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Original Article

Identification of Family with Sequence Similarity 110 Member C (FAM110C) as a Candidate Diagnostic and Prognostic Biomarker for Glioma

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Abstract

Background: Gliomas are the most frequent and dangerous primary cerebral tumors. Therefore, there is a need to develop molecular targets for the diagnosis and treatment for glioma.

Methods: In September 2020, we retrieved the expression matrix of glioblastoma (GBM) sufferers and pertinent clinical data from the TCGA (The Cancer Genome Atlas) database. Prognostic differences between various families with sequence similarity 110 member C (FAM110C) expression groups were assessed by Kaplan-Meier with log-rank test. The R platform get used to assess the accuracy of FAM110C delivery in predicting the prognosis of PDAC using a time-dependent receptor operating characteristic (ROC) curve. The delivery level of FAM110C was determined by qRT-PCR and western blot. Gene set enrichment investigated possible mechanisms between different FAM110C expression groups in GBM (GSEA). The impact of FAM110C on glioma cell movement was discovered using migration test. The drug's gene-targeting impact was validated by the CCK8 test.

Results: A total of 173 GBM samples were obtained from the TCGA database, with 148 including information on IDH1 mutations and 151 containing information on overall survival. The mRNA expression level of FAM110C was greater in wild-type GBM, according to qRT-PCR data. The connection between FAM110C expression and Hallmark, GO, and KEGG pathway gene sets was investigated using GSEA software. We used migration test to assess the impact of FAM110C on glioma motility in order to confirm the findings of the GSEA analysis.

Conclusion: FAM110C might get used as a possible diagnostic and prognostic biomarker for wild-type GBM, and its inhibition could be used to prevention and treatment wild-type GBM.

Keywords: Glioma; Biomarkers; Molecular targets; Prognosis

Introduction

Gliomas are frequent and serious primary cerebral tumors (1). Despite breakthroughs in surgical methods, radiotherapy, and chemotherapy, for sufferers with gliomas remains dismal, particularly those with glioblastoma multiforme (GBM), the WHO grade IV of gliomas. The median survival time duration for GBM patients is 12-15 months (2). Reinventing itself, invasiveness, production vessel, high death rate, and recurrence are all biological hallmarks of glioma (3,4). Using a combination based on gene expression and histological categorization is more accurate in predicting treatment effect (5). The World Astrocytoma and glioblastoma may be categorized into IDH-mutant and wild-type groups, according to the WHO 2016 entities (6). Furthermore, 1p/19q codeletion has emerged as a key marker for oligodendroglioma, which must be diagnosed using both IDH-mutant and 1p/19q-codeleted cells (7). Because gliomas have unique molecular properties, there is a need to develop molecular targets for the diagnosis and treatment of glioma. Cancer biomarkers can characterize the presence or extent of cancer in the body (8). There was few study described the identification of GBM biomarkers and was verified and effectively used in clinical practice (9). Furthermore, most biomarkers have limitations in the early detection of GBM, and their prognostic utility is hampered by errors. The deficiencies of biomarkers are more likely to be overcome using bioinformatics analysis (10). Researchers may use bioinformatics analysis to dive into combined data from several clinical specimen s from various independent research, providing a data base installation for finding potential biomarkers and upgrading our knowledge of malignancies (11-13).

In this study, we used bioinformatics methods to analyze the GBM candidate biomarker and explored the role of this marker in the diagnosis and prognosis of GBM. The relationship between this marker and GBM proliferation and migration was investigated by biological experiments. Then CMAP was used to screen targeted drugs for markers, and rescue experiments were designed to analyze the effects of targeted drugs.

Methods

Data acquisition

Through the collecting of cancer-related omics data, TCGA offers a huge, free reference library for cancer study, which is publically accessible through the TCGA Data Portal (https:/cancergenome.nih.gov). In September 2020, we retrieved the deliverance matrix of GBM sufferers as well as pertinent clinical data from the TCGA database.

Survival analysis of FAM110C

The median expression value of FAM110C was used to divide the different expression groups in this investigation. The Kaplan-Meier with log-rank examination was used to evaluate prognostic differences between various FAM110C expression groups. The survival package in the R terrace get used to asscss the accuracy of FAM110C expression in predicting the prognosis of PDAC usage a time-dependent receptor operational characteristic (ROC) curve.

Tumor cell lines

U87 and Ln229 belong to the human glioma cell lines obtained from the American type culture collection (ATCC). Glioma cells were cultured in Dulbecco's modified medium containing 10% fetal bovine serum (Corning, Corning, NY). The medium was supplemented with penicillin at a concentration of 100 U/mL and streptomycin at 100 g/mL. Keep the cells at 37 °C in a atmosphere of 5% CO2.

