Transcription Factor ELF1 Activates MEIS1 Transcription and Then Regulates the GFI1/FBW7 Axis to Promote the Development of Glioma

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INTRODUCTION
Malignant gliomas are among the most common primary brain tumors in adults.1 Due to the infiltrative nature of this disease and the localization close to eloquent brain areas, surgical resection fails to cure the disease. Patients diagnosed with a malignant glioma such as glioblastoma have to undergo a fierce clinical course with a survival time of less than 2 years for most patients.2,3 Based on advances in the molecular characterization of these tumors, disease-associated targets, including epidermal growth factor receptor (EGFR) or vascular EGFR (VEGFR), were identified, which led to the development of new approaches using traditional routes of drug development.4–6 According to the World Health Organization (WHO) classification of tumors of the central nervous system, gliomas can be categorized into four grades (grades I–IV), among which grade IV is also called glioblastoma or glioblastoma multiforme (GBM).7 Moreover, a gene expression-based molecular classification of glioblastoma has been presented, including proneural, neural, classical, and mesenchymal subtypes.8 Despite the identification of these different subtypes, no effective targeted therapy for gliomas has been developed in recent decades to improve outcomes. Unfortunately, these strategies failed so far, because the complexity of the disease was underestimated and important factors such as the capability of therapeutics to pass the blood-brain barrier or to penetrate the tumor tissue were not sufficiently considered. This perspective is substantiated by the fact that areas of variant morphology exhibit significant differences in gene expression subtype within a single tumor yet harbor a large number of identical genetic alterations.9

Erythroblast transformation-specific (ETS) family transcription factors play important roles in prostate tumorigenesis, with some acting as oncogenes and others as tumor suppressors. ETS factors compete for binding at some cis-regulatory sequences. Therefore, changes in expression of ETS family members during tumorigenesis can have complex, multimodal effects. Recent research showed that ETS transcription factor 1 (ELF1) could serve as a possible factor for tumor progression.10 Genome-wide mapping in cell lines indicated that ELF1 has two distinct tumor suppressive roles mediated by distinct cis-regulatory sequences. ELF1 and ELF2, closely related transcription factors to ELF4, also exerted a proliferative effect in various cancer cell systems with no immediate prospect of a cure. Comprehensive understanding on the pathogenesis of the disorder contributes to a better outcome. Herein, we aimed to investigate whether transcription factors erythroblast transformation-specific (ETS) transcription factor (ELF1), myeloid ecotropic viral integration site 1 (MEIS1), and growth factor independence 1 (GFI1)/F-box/WD repeat-containing protein 7 (FBW7) mediate progression of glioma. ELF1, MEIS1, and GFI1 were upregulated in glioma cells and tissues, as ELFI was correlated with poor prognosis. Bioinformatics analysis identified the binding between ELF1 and MEIS1 as well as between GFI1 and FBW7, confirmed by chromatin immunoprecipitation (ChIP) experiments. Functional experiment indicated that silencing of ELFI decreased MEIS1 expression and that overexpression of MEIS1 increased GFI1 expression by activating GFI1 enhancer but decreased FBW7 expression. Importantly, silencing of ELF1 decreased the capacities of proliferation, migration, and invasion of glioma cells whereas it increased apoptosis, supported by increased capase-3 and decreased matrix metalloproteinase-9 (MMP-9) and proliferating cell nuclear antigen (PCNA) expression. Moreover, an in vivo experiment confirmed the inhibitory role of silenced ELF1 in tumor growth, with a decreased level of MEIS1 and GFI1. Taken together, our study elucidated a potential mechanism that ELF1 promoted cell progression by increasing GFI1 and METS1 as well as decreasing FBW7 expression in glioma.

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In our study, we aimed to investigate the mechanism underlying ELF1 mediating the progression of glioma. Our results revealed that interference of ELF1 in glioma reduced its ability to recruit the transcription factor MEIS1 and further impaired the activation ability of MEIS1 to GFI1 enhancer in glioma cells. Additionally, an animal model was also established to detect the impact of ELF1/MEIS1/GFI1 on tumor growth.

RESULTS

ELF1 Is Highly Expressed in Glioma Tissues and Correlates with WHO Grading and KPS Score of Patients

Datasets GEO: GSE12657, GSE35493, GSE104291, and GSE50161 were analyzed by R language, and we found 1,507, 4,173, 2,784, and 4,554 differentially expressed genes, respectively. We found that there were 578 genes expressed in these four datasets through coexpression analysis using the RobustRankAggreg pack (Figure 1A). Eight key transcription factors were obtained from hTfTarget and Cistrome, including BCL11A, EZH2, FOXM1, HDAC1, ELF1, STAT4, CBX3, and VEZF1 (Figure 1B), among which ELF1 has been implicated as being associated with glioma.15 The expression data from datasets GEO: GSE35493, GSE35493, GSE104291, and GSE50161 were presented in a boxplot where ELF1 was indicated as highly expressed in glioma, while Gene Expression Profiling Interactive Analysis (GEPIA) analysis of GBM data from GTEx dataset also identified the high expression of ELF1 (Figure 1C).

