZ treatment. Thus, targeting FUT8 or using fucosylation inhibitors could have synergetic effects with TMZ therapy, and similar to those drugs targeting multiple RTKs. This study opens up avenues for treating cancers by targeting not only the receptors themselves but also their fucosylation regulators.

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Informed consent was obtained from all subjects involved in the study.

Disclosure of conflict of interest

None.

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Table S1. Information of glioma cell lines

Name	Collection Number	Domain	Source
Ln18	ATCC, CRL-2610	Cell Line	Homo sapiens (human) brain; glioblastoma
U251-MG	Kerafast, Inc. Number: EDK001	Cell Line	Homo sapiens (human) brain; glioblastoma
A172	ATCC, CRL-1620	Cell Line	Homo sapiens (human) brain; glioblastoma
U118 (U-118 MG)	ATCC, HTB-15	Cell Line	Homo sapiens (human) brain; glioblastoma
GBM8401	Obtained from Dr. Wei-Hwa Lee, Tri-service General Hospital, Taipei, Taiwan. BCRC Number: 60163	Cell Line	Homo sapiens (human) brain; glioblastoma
GL261	Leibniz Institute DSMZ, Number: ACC802	Cell Line	Mus musculus (Mouse) Breed/subspecies: C57BL/6. Mouse glioblastoma