Identification of RNA-Binding Protein LARP4B as a Tumor Suppressor in Glioma

Hideto Koso¹, Hungtsung Yi¹, Paul Sheridan², Satoru Miyano², Yasushi Ino³, Tomoki Todo³, and Sumiko Watanabe¹

Abstract

Transposon-based insertional mutagenesis is a valuable method for conducting unbiased forward genetic screens to identify cancer genes in mice. We used this system to elucidate factors involved in the malignant transformation of neural stem cells into glioma-initiating cells. We identified an RNA-binding protein, La-related protein 4b (LARP4B), as a candidate tumor-suppressor gene in glioma. LARP4B expression was consistently decreased in human glioma stem cells and cell lines compared with normal neural stem cells. Moreover, heterozygous deletion of LARP4B was detected in nearly 80% of glioblastomas in The Cancer Genome Atlas database. LARP4B loss was also associated with low expression and poor patient survival. Overexpression of LARP4B in glioma cell lines strongly inhibited proliferation by inducing mitotic arrest and apoptosis in four of six lines as well as in two patient-derived glioma stem cell populations. The expression levels of CDKN1A and BAX were also upregulated upon LARP4B overexpression, and the growth-inhibitory effects were partially dependent on p53 (TP53) activity in cells expressing wild-type, but not mutant, p53. We further found that the La module, which is responsible for the RNA chaperone activity of LARP4B, was important for the growth-suppressive effect and was associated with BAX mRNA. Finally, LARP4B depletion in p53 and NF1-deficient mouse primary astrocytes promoted cell proliferation and led to increased tumor size and invasiveness in xenograft and orthotopic models. These data provide strong evidence that LARP4B serves as a tumor-suppressor gene in glioma, encouraging further exploration of the RNA targets potentially involved in LARP4B-mediated growth inhibition.

Introduction

Glioblastoma is the most common form of malignant brain cancer in adults. Patients with glioblastoma invariably have a bad prognosis, with a mean survival of around 1 year (1). Thus, more studies are needed to better combat this disease. Genome sequencing has revealed a plethora of mutations in glioblastoma (2); however, the pathologic relevance of these mutations is unclear. Transposon-based insertional mutagenesis is useful as it allows for an unbiased forward genetic screen for cancer genes in mice (3, 4), thereby providing a functional readout to complement cancer genome-sequencing studies. We used transposon mutagenesis to better understand the genes and pathways that are able to transform neural stem cells (NSC) into glioma-initiating cells (5), and identified La-related protein 4b (Larp4b) as a candidate tumor-suppressor gene in glioblastoma.

The La-related proteins (LARP) are characterized by the presence of a La motif (LaM), which is a highly conserved domain similar in structure to the winged-helix motif commonly found in DNA-binding transcriptional factors (6). The La motif is immediately followed by one or several RNA-recognition motifs (RRM), and this tandem arrangement is known as the La module. The functions of the La module have been best studied in the genuine La proteins (7). The genuine La protein La module binds to 3’ oligo (uridylic acid) stretches (UUU-3’OH), which are commonly found in newly synthesized RNA polymerase III transcripts, such as tRNA and 5S rRNA precursors. La engagement is thought to protect RNA trailers from 3’-exonuclease digestion and to assist their folding via its RNA chaperone activity.

Seven different LARPs have been identified in humans (LARP1, LARP1B, LARP4, LARP4B, LARP5, LARP6, and LARP7) based on their homology with genuine La proteins (8). However, the functions and binding modes of the La module in LARPs are not well understood. One of the best-characterized human LARPs is LARP7. By stabilizing 7SK RNA, LARP7 inhibits the cyclin-dependent kinase (CDK) activity of p-TEFb (9, 10), and functions as a tumor-suppressor gene in several types of human cancer (11, 12). LARP4B is a cytosolic protein, which interacts with the cytosolic polyA-binding protein 1 (PABPC1) and the receptor for activated C kinase (RACK1), a component of the 40S ribosomal subunit (13). Schaffler and colleagues also showed that overexpression of LARP4B stimulated protein synthesis, whereas a knockdown of LARP4B impaired translation of a large number of mRNAs, suggesting a stimulatory role for LARP4B in translation (13). In the present study, we identified LARP4B as a potential tumor-suppressor gene in gliomas. An analysis of cancer database information suggested that LARP4B participates in the pathogenesis of human glioma, and cellular analyses revealed its function as a tumor suppressor.
Materials and Methods

Retroviral expression vectors

pMXs-ires-Puro (pMXs-IP; Cell Biolabs, Inc.) and MSCV-LTRmiR30-PIG (LMP; Thermo Scientific) were used for the overexpression and knockdown experiments, respectively. Details on the vector construction strategies are described in Supplementary Materials and Methods.

