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LABORATORY INVESTIGATION

Functional analysis of the DEPDC1 oncoantigen in malignant glioma and brain tumor initiating cells

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Abstract DEP domain containing 1 (DEPDC1) is a novel oncoantigen expressed in cancer cells, which presents oncogenic activity and high immunogenicity. Although DEPDC1 has been predicted to be a useful antigen for the development of a cancer vaccine, its pathophysiological roles in glioma have not been investigated. Here, we analyzed the expression and function of DEPDC1 in malignant glioma. DEPDC1 expression in glioma cell lines, glioma tissues, and brain tumor initiating cells (BTICs) was assessed by western blot and quantitative polymerase chain reaction (PCR). The effect of DEPDC1 downregulation on cell growth and nuclear factor kappa B (NFκB) signaling in glioma cells was investigated. Overall survival was assessed in mouse glioma models using human glioma cells and induced mouse brain tumor stem cells (imBTSCs) to determine the effect of DEPDC1 suppression in vivo. DEPDC1 expression was increased in glioma cell lines, tissues, and BTICs. Suppression of endogenous DEPDC1 expression by small interfering RNA (siRNA) inhibited glioma cell viability and induced apoptosis through NFκB signaling. In mouse glioma models using human glioma cells and imBTSCs, downregulation of DEPDC1 expression prolonged overall survival. These results suggest that DEPDC1 represents a target molecule for the treatment of glioma.

Keywords DEPDC1 · Oncoantigen · Malignant glioma · Vaccine therapy · Molecular target therapy

Introduction

Malignant gliomas exhibit aggressive infiltrative growth patterns and are the most prevalent malignant tumors of the central nervous system. The standard therapy for glioblastoma (GBM) patients is a combination of surgery, radiation, and chemotherapy using temozolomide (TMZ), and despite advancement, the prognosis remains very poor [1]. Recent treatment advances involving molecular targeting therapy and new modalities have also had a limited impact [2–4].

While the initial treatment shows a certain results, remaining tumors recur and become resistant to the drug in GBM patients. Brain tumor initiating cells (BTICs)/brain tumor stem cells (BTSCs), in particular, are thought to be important for tumor maintenance, survival, and recurrence [5–8]. Therefore, identification of new drug targets for tumors and their BTICs/BTSCs are essential for developing effective therapies against GBM [9–14].

Oncoantigens are tumor associated molecules that have oncogenic activities and a high ability to induce cell-mediated and antibody-mediated immune responses [15]. However, there are few reports analyzing of oncoantigens in glioma. DEP domain containing 1 (DEPDC1), a novel oncoantigen, was first reported to be upregulated in bladder cancer [16] and a cancer peptide vaccine has been used to target this protein [17]. More recently, DEPDC1 was reported to be overexpressed in other malignant tumors