Transcription Factor ELF1 Binds to the MEIS1 Promoter to Promote Its Transcription and Promote the Development of Glioma

In the above studies, we have identified that ELF1 was highly expressed in glioma tissues and can significantly inhibit the proliferation, migration, and invasion of glioma cells after specific interference treatment. Then, we continued our review of relevant literature and found that ELF1, a transcription factor, can be combined into MEIS1 promoter regions, thus affecting the transcription.16 The normalized data of ELF1 and MEIS1 of 84 samples from datasets GEO: GSE12657, GSE35493, GSE104291, and GSE50161 identified a positive correlation between ELF1 and MEIS1 (Figure 3A). GEPIA analysis confirmed the positive correlation upon analysis of GBM data from The Cancer Genome Atlas (TCGA) dataset (Figure 3B). The analysis of the four datasets also revealed that MEIS1 was significantly highly expressed in glioma tissues in the GEO: GSE35493 and GSE35492 datasets, while it was highly expressed in GEO: GSE104291 and GSE50161, but not significantly (p > 0.05) (Figure 3C). Based on this evidence, we hypothesized that ELF1 may affect glioma development by promoting the transcription of MEIS1. We initially detected MEIS1 expression in glioma tissues through the qRT-PCR and western blot analysis and identified the elevated expression of MEIS1 in glioma tissues relative to normal brain tissues (KPS) scores in patients; however, there was no significant correlation between expression of ELF1 and patient’s age, sex, tumor size, and tumor recurrence (Table 1). After Kaplan-Meier analysis, the log-rank test of survival data showed that expression of ELF1 was negatively correlated with survival time and prognosis of patients (Figure 1F).

Silencing ELF1 Inhibits the Proliferation, Migration, and Invasion of Glioma Cells and Promotes Cell Apoptosis

In view of the significant upregulation of ELF1 in glioma tissues, in order to determine how it affected the proliferation, migration, and invasion abilities of glioma cells, we performed functional experiments on glioma cells. We constructed small interfering RNA (siRNA) specific to ELF1 (si-ELF1-1, si-ELF1-2, si-ELF1-3), and si-ELF1 exhibited the most significant interference on ELF1 expression, according to the results from qRT-PCR (Figure S4A). After transfection with si-ELF1, the expression of ELF1 was significantly decreased in glioma cells A172, U251, and T98G according to results of qRT-PCR and western blot analysis (Figure 2A; Figure S1A). From the results of a Cell Counting Kit-8 (CCK-8) assay, transwell assay, and annexin V/propidium iodide (PI) dual staining, glioma cell abilities of proliferation, migration, and invasion were significantly inhibited and cell apoptosis was induced after interference of ELF1 (Figures 2B–2E; Figures S1B–S1E). Proliferation-related factor proliferating cell nuclear antigen (PCNA), invasion-related factor metalloproteinase-9 (MMP-9), and apoptosis-related factor cleaved caspase-3 expression was detected by western blot analysis, and results showed that compared with the si-negative control (NC) group, expressions of PCNA and MMP-9 in the si-ELF1 group were significantly decreased, while expressions of cleaved caspase-3 were significantly increased (Figure 2F; Figure S1F).
The binding site between ELF1 and MEIS1 promoter obtained by the JASPAR dataset (Figure 3E; Table S1) was detected by a chromatin immunoprecipitation (ChIP) experiment. We applied magnetic protein A beads to precipitation and found that compared with the immunoglobulin G (IgG) group, the promoter DNA of binding MEIS1 in the ELF1 group was significantly increased (Figure 3F; Figure S2A). When si-ELF1 plasmids were transfected into glioma cells, mRNA and protein expressions of MEIS1 were significantly decreased (Figure 3G; Figure S2B).

Furthermore, to determine whether ELF1 was involved in the development of glioma through affecting the transcription of MEIS1, we transfected si-NC + overexpression (oe)-NC, si-ELF1 + oe-NC, and si-ELF1 + oe-MEIS1 into glioma cells A172, U251, and T98G, followed by transwell and CCK-8 assays, as well as annexin V/PI staining. It was clear that treatment with si-ELF1 + oe-NC decreased cell proliferation, migration, and invasion ability, whereas it increased apoptosis, in comparison with that of the control group. Based on si-ELF1, addition of oe-MEIS1 reversed the effect of ELF1.
interference on glioma cells, promoting the growth, migration, and invasion of glioma cells, and reducing cell apoptosis (Figures 3H–3K; Figures S2C–S2F).