Glioma cell lines

Five human glioma cell lines were obtained from the ATCC: U-87MG (ATCC HTB-14), U-118MG (ATCC HTB-15), U-138MG (ATCC HTB-16), T98G (ATCC CRL-1690), and A172 (ATCC CRL-1620). U-251MG was obtained from the ICRB Cell Bank (IFOS0288). All cell lines tested negative for mycoplasma contamination. The lines were authenticated by standard morphologic examination using microscopy. The glioma cell lines were cultured in DMEM containing 10% FBS and penicillin-streptomycin.

Glioma stem cells

Information regarding the patient-derived glioma stem cells (TCS-01, TCS-02, and TCS-04; ref.14) is provided in Supplementary Table S1. Glioma stem cells were cultured in DMEM/F12 supplemented with B27 (Invitrogen), 20 ng/mL EGF, and 20 ng/mL FGF (both from PeproTech).

Preparation of primary astrocytes

Primary astrocytes were harvested from the brains of neonatal C57BL6J mice as described previously (15). After 1 to 2 weeks, growing cells were retrovirally transduced with dominant-negative p53 (p53DN; ref.15) and used as immortalized astrocytes.

Retroviral infection

Retrovirus was generated with Platinum-A packaging cells (Cell Biolabs, Inc.) and used to infect target cells as described previously (5). Infected cells were selected with puromycin (2 μg/mL) for 2 days and used for proliferation assays. Details on the method used to serially infect target cells with different retroviruses are provided in Supplementary Materials and Methods.

Cell proliferation assay

LARP4B-transduced or control cells (5 x 10^4) were plated in 6-well plates in triplicate. The total number of cells was counted on day 5, and the fold change was calculated. Viable cell counting was performed using the Trypan Blue exclusion method.

Western blot analysis

HEK293T cells (ATCC) were transfected using Genelucite transfection reagent (Millipore). After 48 hours, the transfected cells were collected and used for Western blotting. Primary antibodies against HA (Covance), actin (Sigma), Larp4b (Abcam), and horseradish peroxidase–linked secondary antibodies (GE Healthcare) were used. Band intensities were quantified using ImageJ software.

Real-time PCR analysis

To analyze the expression levels of the candidate genes in Table 1, total RNA was extracted from NSCs, glioma stem cells, and cell lines using Sepazol (Nacalai Tesque, Inc.). cDNA was synthesized with ReverTra Ace (Toyobo), and qPCR was performed using TaqMan assays (Invitrogen; Supplementary Table S2). All other qPCR experiments were performed with the primers shown in Supplementary Table S3 using the LightCycler 96.

Table 1. Genomic alterations in candidate tumor-suppressor genes in human glioblastoma

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<tr>
<th>Gene</th>
<th>%Homo_Del</th>
<th>%Hetero_Del</th>
<th>%Diploid</th>
<th>%Low_Amp</th>
<th>%High_Amp</th>
<th>%Mut</th>
<th>Expression</th>
<th>P val (adjusted)</th>
<th>Cox P val (adjusted)</th>
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Abbreviation: N/A, data not available in the TCGA dataset.

*The percentage (%) of tumors with homozygous deletion (Homo_Del), heterozygous deletions (Hetero_Del), diploid, low amplification (Low_Amp), high amplification (High_Amp), and somatic mutation (Mut). The total number of tumor samples analyzed for copy-number alterations and mutations are 565 and 283, respectively.

*Correlation between number alterations and expression changes was analyzed between those tumor samples in which it is deleted versus amplified/normal ones. P values were calculated according to the two-sample t test and adjusted for multiple testing using the Benjamini–Hochberg method in the R project function p.adjust. The total number of tumor samples analyzed for expression changes is 147.