Establishment of stable cell lines and lentivirus production

Ln229 and U87 are mutant or wild-type infecting IDH1-R132H virus. IDH1-R132H lentivirus of mutant and wild-type were obtained from Shanghai Gene Chemical (Shanghai, China).The virus-

containing culture medium was introduced to the cells. We removed the supernatant after 48 hours of infection and replaced it with new culture medium. Puromycin should be used to filter cells. GFP fluorescence and Western blot analysis were used to demonstrate IDH1-R132H expression.

Cell culture and transfection

The medium was supplemented with fetal bovine serum from Gibco Invitrogen at a concentration of 10% or 20%. (Paisley, UK). In this investigation, we employed a small interfering RNA (siRNA) targeting FAM110C produced and synthesized by Guangzhou Ruibo Biological Co., Ltd. The siRNA sequence acquired in the trials is detailed in Annex. As a negative control, non-targeting siRNA (si-NC) was employed. Thermo Fisher's Lipofectamine® 2000 transfection reagent (Cat. #11668019) was used for siRNA transfection according to practical guidelines. We conducted in vitro experiments after 24 hours. To overexpress FAM110C, the previously constructed plasmid pcDNA3.1- FAM110C was used. Following transfection, all cells were treated with G418 at a dose of 500 g/ml manufactured by Santa Cruz Biotechnology (Cat.#sc-29,065) for 4 weeks to obtain stable and consistent cell lines. As a negative control data source, we used an empty vector.

RNA extraction and qRT-PCR

Total RNA from cell lines was extracted using TRIzol (Biyuntian, China) according to the manufacturer's instructions. Transcription was performed using a reverse transcriptase kit (Biyuntian, China) using 1 μg of total RNA as template. Then, the cDNA samples were amplified by qRT-PCR with GAPDH as the reference sample. Each sample was detected 3 times, and the relative expression level was determined by 2-ΔΔCT technique.

Western blot

Thirty μg of protein was collected for SDS-PAGE and then transferred to PVDF membrane (Biyuntian, China). It was then blocked with 5% nonfat milk and immunoblotted with primary antibody overnight. The membranes were then incubated with anti-rabbit IgG antibody (Biyuntian; China). Determined by ECL (Milibo, USA).

Cell proliferation assay

The Cell Counting Kit-8 (CCK-8) test get used to determine cell viability. Cells were extracted and counted in a quick manner. In 96-well plates, we implanted the appropriate amount of tumor cells. Cells were treated with varying amounts of TMZ or DMSO. After a specific time, we injected the CCK-8 solution into the wells. In the microplate reader, glioma cells were found at 450 nm of absorbance.

Migration assay

Migration experiments were performed using transwell chamber (Corning Costar, USA) under the instructions. Treated glioma cells were added to the top compartment of DMEM and DMEM with 30% FBS in the bottom compartment. Cells were fixed with 4% paraformaldehyde and stained with crystal violet (Biyuntian, China). Olympus microscope observations.

GBM genome-wide dataset and FAM110C gene set enrichment analysis

Gene set enrichment analysis (GSEA) was performed using the c2 and c5 molecular signature database gene sets as a reference to further investigate the possible mechanisms between the FAM110C expression groups in GBM.

Statistical analysis

SPSS 23.0 (IBM Corp., Armonk, NY, USA) or Prism 7 were used for statistical analysis (Inc., USA). The results were reported as the mean SD of three separate studies. Student's t-test or ANOVA were used to contrast two or three groups, respectively. For FAM110C in GBM, the Connectivity Map (CMap) was utilized to find prospective small molecule targeted therapies (14, 15).

Results

FAM110C is highly expressed in wild-type GBM and is correlative with poor prognosis

Expression data of 173 GBM samples were retrieved using the TCGA database, of which 148 samples had IDH1 mutation information (7 mutant, 141 wild-type) and 151 samples had overall survival information. According to differential expression analysis, FAM110C was strongly elevated in wild-type GBM patients (P = 0.0053, Fig. 1A), The greater the expression of FAM110C, the worse the prognosis of patients with wild-type GBM (P =0.03, HR=1.55 [95 percent CI 1.04,2.31], Fig. 1B). Furthermore, we discovered that FAM110C expression could correctly predict wild-type GBM patients' survival at 1, 3, and 5 years (ROC-1 year:0.647; ROC-3 years:0.709; Roc-5 years:0.932, Fig. 1C).