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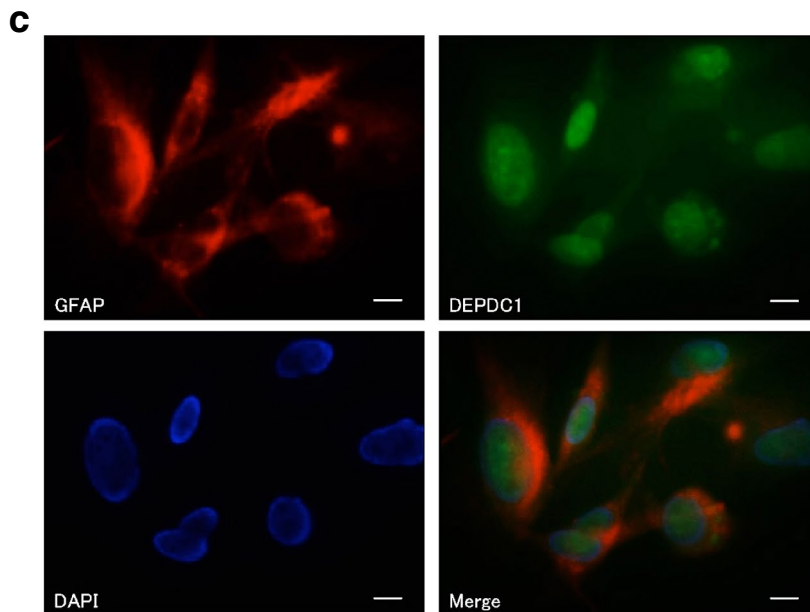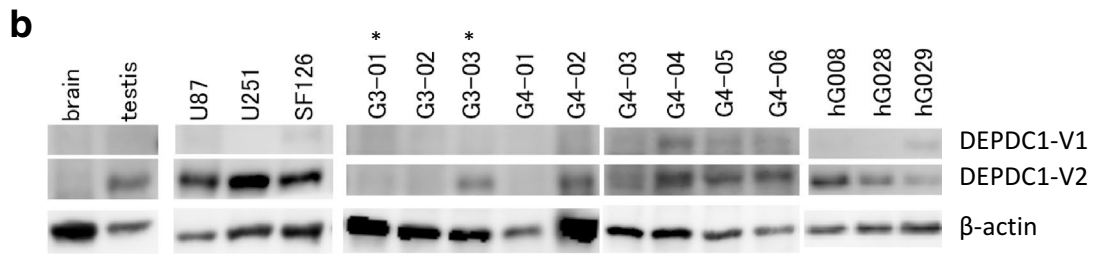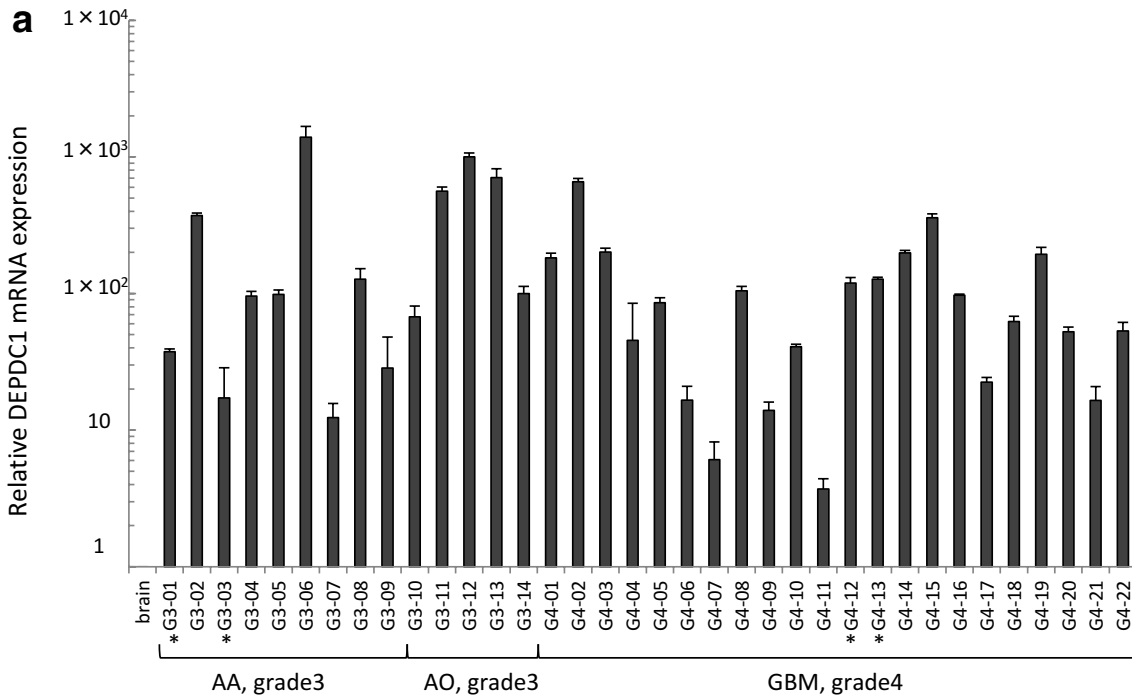
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◄**Fig. 1** DEPDC1 is highly expressed in glioma cell lines and tissue. **a** Quantitative PCR analysis of *DEPDC1* gene expression in glioma tissue and normal brain tissue. DEPDC1 expression level was normalized to GAPDH level in each sample and calculated as the threshold cycle (CT) value in each sample divided by the CT value in the normal brain. Mean ± SD (*bars*) for at least three independent experiments are shown. *Recurrent case. **b** Western blot analysis of DEPDC1 expression in normal brain tissue (negative control), testis tissue (positive control), three glioma cell lines (U87, U251, and SF126), nine glioma tissues, and three BTICs. DEPDC1 has two transcriptional variants, DEPDC1 isoform a (DEPDC1-V1) and isoform b (DEPDC1-V2). β-Actin loading controls from the same blots are shown in the *lower panels*. *Recurrent case. **c** Immunofluorescent cell staining of DEPDC1 in U251 glioma cells. U251 cells were immunoprobed using DEPDC1 and GFAP antibodies, followed by FITC and RITC-conjugated secondary antibodies. Nuclei were stained with DAPI. *Scale bar* 10 µm

including breast cancer, multiple myeloma, hepatocellular carcinoma, and colorectal cancer [18–21], but was not expressed in any of normal human organs except the testis [16]. However, the pathophysiological roles of DEPDC1 have not been investigated in glioma.