*Correlation between copy-number alterations and clinical status (Kaplan–Meier survival) was analyzed between those tumor samples in which it is deleted versus amplified/normal ones. P values were calculated according to the Cox regression analysis and adjusted for multiple testing using the Benjamini–Hochberg method in the R project function p.adjust. The total number of tumor samples analyzed for Cox regression analysis is 565.

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Immunohistochemical analysis

Actin staining was performed with rhodamine-phalloidin (Wako). For analysis of the brain, perfusion fixation was performed prior to dissection, and the brain was fixed in 4% PFA. For immunostaining, sections were incubated with primary antibodies against GFP (Abcam), S100b (Sigma), Gfap (Sigma), or CD31 (BD), and then incubated with the appropriate secondary antibodies. EdU staining was performed using Click-iT Assay Kits (Invitrogen) according to the manufacturer’s protocol. Nuclei were counterstained with DAPI. Stained cells were observed with an inverted fluorescence light microscope (Zeiss), and images were acquired with AxioImager software.

Cell-cycle analysis

Cells were harvested on day 5 after plating, fixed in 70% ethanol, and incubated with 33 μg/ml ribonuclease A. Next, the cells were resuspended in 1% propidium iodide (PI) to label the DNA. DNA content was measured using a FACSCalibur instrument (BD) and analyzed with FlowJo software.

Cell apoptosis analysis

Cells were harvested at day 5 after plating and stained with Annexin V–FITC (BioVision) and PI. Stained cells were analyzed by using FACSCalibur (BD), and the data were analyzed with CellQuest software.

RNA immunoprecipitation-PCR

Cells were harvested 2 days after plating and lysed as described previously (16). Immunoprecipitation was performed using anti-HA magnetic beads (MBL). Coprecipitated RNA was isolated from the beads and input control using phenol extraction. cDNA was generated, and qPCR was performed with the primers listed in Supplementary Table S3.

Animal experiments

A total of 5 × 10⁶ of immortalized astrocytes transduced with either shRNA against luciferase (shLuc) or Larp4b were injected subcutaneously into the flank of three recipient C57BL6/J mice. The size of each tumor was measured every week until week 9, and the tumor volume was estimated by multiplying the length, width, and height. For intracranial transplantation, 2 × 10⁵ cells were injected into the striatum of C57BL6/J mice on postnatal day 7 (P7). After 2 or 8 weeks, histologic analyses were performed. All procedures were performed with institutional approval (IMSUT).

Results

LARP4B is a candidate tumor-suppressor gene in glioma

We previously identified more than 100 candidate cancer genes for glioma using transposon-based insertional mutagenesis (5). Among these, we focused on 88 genes predicted to function as tumor-suppressor genes based on transposon insertion patterns (Supplementary Table S4). To investigate the possibility that these genes function as tumor suppressors in human glioma, we first examined DNA copy-number alterations using 577 human glioblastoma samples in the TCGA database. Copy-number loss was more frequently observed than copy-number gain for 54 genes (Supplementary Table S4). A total of 17 genes had copy-number loss in more than 20% of cases (Table 1), and these genes included well-established tumor-suppressor genes such as PTEN and QKI (17, 18), as well as genes that had been previously unreported as tumor suppressors. Notably, four genes (VTI1A, DDX50, MLLT10, and LARP4B) showed heterozygous deletion in more than 80% of glioblastoma samples (Table 1, %Hetero_Del). We next analyzed the relationship between copy-number loss and gene expression levels. Tumors with copy-number loss had much lower expression levels of these genes than did tumors with no copy loss (Table 1, Expression p-val). We also investigated the correlation between copy-number changes and patient survival, and found that a low copy-number of five genes (PTEN, VTI1A, DDX50, MLLT10, and LARP4B) predicted poor patient survival (Table 1, Cox p-val). We next compared the expression levels of the 17 genes in Table 1 between normal NSCs and glioma. We used two NSC lines differentiated from two human iPS cells (tkCB_Sev9 and tkDA_3-4; ref 19) and one commercially available NSC line derived from an embryonic stem cell (ES) line. We also used three glioma stem cells (14) and six glioma cell lines. Real-time PCR analysis revealed that the expression levels of 15 genes were significantly decreased in glioma cells compared with normal NSCs (Fig. 1A and B), consistent with their putative tumor-suppressor functions.