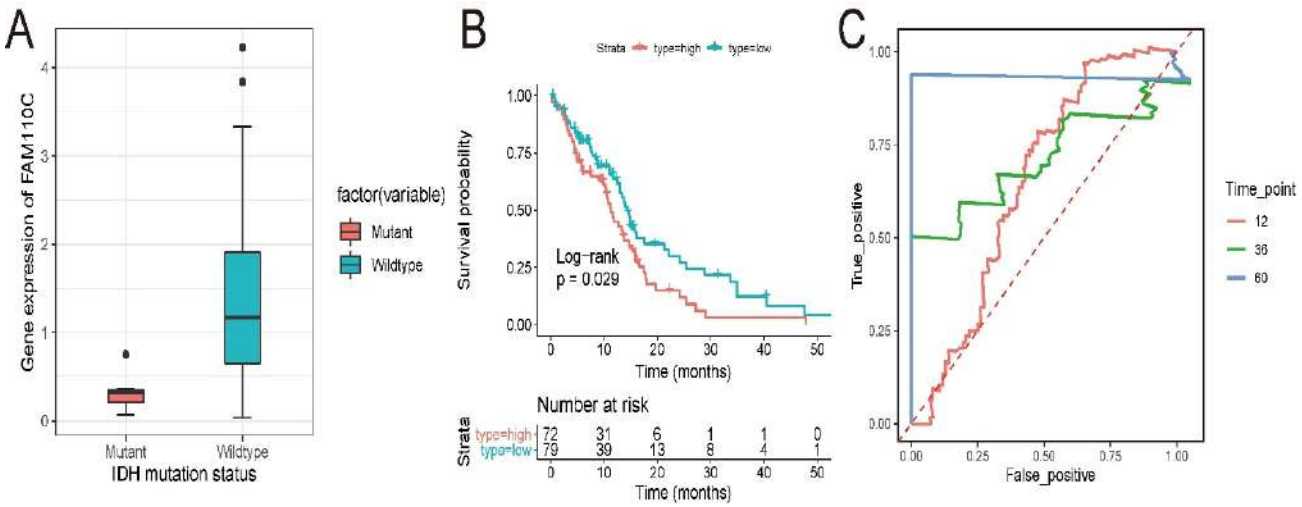


Fig. 1: FAM110C as a good biomarker for the diagnosis and prognosis of wild-type GBM patients in TCGA dataset. (A). FAM110C was highly expressed in patients of wild-type GBM. (B). Survival analysis demonstrated that the higher the expression of FAM110C, the worse the prognosis of patients. (C). ROC curves showed that expression of FAM110C could also accurately predict the survival rate of wild-type GBM patients

FAM110C is a risk biomarker for GBM progression

GBM data on the CGGA website (http://www.cgga.org.cn/analyse/RNA-data-expression-distribution-result.jsp) revealed that FAM110C expression increased with grade (P = 6.7E 05, Fig. 2A) and was very significantly expressed in IDH1 wild type (P = 1.5E 19, Fig. 2B). According to multivariate analysis (Fig. 2C), FAM110C was also strongly expressed in the wild type in the WHO ii, WHO iii, and WHO iv.