Western blot analysis was then conducted to analyze proliferation factor PCNA, invasion-related protein MMP-9, and apoptosis key factor cleaved caspase-3 upon treatments. The expression of PCNA and MMP-9 in the si-ELF1 + oe-NC group was decreased and the expression of cleaved caspase-3 was increased. Similarly, overexpression of MEIS1 increased the expression of PCNA and MMP-9, and decreased cleaved caspase-3, restoring the expression of these key factors (Figure 3L; Figure S2G). These results suggested that the transcription factor ELF1 may be involved in glioma development by enhancing MEIS1 transcription in glioma.

**MEIS1 Promotes Glioma Development by Regulating the Activity of the GFI1 Promoter**

Multi Experiment Matrix (MEM) analysis, a visualization tool gathering publicly available gene expression data and ranking genes by their similarity, in the current study pointed to a co-expression relationship between MEIS1 and GFI1 (Figure 4A). To confirm this relationship in tissues, we then detected GFI1 expression in the glioma and normal control tissues by qRT-PCR and western blot analysis, and found that expression of GFI1 in glioma tissues was higher (Figure 4B). When glioma cells were transfected with overexpression of MEIS1, as demonstrated by western blot analysis, GFI1 expression in cells was increased (Figure 4C; Figure S3A). Then, the ChIP experiment was used to verify the relationship between MEIS1 and GFI1. Overexpression of MEIS1 in A172, U251, and T98G cells led to the enrichment of H3K4me1, H3K27ac, and MEIS1 in the GFI1 promoter and enhancer region with similar results observed in the three cell lines. These results indicated that MEIS1 promoted the expression of GFI1 by activating the enhancer of GFI1 (Figures 4D and 4E; Figures S3B and S3C).

To further explore the impact between MEIS1 and GFI1 on gliarial development, we treated U251 cells with overexpressed MEIS1 or interfered GFI1 simultaneously, followed by a CCK-8 assay, transwell assay, and flow cytometry. Compared with the oe-NC + si-NC group, cell proliferation, migration, and invasion were increased but the apoptosis rate was decreased in the oe-MEIS1 + si-NC group. The interference plasmids of GFI1 (si-GFI1-1, si-GFI1-2, si-GFI1-3) were established and si-GFI1-1 with greatest efficiency of interference was selected to transfect to oe-MEIS1-treated cells (Figure 4M). However, oe-MEIS1 + si-GFI1 reversed the effect of overexpressing MEIS1 on cell progression (Figures 3D–3G and 4F–4I). These results were supported by following the detection of MMP-9, cleaved caspase-3, and PCNA in the cells upon treatment. As displayed in Figure 4J and Figure S3H, interference with GFI1 could reverse the effect of overexpression of MEIS1 on all related proteins, inhibiting the expression of PCNA and MMP-9, and promoting the expression of cleaved caspase-3.

In the occurrence of cervical cancer, as reported, GFI1 can inhibit the expression of F-box/WD repeat-containing protein 7 (FBW7) through its correlation with FBW7.17 Since the GEO: GSE12657 dataset does not include the expression data for FBW7 (named FBXW7 in NCBI), we only normalized the data concerning FBW7 from the other three datasets to assess the correlation between GFI1 and FBW7. We found that there was a negative correlation between GFI1 and FBW7 (Figure 4K). Analysis from the datasets GEO: GSE35493, GSE104291, and GSE35493 indicated poorly expressed expression of FBW7 in glioma, consistent with the results from TCGA and GTEx datasets analyzed by GEPIA (Figure 4L). GFI1 and FBW7 had a significantly co-expressed relationship, as evidenced by MEM analysis (Figure 4M). In addition, we continued to detect the expression of FBW7 in glioma cells by western blot analysis after transfection with MEIS1 or GFI1. As shown in Figure 4N and Figure S3I, compared with the oe-NC + si-NC group, FBW7 expression was significantly lower in the oe-MEIS1 + si-NC group, but additional treatment with si-GFI1 hardly altered expression of FBW7. This evidence elucidated a mechanism that MEIS1 inhibited FBW7 expression through mediating the activity of GFI1 and thereby promotes proliferation, migration, and invasion and inhibits apoptosis of glioma cells.