We next focused on human LARP4B, which is located at 10p15.3 and is frequently deleted in glioblastoma (Fig. 1C) because its role in glioma has not yet been reported. Transposon insertions in the Larp4b gene locus were distributed throughout the gene, with little orientation bias (Fig. 1D), suggesting a tumor-suppressor role for Larp4b. In addition, when we looked at the TCGA database, heterozygous deletion of LARP4B was found in 80.9% of glioblastoma cases, whereas homozygous deletion was only found in 0.7% (Table 1). DNA copy-number status was correlated with LARP4B expression levels in glioblastoma samples (Fig. 1E). Furthermore, LARP4B loss was inversely correlated with patient survival (Fig. 1G), indicating that decreased LARP4B expression is a negative prognostic factor for human glioblastoma patients. Analysis of the methylation status of the LARP4B gene locus using the TCGA dataset (2) revealed that LARP4B was methylated in all of the 59 tumors with heterozygous LARP4B deletion had LARP4B DNA methylation, suggesting that the remaining allele may be transcriptionally silenced. An analysis of low-grade glioma samples in the TCGA database (Supplementary Table S5) also revealed that LARP4B loss was correlated with decreased expression and poor patient survival in low-grade glioma (Fig. 1F and H). Other LARP family genes (LARP1, 1B, 4, 6, and 7) were not significantly downregulated in glioma compared with NSCs (Supplementary Fig. S1A and S1B). These LARP family genes are mostly (~80%) diploid in glioblastoma.

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Koso et al.
Supplementary Table S6 and Supplementary Fig. S1C), and copy-number losses of these genes were not correlated with poor patient survival (Supplementary Fig. S1D). These data are consistent with the hypothesis that LARP4B serves as a tumor suppressor in human glioma.

LARP4B overexpression suppressed proliferation of glioma cell lines

To further investigate the tumor-suppressor function of LARP4B, we first examined whether transduction of LARP4B suppresses glioma cell growth by overexpressing LARP4B in human glioma cell lines. We generated an N-terminal HA-tagged LARP4B (Fig. 2A and B). We then retrovirally transduced either HA-LARP4B or the empty vector control into the glioma cell lines. The same number of infected cells was plated, and the cell number was determined on day 5. The number of control U87MG glioma cells increased continuously, resulting in a nearly 10-fold increase in total cell number (Fig. 2C and D; Supplementary Fig. S2A). In sharp contrast, proliferation of U87MG cells transduced with HA-LARP4B was greatly suppressed; the total cell number remained almost equal to the initial number (Fig. 2C and D). Similar growth suppression was observed for three other glioma cell lines, U87MG, U118MG, and A172, and two glioma stem cells, TGS-01 and TGS-04 (Fig. 2C and D; Supplementary Fig. S2A and S2B).
However, such growth suppression was moderate for U251MG glioma cells, and T98G glioma cells continued to proliferate even after the transduction of LARP4B (Fig. 2C and D), indicating that the growth-suppressive effect of LARP4B is cell-line dependent.

LARP4B suppressed glioma cell growth by inducing mitotic arrest and apoptosis

The strong growth suppression of glioma cells by LARP4B suggested that glioma cells may have entered senescence after LARP4B transduction. One of the cardinal features of the senescent phenotype is a change in cellular morphology (20). Actin staining of the glioma cell lines in which LARP4B suppressed cell growth confirmed a flat and enlarged morphology (Fig. 3A). Notably, LARP4B overexpression increased the number of multi-nucleated cells in the U138MG cell line (Control: 5.8%, LARP4B: 30.1%, P < 0.001; Fisher exact test), suggesting that mitosis was deregulated by LARP4B. The proportion of multi-nucleated cells was not significantly altered in other lines. We next performed cell-cycle analysis (Gating strategies are shown in Supplementary Fig. S3A). The proportion of cells in the G2–M phase was significantly increased by LARP4B overexpression in U87MG, U118MG, and U138MG glioma cell lines (Fig. 3B and D). Hyper-diploid cells were increased in U87MG by LARP4B (Fig. 3B and E), suggesting a premature exit from mitosis (21). Annexin V and PI staining showed that the fraction of Annexin V–positive and PI-negative apoptotic cells increased in three cell lines (U87MG, U118MG, and A172; Fig. 3C and F; gating strategies are shown in Supplementary Fig. S3B). Taken together, these data...
Figure 3.
LARP4B overexpression induces mitotic arrest and apoptosis of glioma cell lines. A, glioma cell lines transduced with HA-LARP4B were stained with rhodamine-phalloidin to visualize actin filaments. Arrowheads, multinucleated cells. Scale bars, 50 μm. B, cell-cycle analysis. Green, yellow, and blue histograms indicate G1, S, and G2–M phases of the cell cycle, respectively. Percentage of hyper-diploid cells is indicated in U87MG. C, analysis of apoptosis. Horizontal and vertical axes represent Annexin V expression and PI staining, respectively. D, the fractions of cells in the G2–M phase are shown for control and LARP4B-transfected glioma cells. E, percentage of hyper-diploid cells in U87MG cells (B). F, the fractions of apoptotic cells (red squares in C) are shown for control and LARP4B-transfected glioma cells. Data represent mean ± SEM (n = 3 per group). Student t test, *, P < 0.05; **, P < 0.01; ***, P < 0.001. Data are representative of two independent experiments. Gating strategies are shown in Supplementary Fig. S3A and S3B.
indicate that LARP4B suppressed glioma cell growth by inducing mitotic arrest and apoptosis.