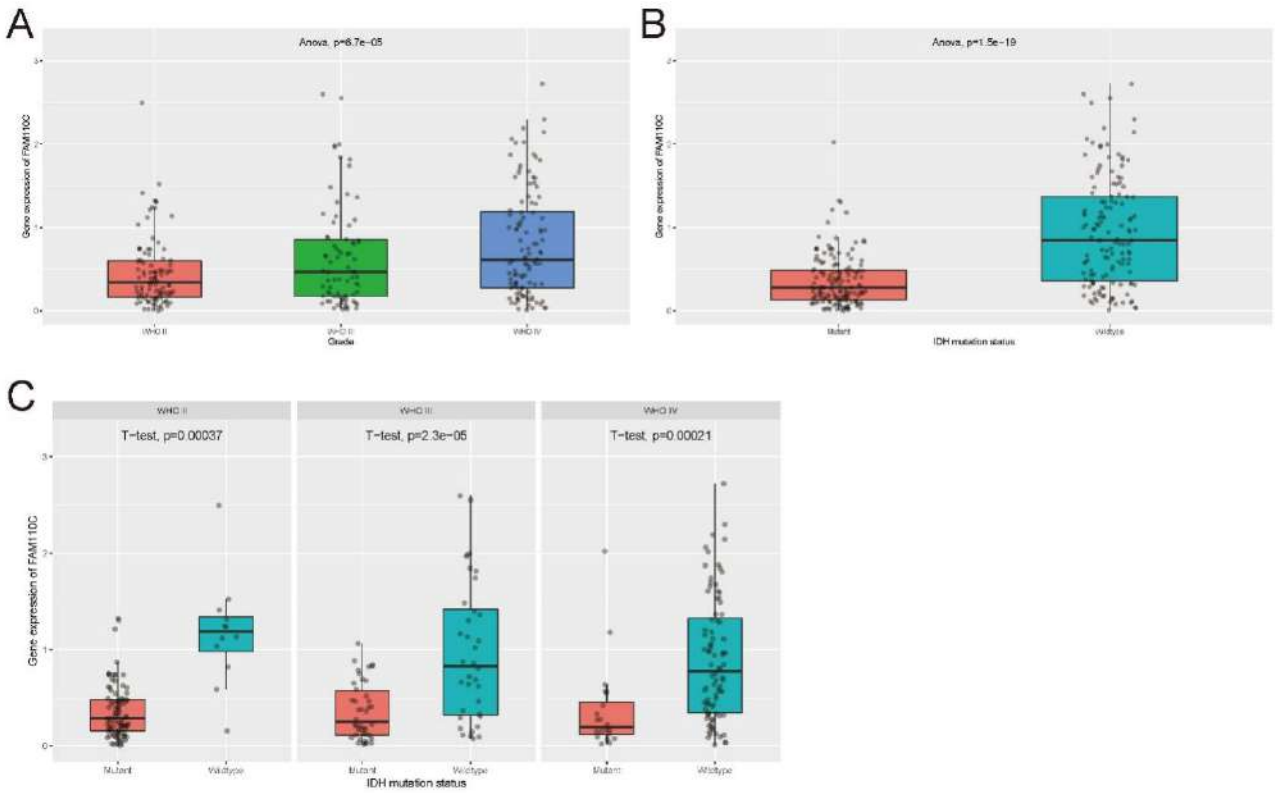


Fig. 2: FAM110C as a risk biomarker for GBM progression. (A-B). Data of GBM in CGGA website showed the expression level of FAM110C increased with the increase of grade and was also highly expressed in IDH1 wild type. (C). multivariate analysis showed that FAM110C was highly expressed in wild type in WHO ii, WHO iii and WHO iv

FAM110C was highly expressed in wild-type GBM cell lines

Ln229 and U87 cell lines expressing IDH1-R132H were created to corroborate the findings of bioinformation analysis. Western blot verified IDH1-R132H expression (Fig. 3A). According to QRT-PCR data (Figure 3B), the mRNA expression level of FAM110C was greater in wild-type GBMs. The expression of FAM110C was also validated using a western blot (Fig. 3C).

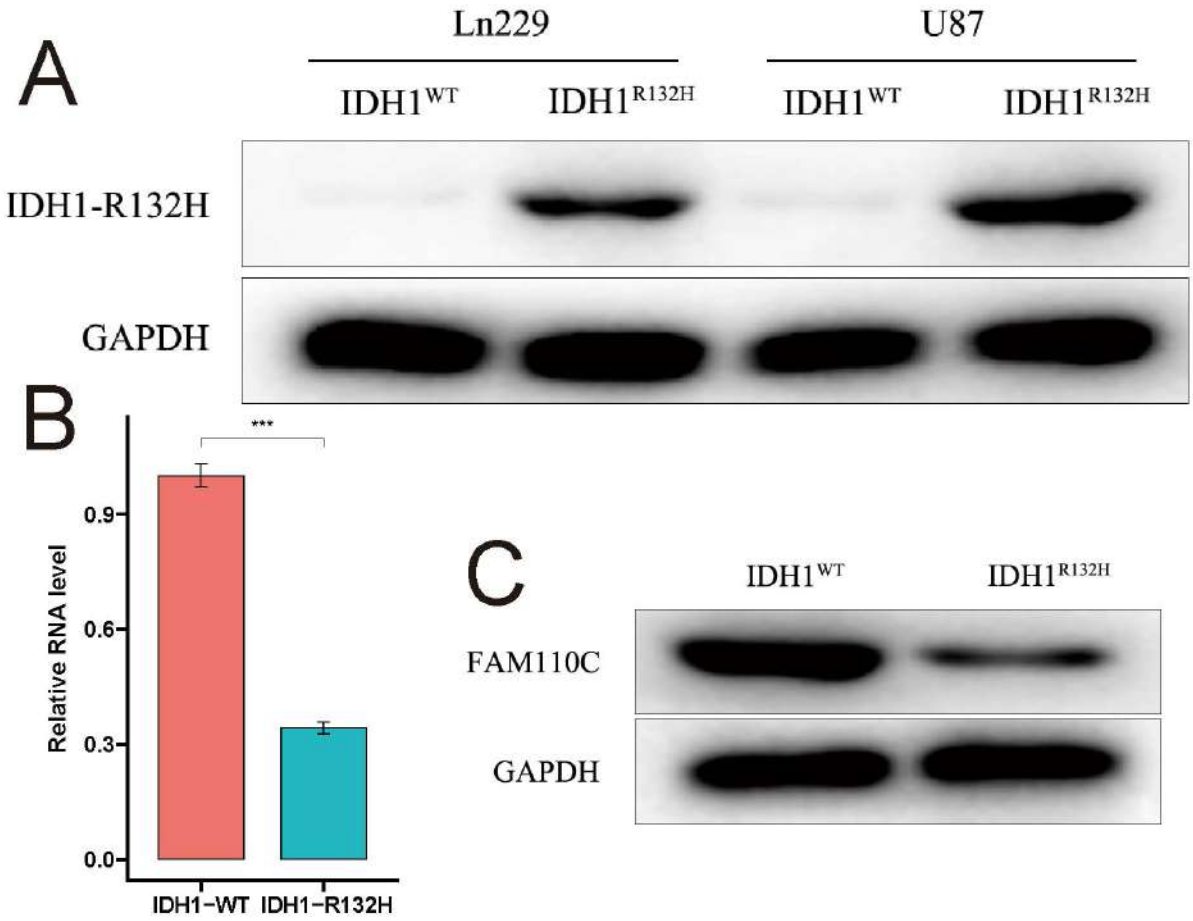


Fig. 3: FAM110C was highly expressed in wild-type GBM cell lines(A). IDH1-R132H expression was detected by western blot. (B) RT-PCR results revealed that the mRNA expression level of FAM110C was higher in wild-type GBM. (C). Western blot showed FAM110C expression was higher in wild-type GBM. ***$P < 0.001$