Induced mouse brain tumor stem cells (imBTSCs) are purified from tumors formed by cells established through the transduction of Ink4a/Arf–null neural stem/progenitor cells with a retroviral vector for the oncoprotein H-RasV12. imBTSCs retain stem cell characteristics such as self-renewal and differentiation ability [22, 23]. With these cells, the syngeneic mouse glioma model shows hallmark features of glioblastoma including cellular and histological heterotypia, diffuse brain infiltration, hemorrhage, and necrosis [22]. This model is immunocompetent and shows a predictable and reproducible growth pattern. Using this model it is possible to perform detailed functional analyses of target molecules.

In this study, we analyzed the expression and function of DEPDC1 in malignant glioma and BTICs in vitro. Furthermore, to determine the usefulness of DEPDC1 for molecular target therapy in vivo, we studied a mouse glioma model bearing human glioma cells or imBTSCs.

Materials and methods

Tissue samples and cell lines

All tumor tissue specimens were obtained from glioma patients who underwent surgery at the Department of Neurosurgery, Keio University School of Medicine. Written informed consent for the study was obtained from all participants. The study was approved by the local ethical review board of Keio University (No. 12-21-2). Tumors obtained from surgical cases were classified according to the 2007 World Health Organization (WHO) criteria. G3-01, G3-03, G4-12 and G4-13 were recurrent cases.

Human adult brain-tissue and testis samples were obtained from Biochain.

Glioblastoma cell lines (U87, U251, and SF126) were cultured in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) and antibiotics [50 IU/mL benzyl penicillin G potassium and 100 mg/mL streptomycin sulfate (Meiji)] at 37 °C in 5% CO_2. U87 cells were obtained from American Type Culture Collection (ATCC). U251 cells were purchased from the RIKEN Cell Bank. SF126 cells were obtained from the Japanese Collection of Research Bioresources Cell Bank. Short tandem repeat (STR) DNA profiling of U87 and U251 cells was performed using the Cell ID System (Promega), showing that U87 cells used in this study are similar to ATCC and European Collection of Authenticated Cell Cultures (ECACC) U87 cells and U251 cells are similar to ECACC U251 cells. Human BTICs (hBTICs) were obtained and cultured as previously reported [10]. imB-TSCs were cultured as previously described [23].

Western blot analysis

Cell lysates and tumor tissue specimens were prepared using RIPA buffer (Thermo Scientific) containing protease inhibitors (Cocktail Tablet; Roche Diagnostics). Total proteins of human adult normal brain and human adult normal testis were purchased from Cosmo Bio as negative and positive controls, respectively. The protein concentration of each sample was determined using a Bio-Rad protein assay kit (Bio-Rad). Identical amounts of proteins were electrophoresed in 7.5 or 10% Mini-PROTEAN TGX Precast Gels (Bio-Rad) and transferred to a nitrocellulose membrane. Blots were incubated with either mouse anti-DEPDC1 monoclonal antibody (1:200; Oncotherapy) or mouse anti-β-actin antibody (1:5000; Sigma). Immune complexes were detected with horseradish peroxidase-conjugated secondary antibodies (1:10,000; anti-rabbit; MBL International; anti-mouse; GE Health Care Biosciences) and an enhanced chemiluminescence detection system (LAS 4000 mini; GE Health Care).