p53 activity is partially required for LARP4B-mediated growth suppression

We next investigated the molecular mechanisms involved in LARP4B-mediated growth suppression. Mammalian CDK activity is essential for driving cell-cycle phases, and CDK activity is regulated by two families of inhibitors: INK4 proteins and the Cip and Kip family proteins (22). We first examined the expression of all INK4 genes and all Cip and Kip family genes in glioma cells transduced with LARP4B. We found that CDKN1A (also known as p21CIP1) was upregulated in three of four glioma cell lines and two glioma stem cells after LARP4B transduction (Fig. 4A); however, the expression of other CDK inhibitors was not affected. In addition to mitotic arrest, LARP4B induced apoptosis in glioma cell lines. Apoptotic cell death is regulated by proapoptotic Bcl2 family members: BAX and its close relative BAK1 (23). qPCR analysis revealed that BAX expression was significantly increased in all glioma cell lines and stem cells transduced with LARP4B (Fig. 4B). Cell-cycle arrest and apoptosis are often regulated by the activation of the p53 pathway in many types of cancer, and CDKN1A and BAX are both target genes of the p53 transcriptional factor (24), raising the possibility that p53

![Figure 4](https://cancerres.aacrjournals.org/content/76/8/2260/F4.large.jpg)

Figure 4. p53 activity is partially required for the growth suppression of LARP4B. A and B, CDKN1A and BAX expression levels were analyzed on day 1. GAPDH was used as a normalization control. C and D, glioma cell lines transduced with either p53DN or the vector control were then transduced with either HA-LARP4B or the vector control. The morphology of the cells was observed on day 5 (C). Scale bars, 200 μm. The fold change was calculated on day 5 (D). Data represent mean ± SEM (n = 3 per group). Student t test, *, P < 0.05; **, P < 0.01; †††, P < 0.001. Data are representative of two independent experiments.
activity may be involved in LARP4B-mediated growth suppression. To test this possibility, we examined the effect of a p53 knockdown on LARP4B-mediated growth suppression by transfecting the dominant-negative form of p53 (p53DN; ref. 25) into the four LARP4B-sensitive glioma cell lines. The proliferation of both p53 wild-type (WT) cell lines (U87MG and A172; refs. 26, 27) was partially rescued by p53DN after LARP4B transduction (Fig. 4C and D), indicating that p53 activity is partially required for the LARP4B-mediated growth suppression in these cell lines. In contrast, one of the p53-mutant cell lines (U118MG and U138MG; refs. 26, 27) did not respond to p53DN (Fig. 4C and D), suggesting that the growth-inhibitory effect of LARP4B is p53 mutation-dependent (28).

The RNA-binding ability of LARP4B contributes to its growth-inhibitory function

LARP family proteins are characterized by their La module, which enables the binding of LARP proteins to RNA. To examine whether the La module is essential for the growth-suppressive functions of LARP4B, we created a deletion mutant that lacked the La module (LARP4BΔLM; Fig. 5A and B). Compared with the case with full-length LARP4B, growth suppression was greatly reduced by the deletion of the La module (Fig. 5C and D), indicating that LARP4B inhibits glioma cell growth through an interaction with RNA. As deletion of the La module did not completely rescue LARP4B’s growth suppression, LARP4B may also interact with proteins that contribute to the inhibition of glioma cell growth.