FAM110C promoted glioma cell migration and invasion

FAM110C was knocked down in Ln229 and U87 cell lines wide-type GBM cell lines to investigate the impact of FAM110C on wild-type GBM cell growth. FAM110C was effectively suppressed (Fig. 4A), which inhibited the development of wild-type GBM cells (Fig. 4B). The relationship between FAM110C expression and Hallmark, GO and KEGG pathway genomes was examined using GSEA software. The interaction of cytokines with their receptors, chemokine signaling pathways, and cell adhesion molecules were all considerably enriched (Fig. 4C-E). We used a migration assay to assess the impact of FAM110C on glioma motility to confirm the results of the GSEA analysis. FAM110C knockdown dramatically reduced the migration of Ln229 and U87 glioma cells in migration experiments (Fig. 4F-H).

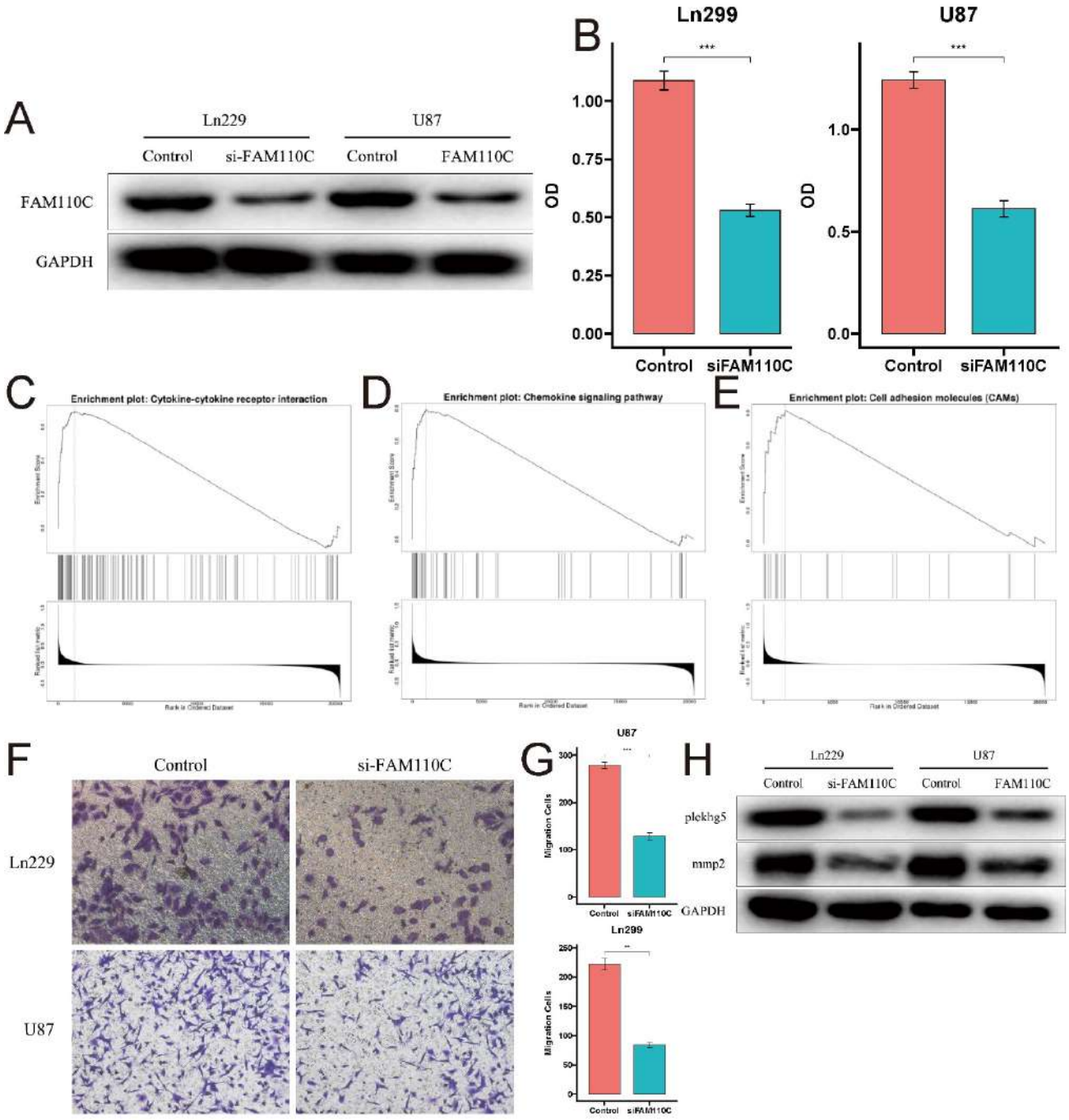


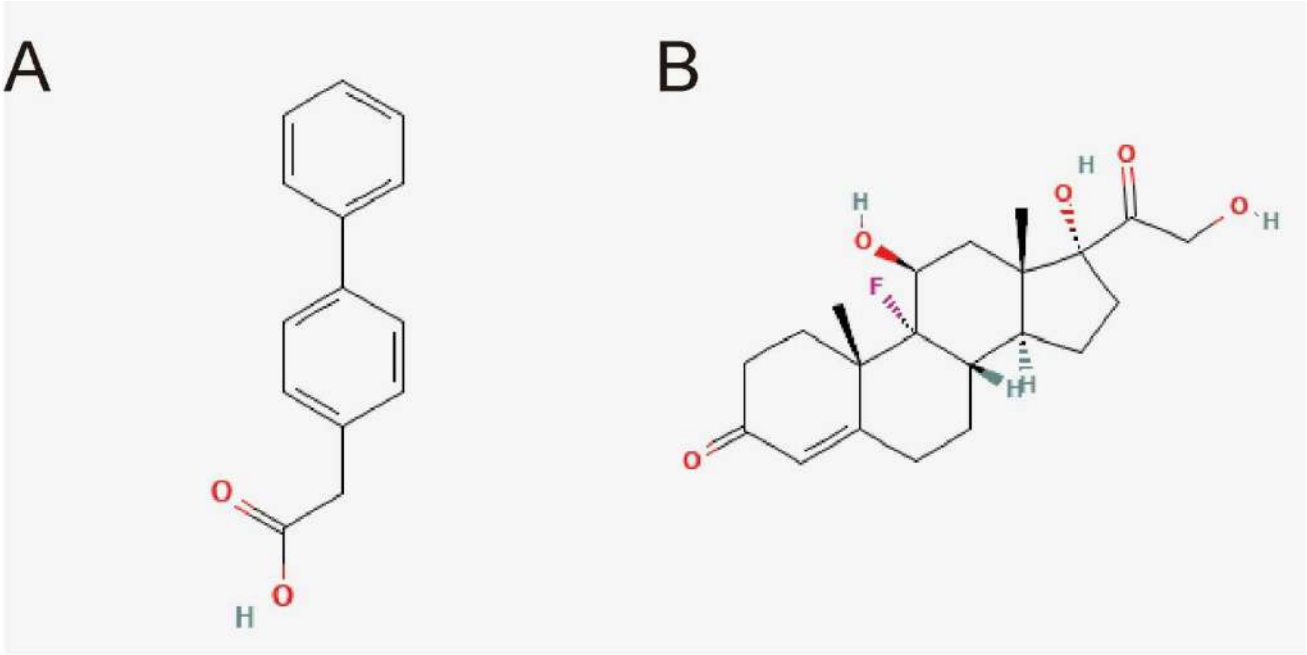
Fig. 4: FAM110C promoted glioma cell migration and invasion

(A) In Ln229 and U87 cells, FAM110C was effectively suppressed. (B) Knocking down FAM110C inhibited the development of Ln229 and U87 cells. (C-E) GSEA analysis revealed considerable enrichment in cell. (F-H) A migration experiment demonstrated that knocking down FAM110C greatly reduced the migration of Ln229 and U87 cells. $^{**}P < 0.01$, $^{***}P < 0.001$