RNA isolation and real-time quantitative reverse-transcription polymerase chain reaction

Total RNA from human normal brain was used as a control and purchased from Clontech. Total RNAs from tumor tissue samples were isolated using TRIzol Reagent (Life Technologies), and the concentration was determined with a NanoDrop spectrophotometer (Thermo Scientific). cDNA was synthesized from 10 µg total RNA using reverse transcriptase XL (AMV; Takara Bio). Real-time quantitative reverse-transcription polymerase chain reaction (qPCR) was performed on a StepOnePlus (Applied Biosystems,
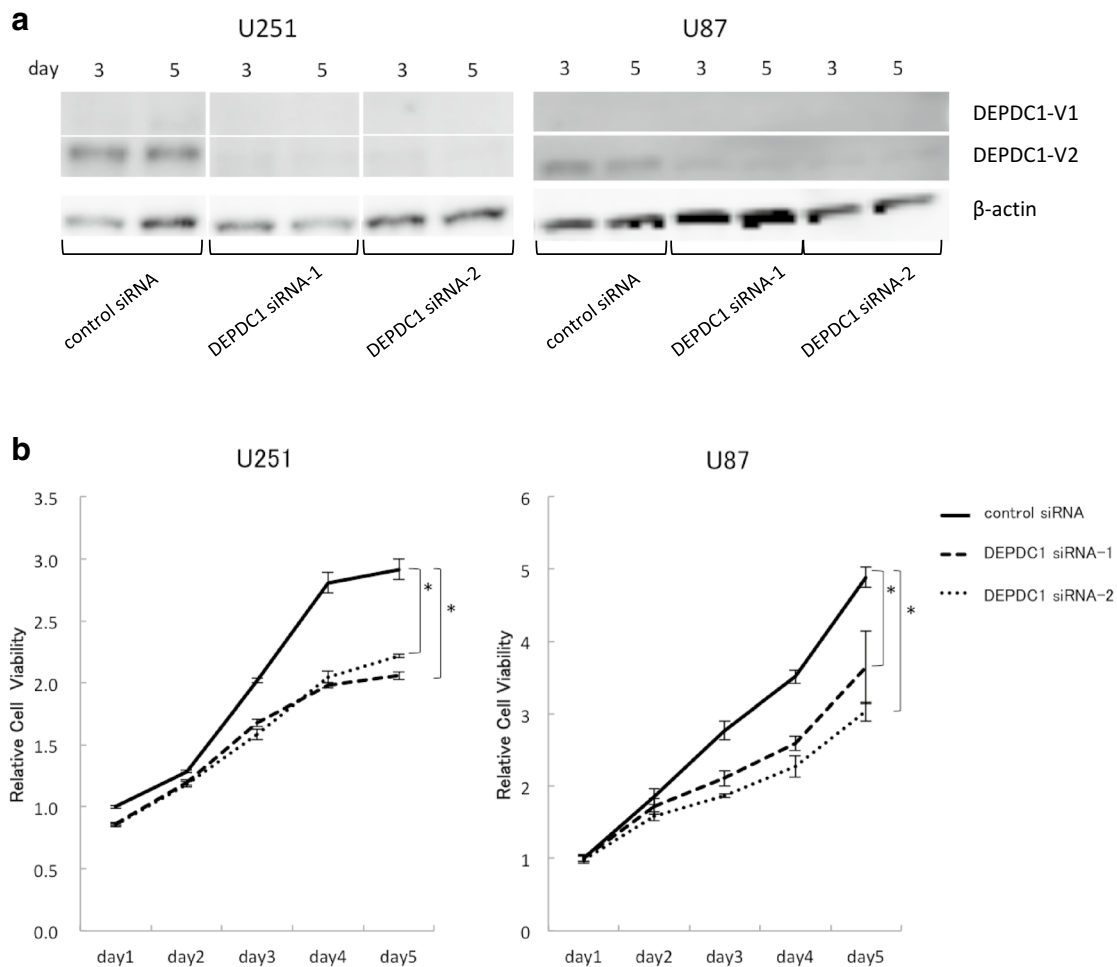
Fig. 2 Inhibition of glioma cell proliferation by DEPDC1 downregulation. **a** Western blot analysis of DEPDC1 isoform a (DEPDC1-V1) and isoform b (DEPDC1-V2) in U251 and U87 glioma cells 3 and 5 days after siRNA transfection. β-Actin was used as a loading control. **b** The effect of DEPDC1 siRNAs on cell proliferation in U251 and U87 glioma cells. Cell viability was analyzed daily up to and including the fifth day after transfection. Results are presented as fold relative to the cell viability of control siRNA-treated cells at day 1. Data are mean ± SD (*bars*) of three experiments. *P < 0.01