LARP4B associates with BAX mRNA

A recent study identified RNA targets of LARP4B in HEK293 cells using PAR-CLIP, a transcriptome-wide crosslinking method for RNA-binding proteins (16). CDKN1A and BAX were included in the list of candidate RNA targets of LARP4B. To validate specific interactions between LARP4B and CDKN1A and BAX mRNAs, we used RNA immunoprecipitation followed by RT-PCR analysis (RIP-PCR). LARP4B strong targets (EEF2 and RALY) and low-ranked targets (DDX6 and TOP2A) were used as positive and negative control, respectively. Student t test, **, P < 0.01; ††, P < 0.001. Data represent mean ± SEM (n = 3 per group). Data are representative of two independent experiments.
indicating that a direct interaction between LARP4B and BAX mRNA may have contributed to BAX upregulation.

**Larp4b knockdown promoted tumor formation of primary astrocytes**

To further confirm the tumor-suppressor function of Larp4b, we next examined whether a Larp4b knockdown promoted tumor formation. Primary astrocytes were immortalized by p53DN and then retrovirally transduced with shRNA against the Nf1 tumor-suppressor gene (5), which is frequently mutated in human glioblastoma (29). The p53 and Nf1-deficient astrocytes were then infected with a retrovirus that expressed shRNA against Larp4b. Decreased expression of Larp4b was confirmed by qPCR and Western blot experiments (Fig. 6A and B). Immunostaining confirmed the expression of S100b and Gfap, markers for astrocytes in these cells (Fig. 6C). Analysis of *in vitro* proliferation showed that Larp4b silencing increased proliferation of primary astrocytes (Fig. 6D and E). We next analyzed the effects of Larp4b knockdown on *in vivo* proliferation. Five million cells were subcutaneously transplanted into C57BL6/J mice. Compared with the control p53 and Nf1-deficient astrocytes, the knockdown of Larp4b increased the average tumor size (Fig. 6F), although the difference was not significant for sh1. Intracranial injection of these astrocytes revealed EdU incorporation and S100b expression at 2 weeks after transplantation (Supplementary Fig. S4A and S4B). Notably, tumor invasiveness was increased by Larp4b knockdown (Supplementary Fig. S4C). After 8 weeks, proliferative lesions were induced by Larp4b knockdown (Supplementary Fig. S4D and S4E). The maximal diameter and the cross-sectional area of the lesions were both increased by Larp4b knockdown (Supplementary Fig. S4F), although the difference was significant only for sh2. Kaplan–Meier survival analysis revealed that Larp4b silencing (sh2) significantly shortened lifespan (Supplementary Fig. S4G). These data indicate that Larp4b loss cooperates with the deficiency of p53 and Nf1 in promoting tumor cell growth.

**Discussion**

In this study, we identified LARP4B as a tumor-suppressor gene in glioma. The pattern of transposon insertions in glioma suggests a tumor-suppressor role for Larp4b. Genetic examination of LARP4B in human gliomas available in the TCGA database showed that a heterozygous deletion of LARP4B is a common event in glioma, supporting the notion that LARP4B may function as a tumor suppressor. Importantly, LARP4B loss was correlated with decreased LARP4B expression, and was negatively correlated...
with patient survival, indicating that LARP4B loss has a prognostic value.

We found that Larp4B has been included as a potential cancer-related candidate gene in other transposon mutagenesis screens, including liver cancer (30), colorectal cancer (31), pancreatic cancer (32), malignant peripheral nerve sheath tumors (33), and medulloblastoma (34), raising the possibility that Larp4b is involved in the development of a wide variety of cancers. The patterns of transposon insertions in gastrointestinal tumors (31) and liver cancer (30) support its role as a tumor suppressor (Supplementary Fig. S5A and S5B). A recent study used shRNA to almost completely knockdown endogenous Larp4b in leukemia stem cells and found that proliferation was impaired (35), which is in contrast to our finding that decreased Larp4b levels promoted proliferation of primary astrocytes as well as tumor formation. The discrepancy between these phenotypes may be explained by the idea that a certain Larp4b expression level is critical, and complete depletion of Larp4b might be toxic, even to cancer cells. Another possibility is that Larp4b may function differentially in different types of cancer. In fact, transposon mutagenesis screens have identified Larp4b as a candidate cancer gene for many types of solid tumors (5, 30–34); however, Larp4b has not been identified in acute myeloid leukemia (AML; ref. 36). Moreover, LARP4B is diploid in approximately 98% of human AML cases (Supplementary Table S7), supporting the notion that LARP4B does not act as a tumor suppressor in AML.