Identification of FAM110C targeted drugs

We employed a CMap analysis to find two small molecule medicines that might be used as possible targeted therapy drugs for FAM110C in wild-type GBM, providing a new approach for clinical treatment. Fig. 5A and B demonstrate the molec-

ular chemical structures of the two drugs. Felibinac (Mean connective score = -0.554; *P*=0.001; Fig. 5C) and fludrocortisone (Mean connective score = -0.561; *P*=0.001; Fig. 5C) are the two drugs.



C

Cmap name	Mean	N	Enrichment	P	Specificity	%Non-null
Felbinac	-0.554	4	-0.849	0.00095	0.0142	100
Fludrocortisone	-0.488	8	-0.74	0.00012	0.007	87

Fig. 5: Identification of FAM110C targeted drugs

(A-B) CMap analysis showed that the molecular chemical structures of the two drugs. (C) The target drugs are felbinac and fludrocortisone

Targeted drugs inhibit GBM proliferation by reversing FAM110C

In two GBM cell lines, the impacts of felbinac and fludrocortisone on cell proliferation were investigated (Fig. 6A, B). Felibinac and fludrocortisone decreased cell proliferation in cell lines in a manner of dose-dependent, with IC50 values ranging from 4.48 mmol/L to 17.37 mmol/L for TH588, and from 0.31 mmol/L to 16.26 mmol/L for TH1579, respectively (Fig. 6C). The drug's gene-targeting impact was validated by the CCK8 test (Fig. 6D).