Thermo Scientific) using Fast SYBR Green Master Mix (Applied Biosystems). The sequences of each set of primers were shown in Supplementary Table S1. The threshold-cycle value was defined as the value obtained in the PCR cycle when the fluorescence signal increased above the background threshold.

siRNA/lentivirus-mediated shRNA gene knockdown

Two small interfering RNA (siRNA) oligonucleotide sequences corresponding to human DEPDC1 were used: siRNA1 (sense strand, 5′-GAAGUUACAAGGCAACAG ATT-3′; antisense strand, 5′-UCUGUUGCCUUGUAA CUUCTT-3′) and siRNA2 (sense strand, 5′-CGAGAUGUA UUCAGAACAATT-3′; antisense strand, 5′-UUGUUC UGAAUACAUCUCGTT-3′). Predesigned RNAi (sense strand, 5′-GUACCGCACGUCAUUCGUAUC-3′; antisense strand, 5′-UACGAAUGACGUGCGGUACGU-3′) was used as a control. The final concentration of 10 nM siRNA was incubated with Lipofectamine RNAiMax (Invitrogen) according to the manufacturer's instructions.

DEPDC1 shRNA(h) lentiviral particles (sc-78,918-V; Santa Cruz Biotechnology) were used to inhibit human DEPDC1 expression in U87 and U251 cells. DEPDC1 shRNA(m) lentiviral particles (sc-143,006-V; Santa Cruz Biotechnology) were used in imBTSCs. These lentiviral particles are a pool of