**Interference with ELF1 Can Inhibit Glioma Progression In Vivo by the MEIS1/GFI1/FBW7 Axis**

To confirm the in vivo anti-tumor effect of ELF1, we developed a mouse model. First, we silenced ELF1 expression in U251 cells and

### Table 1. The Relationship between ELF1 Expression and the Clinicopathological Characteristics of Glioma Patients

| Index No. | Low Expression (n = 29) | High Expression (n = 31) | p Values |
|-----------|------------------------|-------------------------|----------|
| Sex       |                        |                         |          |
| Male      | 38                     | 20                      | 18       | 0.431    |
| Female    | 22                     | 9                       | 13       |          |
| Age (Years) |                        |                         |          |
| ≥ 63      | 35                     | 16                      | 19       | 0.794    |
| <63       | 25                     | 13                      | 12       |          |
| Tumor Diameter (mm) |                  |                         |          |
| ≥ 5       | 33                     | 15                      | 18       | 0.796    |
| <5        | 27                     | 14                      | 13       |          |
| TNM       |                        |                         |          |
| I–II      | 29                     | 28                      | 1        | < 0.001  |
| III–IV    | 31                     | 1                       | 30       |          |
| KPS       |                        |                         |          |
| ≥ 70      | 35                     | 27                      | 8        | < 0.001  |
| <70       | 25                     | 2                       | 23       |          |
| Relapse   |                        |                         |          |
| Yes       | 37                     | 14                      | 23       | 0.062    |
| No        | 23                     | 15                      | 8        |          |

TNM, tumor-node-metastasis.
Figure 2. Silencing ELF1 Inhibits Proliferation, Migration, and Invasion and Promotes Apoptosis of A172 and U251 Glioma Cells

(A) Expression of ELF1 in A172 and U251 glioma cells upon si-NC or si-ELF1 was tested by qRT-PCR and western blot analysis. (B) CCK-8 assay of proliferation of A172 and U251 cells upon treatment with si-NC or si-ELF1. (C) Transwell assay of migration of A172 and U251 cells upon treatment with si-NC or si-ELF1 (original magnification, ×200). (D) Transwell assay of migration of A172 and U251 cells upon treatment with si-NC or si-ELF1 (original magnification, ×200). (E) Apoptosis rate of A172 and U251 cells was determined by annexin V/PI flow cytometry. (F) Western blot analysis was used to detect the expression of proliferation-related factor PCNA, invasion-related factor MMP-9, and apoptosis-related factor cleaved caspase-3 of A172 and U251 cells upon treatment with si-NC or si-ELF1. The above values are all measurement data, expressed as mean ± standard deviation. *p < 0.05 compared with the si-NC group. An unpaired t test was used between the two groups, and the data of each group at different time points were compared. The cell experiment was repeated three times.
Figure 3. ELF1 Binds to the MEIS1 Promoter to Promote the Expression of MEIS1 to Participate in the Development of Glioma

(A) Normalized expression correlation diagram of ELF1 and MEIS1 drawn from GEO: GSE50161, GSE35493, GSE12657, and GSE104291 datasets. (B) Expression correlation diagram of ELF1 and MEIS1 through GEPIA analysis of GBM data from TCGA dataset. (C) Boxplot of MEIS1 expression from dataset GEO: GSE12657, GSE35493, GSE50161, and GSE104291, as well as GBM data from TCGA dataset and GTEx through GEPIA analysis. *p < 0.01. (D) qRT-PCR analysis of MEIS1 expression in clinical glioma tissues (n = 60) and normal brain tissues (n = 24). (E) Binding site of ELF1 and METS1 promoter through the JASPAR dataset. (F) ChIP experiment of METS1...
transplanted the cells into the nude mice. Every week, we checked the weight and volume of the tumors. The results showed that the weight and volume of tumor in mice treated with sh-ELF1 were lower than those in the control group (Figures 5A–5C). In addition, western blot analysis showed that sh-ELF1 treatment significantly reduced the expression of ELF1, MEIS1, and GFI1, accompanied with elevated expression of FBW7 (Figure 5D). These results indicated that interference with ELF1 could inhibit MEIS1/GFI1, thereby promoting expression of FBW7 and retarding tumor growth in vivo.