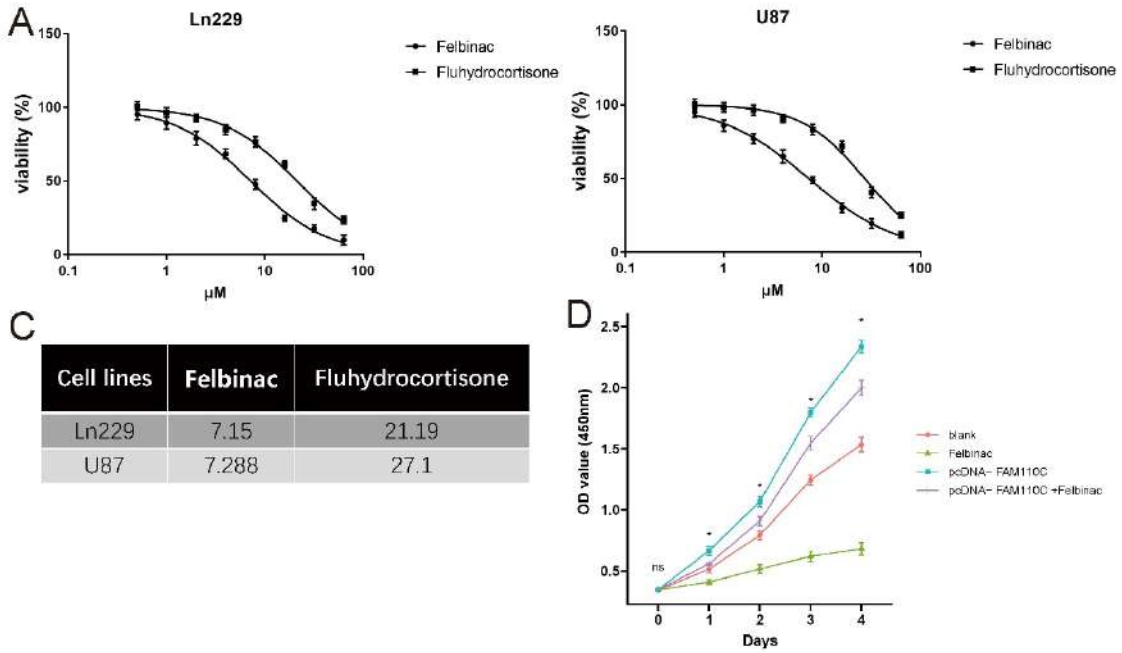


Fig. 6: Targeted drugs inhibit GBM proliferation by reversing FAM110C

(A). The effects of felbinac and fludrocortisone were assessed on cell growth in Ln229 and U87 cells. (B). Inhibits cell growth in a dose-dependent manner. (C) The CCK8 assay confirmed FAM110C targeting effect of the drug. *$P < 0.05$,n.s means no statistical significance

Discussion

The most universal and dangerous primary malignant brain tumor in adults is glioblastoma (GBM) (16). GBM is a molecularly heterogeneous disorder with many subcategories. Much effort has been devoted to this characterization in the field, and the development of drugs targeting genetic abnormalities in each subtypes is now ready (17-19). The efficacy of mTOR pathway inhibition in subependymal giant-cell astrocytoma and the prospect of finding a subtype of GBM susceptible to bevacizumab up-front therapy are two examples, but we need more (20,21).

On the other hand, real-time noninvasive sampling of brain tumor activity is still required to better inform disease prognosis and monitor treatment response (22-24). Imaging and tumor tissue data are now used to diagnose GBM; Nevertheless, there are certain obstacles and limits (25,26). Conventional MRI may help guide surgery, but it cannot tell the differentiate high-grade gliomas and can provide confusing imaging results (27).

Potential oncogenes with diagnostic and prognostic relevance were the focus of this investigation. TCGA data showed that FAM110C was upregulated in GBM and was associated with poor prognosis. . In addition, FAM110C is a useful biomarker for determining the diagnosis and prognosis of individuals with wild-type GBM. Expression of this gene was also confirmed in GBM cell lines. To learn more about FAM110C's significance in GBM, we investigated its biological properties in GBM cells, and we discovered that it encouraged glioma cell motility and invasion. We employed CMAP to create a novel targeted medication for FAM110C, which might be exploited as a possible target for GBM therapy.

Conclusion

The new biomarker FAM110C of GBM was evaluated by bioinformatics analysis, which brings new concepts to the diagnosis and prog-

nosis of GBM. FAM110C contributes to the growth and migration of GBM with IDH1 mutation. For FAM110C, we used CMAP technology to test the targeted drug of FAM110C. The drug played a role in GBM inhibition by reversing the effect of FAM110C, indicating that it may be used as a therapeutic target for GBM therapy.

Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Ostrom QT, Bauchet L, Davis FG, et al (2014). The epidemiology of glioma in adults: a "state of the science" review. *Neuro Oncol,* 16(7):896–913.
2. Wen PY, Kesari S (2018). Malignant gliomas in adults. *N Engl J Med,* 359(5):492–507.
3. Nagarajan RP, Costello JF (2009). Epigenetic mechanisms in glioblastoma multiforme. *Semin Cancer Biol,* 19(3):188–197.
4. Jhaveri N, Chen TC, Hofman FM (2016). Tumor vasculature and glioma stem cells: contributions to glioma progression. *Cancer Lett,* 380(2):545–551.
5. Nutt CL, Mani DR, Betensky RA, et al (2003). Gene expressionbased classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res,* 63(7):1602–1607.
6. Park YW, Kim S, Han K, et al (2023). Rethinking extent of resection of contrast-enhancing and non-enhancing tumor: different survival impacts on adult-type diffuse gliomas in 2021 World Health Organization classification. *Eur Radiol,* Aug 23.
7. Hussein AA, Forouzanfar T, Bloemena E, et al (2018). A review of the most promising biomarkers for early diagnosis and prognosis prediction of tongue squamous cell carcinoma. *Br J Cancer,* 119(6):724–36.
8. Crocetti E, Trama A, Stiller C, et al (2012). Epidemiology of glial and non-glial brain tumours in Europe. *Eur J Cancer,* 48(10):1532–1542.
9. Bhatt AN, Mathur R, Farooque A, Verma A, Dwarakanath BS (2010). Cancer biomarkers—current perspectives. *Indian J Med Res,* 132:129–149.
10. Comabella M, Montalban X (2014). Body fluid biomarkers in multiple sclerosis. *Lancet Neurol,* 13(1):113–126.
11. Subramanian A, Tamayo P, Mootha VK, et al (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A,* 102(43):15545-15550.
12. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P (2015). The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst,* 1(6):417-425.
13. Mootha VK, Lindgren CM, Eriksson KF, et al (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet,* 34(3):267-273.
14. Lamb J, Crawford ED, Peck D, et al (2006). The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science,* 313(5795):1929-1935.
15. Lamb J (2007). The Connectivity Map: a new tool for biomedical research. *Nat Rev Cancer,* 7(1):54-60.
16. Zhu P, Du XL, Lu G, Zhu JJ (2017). Survival benefit of glioblastoma patients after FDA approval of temozolomide concomitant with radiation and bevacizumab: apopulation-based study. *Oncotarget,* 8(27):44015–31.

17. Teunissen CE, Malekzadeh A, Leurs C, Bridel C, Killestein J (2015). Body fluid biomarkers for multiple sclerosis—the long road to clinical application. *Nat Rev Neurol,* 11(10):585–596.
18. Bossuyt PM, Reitsma JB, Bruns DE, et al (2015). STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *Clin Chem,* 61:1446–1452.
19. Yu X, Li Z (2016). MicroRNA expression and its implications for diagnosis and therapy of tongue squamous cell carcinoma. *J Cell Mol Med,* 20(1):10–16.
20. Krueger DA, Care MM, Holland K, et al (2010). Everolimus for Subependymal Giant-Cell Astrocytomas in Tuberous Sclerosis. *N Engl J Med,* 363(19):1801–1811.
21. Aldape KD (2014). American Society of Clinical Oncology (ASCO) 50th Annual Meeting May 30-June 3. Chicago, IL: Impact of Molecular Signatures on Clinical Outcome.
22. Della Puppa A, Rossetto M, Ciccarino P, Del Moro G, Rotilio A, Manara R, et al (2010). The first 3 months after BCNU wafers implantation in high-grade glioma patients: clinical and radiological considerations on a clinical series. *Acta Neurochir (Wien),* 152(11):1923–31.
23. Ulmer S, Spalek K, Nabavi A, et al (2012). Temporal changes in magnetic resonance imaging characteristics of Gliadel wafers and of the adjacent brain parenchyma. *Neuro Oncol,* 14(4):482–90.
24. Colen RR, Zinn PO, Hazany S, et al (2011). Magnetic resonance imaging appearance and changes on intracavitary Gliadel wafer placement: a pilot study. *World J Radiol,* 3(11):266–72.
25. Rosen BR, Belliveau JW, Vevea JM, Brady TJ (1990). Perfusion imaging with NMR contrast agents. *Magn Reson Med,* 14(2):249–65.
26. Villringer A, Rosen BR, Belliveau JW, et al (1988). Dynamic imaging with lanthanide chelates in normal brain: contrast due to magnetic susceptibility effects. *Magn Reson Med,* 6(2):164–74.
27. Huang RY, Neagu MR, Reardon DA, Wen PY (2015). Pitfalls in the neuroimaging of glioblastoma in the era of antiangiogenic and immuno/targeted therapy - detecting illusive disease, defining response. *Front Neurol,* 6:33.