concentrated, transduction-ready viral particles containing three target-specific constructs that encode 19- to 25-nucleotide (plus hairpin) short hairpin RNAs (shRNAs) designed to knockdown gene expression.
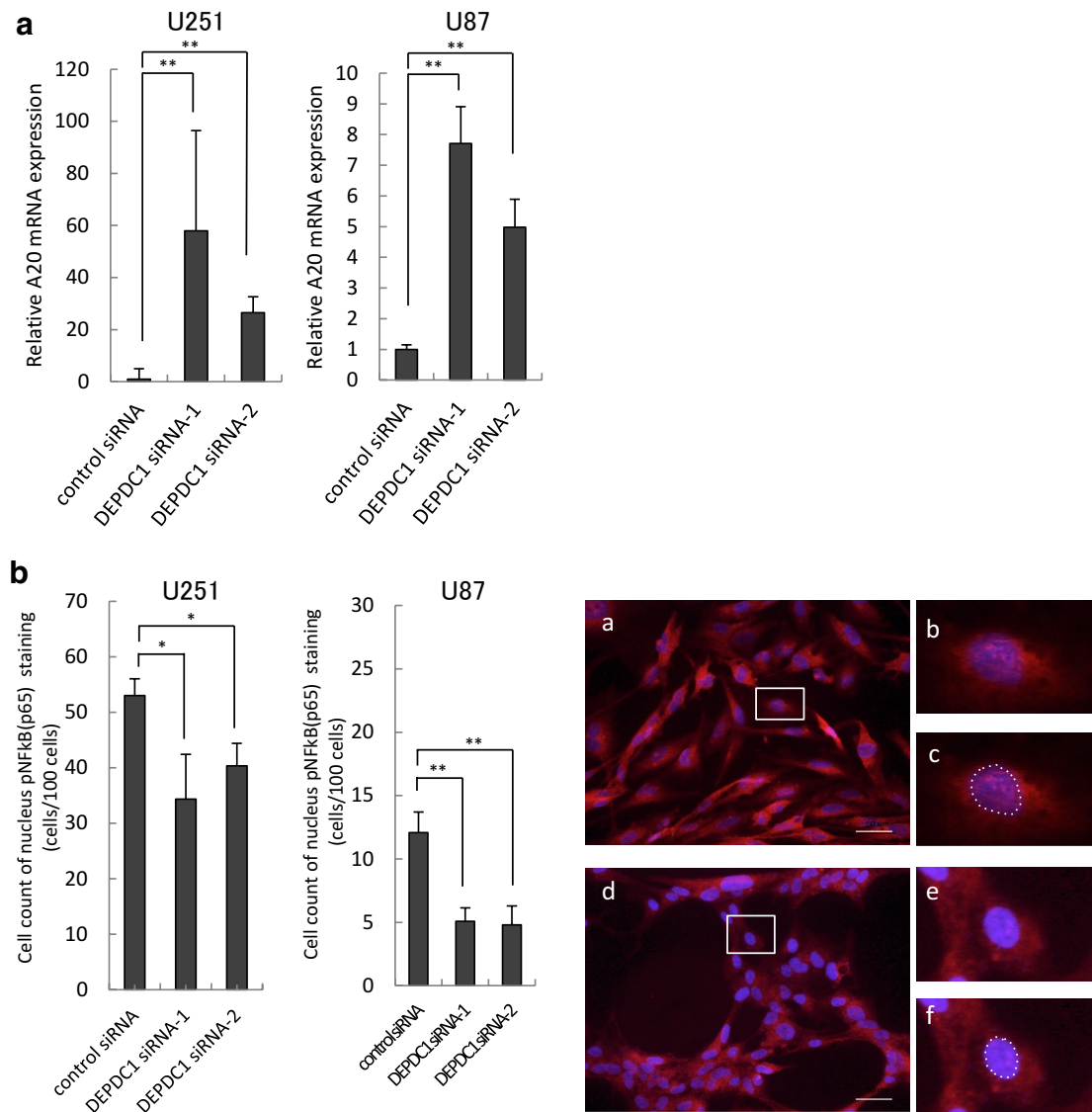
Fig. 3 Blockade of NFκB signaling by inhibition of DEPDC1. **a** Quantitative PCR analysis of *A20* gene expression in siRNA-treated glioma cells. *A20* expression level was normalized to *GAPDH* level in each sample and calculated as the threshold cycle (CT) value of each sample divided by the CT value of control siRNA-treated cells. Mean ± SD (*bars*) for at least three independent experiments are shown. *P < 0.05, **P < 0.01. **b** DEPDC1 knockdown decreases intranuclear pNFκB in glioma cells. U251 and U87 cells were immunoprobed with a pNFκB antibody 4 days after siRNA transfection, followed by rhodamine-conjugated secondary antibody. Nuclei were stained with DAPI. Intranuclear staining of pNFκB was counted in 100 cells. Mean ± SD (*bars*) for at least three independent experiments are shown. Representative images of U251 cells treated with control siRNA (*a, b, c*) and with DEPDC1 siRNA-1 (*c, d, e*) are shown. pNFκB staining was positive in the nucleus (*b*, con-