**DISCUSSION**

Gliomas comprise the most common type of primary malignant brain tumor, and except for pilocytic astrocytoma and subependymal giant cell astrocytoma, nearly all are characterized by a high recurrence rate, high mortality, and short survival times. Only 5.5% of patients typically survive 5 years after diagnosis, and the median overall survival is still dismal at approximately 14.5–16.6 months, even with multimodal therapy comprised of surgery, radiotherapy, and chemotherapy. Therefore, we hypothesized that ELF1 might serve as a factor of tumor promotion. In order to verify our hypothesis, we silenced the ELF1 in A172, U251, and T98G cell lines and found that ELF1 was identified as a potential downstream target of the DNA damage response pathway, and following ionizing radiation, U2OS cells with a siRNA against ELF1 were more likely to escape cell cycle arrest by bypassing the G2-M checkpoint. For the purpose of determining whether ELF1 was involved in the occurrence and development of glioma, we measured the expression of ELF1 in brain tissues of glioma patients and found that ELF1 is highly expressed in glioma tissues and closely correlates with WHO grading and the KPS score of patients. Therefore, we hypothesized that ELF1 might serve as a factor of tumor promotion. In order to verify our hypothesis, we silenced the ELF1 in A172, U251, and T98G cell lines and found that the proliferation activity of cells was significantly decreased. Additionally, silencing of ELF1 dramatically triggered apoptosis in A172, U251, and T98G cell lines. Furthermore, activities of migration and invasion of glioma cells were distinctly impaired by silencing of ELF1. The proliferation-related factor PCNA and invasion-related factor MMP-9 were downregulated, while apoptosis-related factor cleaved caspase-3 was upregulated after interference of ELF1 in glioma cells. The above results suggested that silencing ELF1 inhibits the proliferation, migration, and invasion and promotes cell apoptosis of glioma cells.

MEIS1 is a transcription factor that regulates important functions in cell fate determination during development and cell proliferation. MEIS1 has a key role in the regulation of the stemness state of stem cells and the transcription adjustment of self-renewal genes, as well as involved genes in cell development and differentiation, playing an oncogenic role in several tumors. It has been reported that ELF1 can act as an important positive transcriptional regulator of the Hox cofactor MEIS1. Therefore, we hypothesized that ELF1 may affect glioma development by regulating the transcription of MEIS1. Based on the results from qRT-PCR, the expression of MEIS1 in glioma tissues was significantly upregulated in comparison to normal tissues. In addition, overexpression of MEIS1 reversed the effect of ELF1 interference on promoting the growth, migration, and invasion of glioma cells, and it reduced cell apoptosis in glioma cells. It can be concluded that the transcription factor ELF1 may be involved in promoting glioma progression by regulating MEIS1 transcription.

The GFI1 gene, which is a zinc finger transcription factor essential for development of the erythroid and megakaryocytic lineages, was originally discovered in the hematopoietic system, where it functions as a key regulator of stem cell homeostasis, as well as development of the erythroid and megakaryocytic lineages. A previous study demonstrated that GFI1 expression is controlled by five distinct regulatory regions spread over 100 kb, with Scl/Tal1 and MEIS1 acting as upstream regulators in early hematopoietic cells. To elucidate the underlying mechanism between GFI1 and MEIS1 in glioma development, MEM analysis was used to reveal that a significant relationship of co-expression between MEIS1 and GFI1 existed. Overexpression of MEIS1 in glioma cells would significantly increase the enrichment of H3K4me1, H3K27ac, and MEIS1 in the GFI1 enhancer region, as well as the promoter region of GFI1, H3K4me3, H3K27ac, and GFI1, suggesting that MEIS1 promoted the expression of GFI1 by activating the enhancer of GFI1. Overexpression of MEIS1 activated the enhancer of GFI1, and then inhibited proliferation and migration and triggered apoptosis in glioma cells. Moreover, recent studies showed that the tumor suppressor FBW7, an E3 ubiquitin ligase that mediates ubiquitination and degradation of oncoproteins, participated in and promoted the function of MEIS1/GFI1 in glioma cells. MEIS1 could upregulate the activity of the GFI1 enhancer, followed by inhibition of the expression of FBW7 and then promotion of the proliferation, migration, and invasion and suppression of apoptosis of glioma cells.

Finally, a subcutaneous tumor mouse model of U251 cells (interference of ELF1) was established to confirm the anti-tumor effect of
Figure 4. MEIS1 Regulates GFI1 Enhancer Activity in A172 and U251 Glioma Cells