LARP4B transduction induced strong growth suppression in several glioma cell lines; however, such growth suppression was modest for U251MG glioma cells, and T98G continued to proliferate even after LARP4B transduction. This difference cannot be explained by differential expression of endogenous LARP4B in glioma lines, as LARP4B expression levels in glioma lines were not correlated with the growth-inhibitory effects of LARP4B. A possible cause for this resistance may be defects in the molecular mechanisms regulating mitotic arrest and apoptosis, by either genetic or epigenetic alterations. We found that p53 activity was partially responsible for the growth-suppressive effects of LARP4B in p53 WT cells lines; however, p53 status alone is not sufficient to explain the difference in phenotype because the LARP4B-sensitive cell lines U118MG and U138MG express mutant p53 (26, 27). A previous study showed that CDKN1A (p21^{CIP1}) induction was responsible for the differential response of glioma cell lines to TGFβ-mediated growth suppression (37). The U87MG line is sensitive to TGFβ by inducing CDKN1A expression; however, the T98G and U251MG lines are not sensitive to TGFβ because CDKN1A induction does not occur in these glioma cell lines (37). Notably, T98G and U251MG are less sensitive to LARP4B than the other four lines, and most of the glioma lines that are sensitive to LARP4B expressed CDKN1A at high levels, supporting the notion that CDKN1A induction is responsible for the difference in the response of glioma cell lines to LARP4B.

The LARP4B-mediated growth suppression was partially rescued by p53DN; however, the rescue effect was quite modest even in p53 WT cell lines, indicating that LARP4B is acting mainly through p53-independent pathways. We found that the RNA-binding ability of LARP4B contributed to its growth-inhibitory function, indicating that LARP4B suppresses glioma cell growth through an interaction with RNA. Deregulated expression of RNA-binding proteins is commonly observed in cancer (38), and emerging evidence indicates causative roles for the dysregulation of RNA-binding proteins in cancer progression (39). Alternative splicing of mRNA precursors (pre-mRNA) is one of the mechanisms through which dysregulation of RNA-binding proteins confers growth advantage to cancer cells, because cancer cells favor the production of protein isoforms that promote growth and survival (17, 40). Another mechanism through which RNA-binding proteins regulate cancer progression involves changes in mRNA stability (41). A recent transcriptome-wide analysis of LARP4B target genes showed that LARP4B binds mainly to mature mRNAs at their 3’ untranslated regions, resulting in stabilization of the target mRNAs at the posttranscriptional level with corresponding changes in protein levels (16). As we observed a direct interaction between LARP4B and BAX mRNA, LARP4B transduction may have stabilized BAX mRNA, which in turn caused apoptotic phenotypes in glioma cells. Interestingly, we observed cell-type–specific interactions between LARP4B and CDKN1A mRNA, which can be explained by the difference in expression levels of other interacting proteins, as PABPC1, an LARP4B-interacting partner (13), serves as a scaffold for those proteins, including PAN3, PAIP1, PAIP2, and TNRC6A, which influence the stabilization of mRNAs (42).

In addition to BAX, the PAR-CLIP experiment identified a large number of RNA targets of LARP4B in HEK293 cells (16). A Gene Ontology term enrichment analysis of the top 100 genes ranked by the number of T-to-C transitions identified biologic processes associated with translation (P = 3.7 \times 10^{-6}) and RNA splicing (P = 6.5 \times 10^{-2}) as being highly significant; however, pathways associated with cell-cycle regulation and apoptosis were not identified. Kuspert and colleagues used full-length LARP4B for the PAR-CLIP experiment (16), suggesting that some RNAs identified in their study interacted with LARP4B at regions outside the La module. As the La module is essential for the growth-inhibitory effect of LARP4B, identification of the RNA targets of LARP4B specifically associated with the La module would provide important insight into the mechanisms through which LARP4B induces growth inhibition in glioma.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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