trol siRNA) and negative in the nucleus (*d*, DEPDC1 siRNA-1) of U251 cells. The surface of the nucleus is *circled by a dotted line* (*c, e*). *P < 0.05, **P < 0.01. *Scale bar* 50 μm. **c** DEPDC1 knockdown induces apoptosis in glioma cells. U251 and U87 cells were assayed by annexin V-FITC 5 days after siRNA transfection. Mean ± SD (*bars*) for at least three independent experiments are shown. *Right upper panel* showed U251 cells treated with control siRNA at a magnification of ×40. *Right bottom panel* showed DEPDC1-siRNA1 treated U251 cells at a magnification of ×40. **P < 0.01. *Scale bar* 50 μm. **d** Anti-tumor effect of combined DEPDC1 siRNAs and TMZ treatment on glioma cell growth. TMZ (100, 200, or 500 μM) was added 24 h after siRNA-transfection. Cell viability analysis was performed on 5 day after transfection. Results are presented as fold relative to the cell viability of control siRNA-treated cells. Data are mean ± SD (*bars*) of three experiments. *P < 0.05, **P < 0.01

Control shRNA lentiviral particles (sc-108,080; Santa Cruz Biotechnology) were used to confirm the transduction efficiency. After transduction according to manufacturer's

protocol, a stable cell line expressing shRNA was isolated via selection with 5 μg/mL puromycin. Gene knockdown efficiency was examined by western blot analysis.
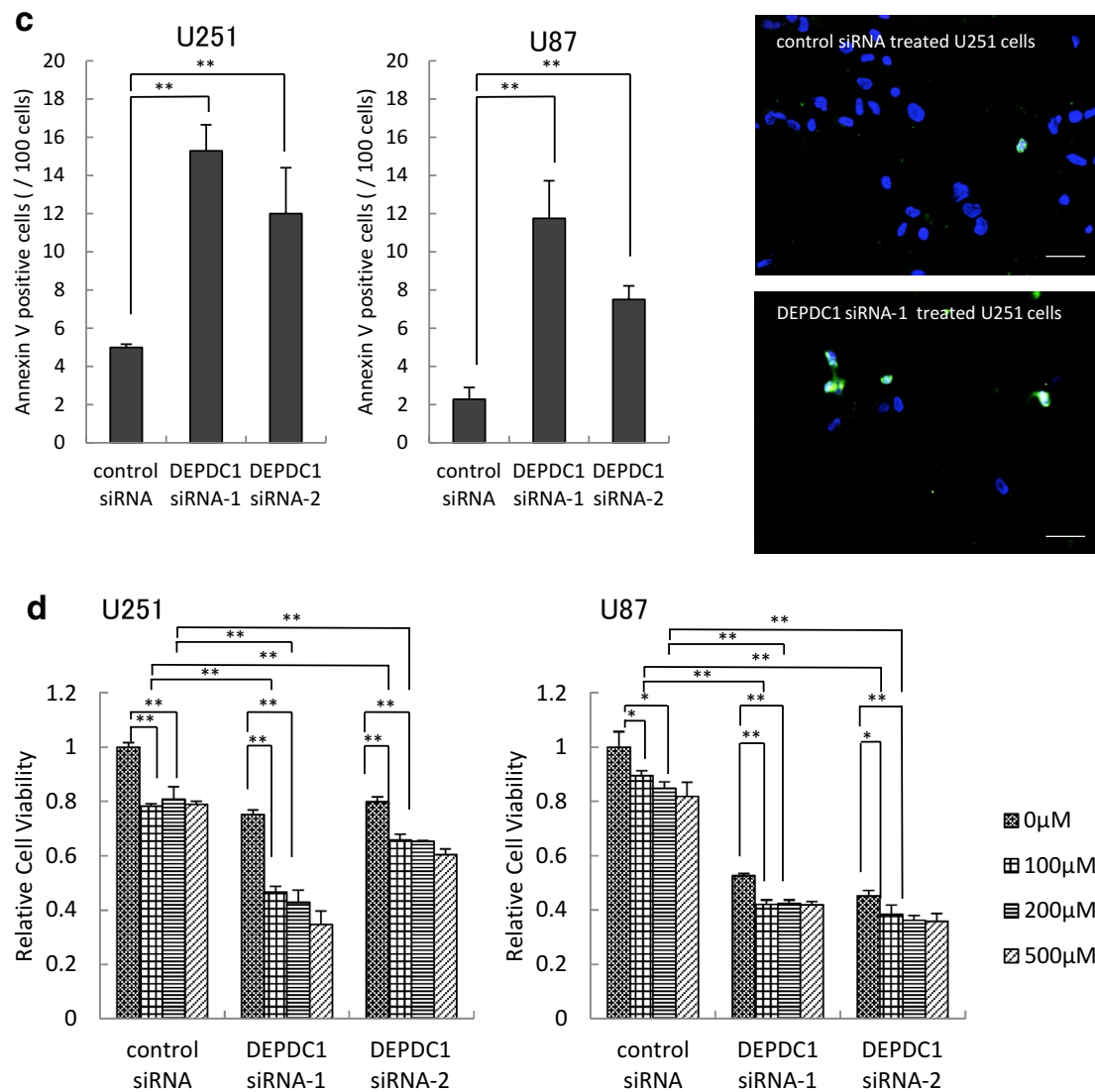
Fig. 3 (continued)

Immunofluorescent cell staining

U87 and U251 cells were seeded on eight well chamber slides (Nunc) at a density of 6×10^3 cells/well. After incubation, cells were fixed with 4% paraformaldehyde, blocked with 1% FBS, and incubated with mouse anti-DEPDC1 antibody (1:66.7; Oncotherapy). The cells were then incubated with Alexa-Fluor anti-mouse IgG. Finally, they were mounted with Vectashield with DAPI (Vector Laboratories).

For phospho-nuclear factor kappa B (pNFκB) expression analysis, cells were fixed 5 days after siRNA transfection, permeabilized, blocked, and incubated with a rabbit anti-pNFκB antibody (1:100; Cell Signaling Technology). The cells were then incubated with Alexa-Fluor anti-rabbit IgG and mounted. Fluorescent images were obtained using

fluorescence microscopy (BX51; Olympus) and camera system (VB7000; Keyence). Intranuclear pNFκB staining was counted in 100 cells.

For apoptosis analysis, cells were stained using Annexin-V-FLUOS Staining Kit (Roche Life Science) 5 days after siRNA transfection. Fluorescent images were obtained and the number of Annexin positive cells in a total of 100 cells was counted.