(A) MEM analysis of co-expression relationship between MEIS1 and GFI1. (B) qRT-PCR analysis of GFI1 expression in glioma tissues and normal brain tissues. (C) Western blot analysis of GFI1 expression in A172 and U251 cells upon treatment with oe-NC or oe-MEIS1. (D) ChIP of enrichment of H3K4me1, H3K27ac, and MEIS1 in the GFI1 enhancer region upon treatment with oe-NC or oe-MEIS1. (E) ChIP detected the enrichment of H3K4me1, H3K27ac, and MEIS1 in the promoter region of GFI1 upon treatment with oe-NC or oe-MEIS1. (F) Flow cytometry of A172 and U251 cell proliferation upon treatment with oe-NC + si-NC, oe-MEIS1 + si-NC, or oe-MEIS1 + si-GFI1. (G) Transwell assay of migration of A172 and U251 cells upon treatment with oe-NC + si-NC, oe-MEIS1 + si-NC, or oe-MEIS1 + si-GFI1. (H) Transwell assay of invasion of A172 and U251 cells upon treatment with oe-NC + si-NC, oe-MEIS1 + si-NC, or oe-MEIS1 + si-GFI1. (I) Flow cytometry of apoptotic rate of A172 and U251 cells upon treatment with oe-NC + si-NC, oe-MEIS1 + si-NC, or oe-MEIS1 + si-GFI1. (J) Western blot analysis of PCNA, MMP-9 and cleaved caspase-3 protein expression in A172 and U251 cells upon treatment with oe-NC + si-NC, oe-MEIS1 + si-NC, or oe-MEIS1 + si-GFI1. (K) Correlation diagram of GFI1 and FBX7 expression through normalization of GEO: GSE50161, GSE104291, and GSE3549 datasets. (L) Boxplot of FBX7 expression from GEO: GSE12657.

(legend continued on next page)
The results showed that the growth and weight of tumors in mice treated with si-ELF1 were significantly lower than those in the control group. Additionally, compared with control group, downregulated expressions of ELF1, MEIS1, and GFI1, and upregulated expression of FBW7, were found in tumor tissues treated with si-ELF1. The results showed that interference with ELF1 would inhibit MEIS1/GFI1, thereby promoting expression of FBW7 and retarding glioma growth in vivo.

In conclusion, the present study demonstrated that upregulated expression of ELF1 in glioma tissues promoted tumor progression by regulating the MEIS1/GFI1/FBW7 axis (Figure 6), suggesting that ELF1 could serve as a promising therapeutic target for glioma.

MATERIALS AND METHODS

Bioinformatics Analysis

Differential expressed genes were screened using the GEO: GSE12657, GSE35493, GSE104291, and GSE50161 datasets downloaded from the GEO dataset (https://www.ncbi.nlm.nih.gov/gds).

The R language limma package25 for microarray data was used for differential expression analysis with the threshold set as |log₂fold change (FC)| >1 and p value <0.05. The contrasts.fit function of the limma package was used to establish a linear model of the dataset, and eBayes was used to assess the significance of the linear model with a t test and calculate the log₂FC value. The expression dataset GEO: GSE12657 contained a total of 12 samples, including 5 normal samples and 7 glioma samples; GEO: GSE35493 contained 7 normal samples and 12 glioma samples; GEO: GSE104291 contained 2 normal samples and 4 glioma samples; and GEO: GSE50161 contained 13 normal samples and 34 glioma samples. Key genes in these expression datasets were obtained by co-expressed analysis through RobustRankAggreg.26 hTFTarget (http://bioinfo.life.hust.edu.cn/hTTarget#!/) and Cis-trome (http://cistrome.org/) were used to screen human transcription factors. The genes at the intersection of key genes and transcription factors were selected as key transcription factors. The possible downstream regulatory pathways were predicted through the existing literature, and the downstream gene promoter sequence of transcription factor was obtained from the University of California Santa Cruz.

Figure 5. ELF1 Inhibits Tumor Growth in Nude Mice

(A) Representative image of tumor formation upon treatment with sh-NC or sh-ELF1 (n = 5). (B) Statistics of tumor volume growth in nude mice upon treatment with sh-NC or sh-ELF1 (n = 5). (C) Tumor weight statistics of nude mice upon treatment with sh-NC or sh-ELF1 (n = 5). (D) Western blot analysis of ELF1, MEIS1, GFI1, and FBW7 upon treatment with sh-NC or sh-ELF1. *p < 0.05 compared with the sh-NC group. Measurement data are expressed as mean ± standard deviation. The data between two groups were analyzed by an unpaired t test. Data at different time points among groups were compared by two-way ANOVA, followed by a Bonferroni post-test. The cell experiment was repeated three times. n = 5.
underwent surgery from January 2014 to January 2015. According to the classification of central nervous system tumors, the gliomas were classified into four grades and the patients in this study consisted of 29 cases of grade I–II, and 31 cases of grade III–IV, with 35 cases of KPS >70 and 25 cases of KPS <70. Excluded criteria were as follows: patients with other malignant tumor concurrent, incomplete clinical information, serious heart disease, kidney disease, and lung dysfunction. In addition, 24 cases of normal brain tissues removed by internal decompression surgery due to severe craniocerebral injury were taken as the control group. All patients did not accept chemoradiotherapy before surgery with 5- to 36-month follow-up until January 2020 by telephone or subsequent visit. By the end of follow-up, two patients were lost to follow-up and the follow-up rate was 96.67%. The 3-year overall survival of each patient was observed. All patients in this study signed an informed consent and were approved by our Medical Ethics Committee to comply with the Declaration of Helsinki.

**Cell Culture and Transfection**

Glioma cell lines A172, U251, and T98G, provided by Stem Cell Bank, Chinese Academy of Sciences, were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in 5% CO₂.

Cells were transfected according to the experimental requirements. When the cell density reached 90% and was in the logarithmic growth phase, cells were digested with trypsin, made into cell suspension (2.5 × 10⁵ cell/mL), and inoculated on six-well plates (2 mL for each well). Lentiviral vectors were constructed using LV5-GFP (lentiviral gene overexpression vector) and the psih1-h1-copgp siRNA vector (lentiviral siRNA fluorescence expression vector gene silencing vector). si-ELF1, si-GFI1, oe-MEIS1, and their NCs were all constructed by Thermo Fisher Scientific (Waltham, MA, USA). Lentivirus was packaged in 293T cells, which were cultured in RPMI 1640 complete medium containing 10% fetal bovine serum and passed every other day. When the A172, U251, and T98G cells were in the logarithmic growth phase, they were digested by trypsin and tritutated, and 2-mL cell suspensions (1 × 10⁸ cells/mL) were inoculated on six-well plates and cultured overnight at 37°C. Then, the virus (1 × 10⁸ transducing units [TU]/mL) was added to the cells for infection, and cells with stable heredity were obtained and collected for subsequent experiments. The sequence of si-ELF1 was 5′-GGATGTTGCTGAAGAAGAA-3′, and the sequence of si-GIF1 was 5′-CGAGCAGACAGCCTCTAA-3′.

**qRT-PCR**

Total RNA (Invitrogen, USA) was extracted according to the instructions of TRizol method, and the RNA was reversely transcribed into cDNA using PrimeScript RT kit (RR037A, Takara, Shiga, Japan) with a system of 10 µL. Then, the reaction liquid was exposed to fluorescence quantitative PCR based on the instructions for the SYBR Premix Ex Taq II kit (RR820A, Takara). The samples were subjected to quantitative real-time PCR using a quantitative real-time PCR system (ABI 7500, Applied Biosystems, Foster City, CA, USA). With GAPDH as an internal control, the 2⁻ΔΔCt method was used to calculate the relative gene expression. Relevant primers were assigned to Shanghai Sangon Biotech (Shanghai, China) (Table 2).

**Protein Extraction and Quantification**

About 1 × 10⁶ cells were treated with 1 mL of cell lysate (containing protease inhibitor) (P0013J, Beyotime Biotechnology, Shanghai, China) for 45 min, and then centrifuged for 30 min at 4°C and 8,000 rpm to collect the supernatant. Then, the protein concentration of each sample was determined using a bicinchoninic acid (BCA) kit...
was added to 200 µL of serum-free medium, after which 50 µL of Matrigel was added to upper chamber and incubated for 2–3 h until the gel became solid. Cells were digested and counted, and the cell suspension was prepared with serum-free medium. Next, 200 µL of cell suspension was added to the upper chamber of each well, and 800 µL of cell suspension was added to the lower chamber containing 20% FBS-conditioned medium. Cells were incubated at 37°C for 20–24 h. After that, a transwell plate was soaked in formaldehyde for 10 min and rinsed with pure water three times. The cells were stained with 0.1% crystal violet at room temperature for 30 min, and the cells on the upper surface were wiped off with cotton balls. Cells on the membrane were observed, imaged, and counted by an inverted microscope. Substrate glue was not required for the transwell migration experiment, and the incubation time was 16 h. Cells from at least four randomly selected microscope regions were counted.

**Cell Apoptosis Assay**

After transfection for 48 h, the cells were digested with 0.25% trypsin and collected in the flow tube, centrifuged, and the supernatant was discarded. Annexin V-fluorescein isothiocyanate (FITC), PI, and HEPES buffer were incorporated into annexin V-FITC/PI dye at a ratio of 1:2:50 according to the instructions of annexin V-FITC apoptosis assay kit (559763, Becton Dickinson, NY, USA). Then, 1 × 10^5 cells were resuspended in 100 µL of dye solution, and the cells were oscillated and mixed. After incubation at room temperature for 15 min, 1 mL of HEPES buffer (PB180325, Porcello, Wuhan, China) was added to the solution for oscillating and mixing. FITC and PI fluorescence were detected by excitation of 525-nm and 620-nm bandpass filters at the wavelength of 488 nm to detect cell apoptosis.

**ChIP**

The EZ-Magna ChIP kit (EMD Millipore) was used for ChIP determination. According to the manufacturer’s protocol, the cells were immobilized with 4% paraformaldehyde and incubated with glycine for 10 min to produce DNA-protein cross-linking. The cells were then lysed with a cell lysis buffer and nucleic acid buffer and treated with sonication to produce 200–300 bp of chromatin fragments (a portion of the DNA as input). Next, lysates were immunoprecipitated by magnetic protein A beads bonded with various antibodies. H3K27ac antibody (ab177178, Abcam) or H3K4me1 (ab176877, Abcam) was added to the target protein group. Negative control was added with rabbit IgG (ab197051, 1:2,000, Abcam, USA) and GAPDH (ab9485, 1:1,000, Abcam) was added to the control group. The precipitated DNA was analyzed by qRT-PCR.

**In Vivo Animal Experiment**

Ten BALB/c male nude mice (age, 4–5 weeks old; weight, 18–22 g) were purchased from Shanghai SLAC Laboratory Animal Co. (Shanghai, China). Lentivirus expressing sh-ELF1 or sh-NC was transfected into the human U251 cell line. Cell suspension (20 µL, 1.0 × 10^6 cells/mL) was inoculated in nude mice at the abdomen subcutaneously (five mice treated with sh-NC, five with sh-ELF1). Tumors were observed weekly and measured with a Vernier caliper. The formula of for calculating tumor volume (TV) is TV = 1/2 × a × b^2, where a is length of tumor and b is width of tumor. Mice

### Table 2. Primer Sequences Used for qRT-PCR

| Targets | Primer Sequence (5’→3’) |
|---------|-------------------------|
| ELF1    | F: 5’-TGTTGCCCAGAGACGAGCCT-3’  |
|         | R: 5’-GGAAAAATACAGTGATCACC-3’  |
| MEIS1   | F: 5’-TCAGCTGGCTTTAAAGAGGA-3’  |
|         | R: 5’-GCTGATGGGTAGACGCCT-3’  |
| GFI1    | F: 5’-AGGTGTGAAACTACCCGAGGAT-3’ |
|         | R: 5’-ACCATGAGGCTTCGGACACT-3’ |
| GAPDH   | F: 5’-GACGCTTCAAGGCTGAGAAC-3’   |
|         | R: 5’-TGGTAAAGACGCGATGGA-3’    |

F, forward; R, reverse.
were exposed to euthanasia at 35 days, and tumors of each group were removed, weighed, and imaged. All of the above experimental animals were approved by the Animal Protection and Use Committee, and all of the animal experiments in this study are in accordance with the management and use principles for local experimental animals.

**Immunohistochemistry**

Paraffin-embedded tumor tissues were subjected to dewaxing, hydration, xylene I and II dewaxing, and gradient alcohol dehydration. Sections were immersed in 3% H2O2 for 10 min, washed with PBS twice for 5 min and rehydrated with antigen (Beyotime, Shanghai, China) at high pressure for 90 s, and then cooled down at room temperature. Sections were blocked with 5% BSA at 37°C for 30 min and incubated with primary rabbit antibody at 4°C overnight. Then, tissues were incubated with HRP-labeled goat anti-rabbit (ab205718, 1:1,000, Abcam) and imaged. Five high-power fields were randomly selected from each section, and 200 cells were counted in each field. The number of positive cells <5% was negative, and the number of positive cells ≥5% was positive. The immunohistochemical results were scored by two reviewers independently in a double-blind fashion.

**Statistical Analysis**

All of the present data were processed using SPSS21.0 software (IBM, Armonk, NY, USA) and expressed as mean ± standard deviation of three independent experiments. The data between two groups were analyzed by one-way analysis of variance (ANOVA) with Turkey’s post hoc test. Data at different time points among groups were compared by two-way ANOVA or repeated-measures ANOVA. Patient survival was calculated by the Kaplan-Meier method, and the relationship between two indexes was analyzed by Pearson’s relation analysis. A log-rank test was used for univariate analysis. p < 0.05 was considered statistically significant.

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.10.015.

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**AUTHOR CONTRIBUTIONS**

M.C. and Y.Z. designed the study. T.Z., M.X., and Y.W. collated the data and carried out data analyses. M.C. and Z.L. produced the initial draft of the manuscript. All authors have read and approved the final submitted manuscript.

**DECLARATION OF INTEREST**

The authors declare no competing interests.

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