Cell viability assay

U251 and U87 cells were plated in 96-well plates at a seeding density of 2×10^3 cells/well. Cell viability was analyzed 3, 4, and 5 days after siRNA transfection using the Cell Titer-Glo Luminescent Cell Viability assay kit (Promega) according to the manufacturer's protocol with a
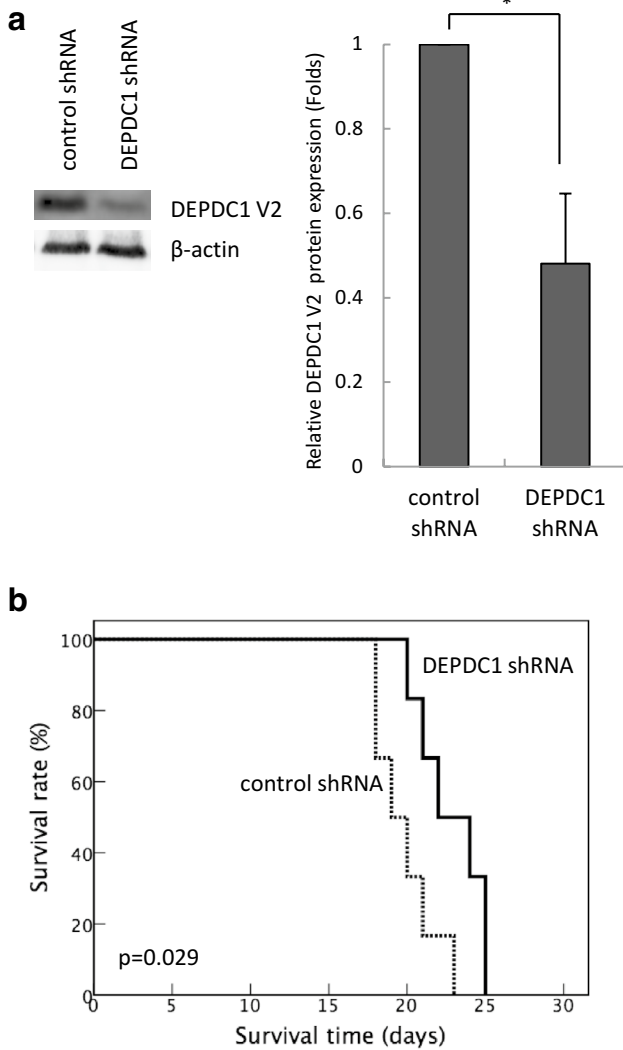
Fig. 4 Anti-tumor effect of DEPDC1 inhibition in vivo. **a** Western blot analysis of DEPDC1 expression in U87 glioma cells stably expressing control shRNA or DEPDC1 shRNA. β-Actin loading controls from the same blots are shown in the *lower panels*. Relative DEPDC1 protein expression level was quantified using Image J software (NIH, USA), and divided by the β-actin intensity in the *same lane*. Mean ± SD (*bars*) for at least three independent experiments are shown. *P < 0.05. **b** Xenogeneic mouse glioma models using U87 expressing control shRNA (n = 6) or DEPDC1 shRNA (n = 6). Mice implanted with U87 expressing DEPDC1 shRNA survived longer than those expressing control shRNAs, as evaluated by Kaplan–Meier analysis (P = 0.029). Representative data of two independent experiments are shown

luminometer (Wallac ARVO 1420 multilabel counter; Wallac Oy). Results are presented as fold relative to the viability of control siRNA-treated cells. To further investigate the efficacy of temozolomide (TMZ) and DEPDC1 inhibition, glioma cell lines were treated with siRNA, and TMZ was added 1 day after transfection. Cells were incubated for 5 days after transfection and cell viability was analyzed.

Mouse orthotopic glioma model bearing human U87 glioma cells or imBTSCs

BALB/c nu/nu mice were purchased from Sankyo Labo Service (Tokyo, Japan), and C57BL/6 J mice were purchased from Charles River Japan (Yokohama, Japan). To generate the xenogeneic mouse glioma model using human glioma cells, female 6 week-old BALB/c nu/nu mice were stereotactically injected in the right frontal lobe (2 mm lateral to the bregma) with 1×10^5 U87 cells stably expressing non-silencing shRNA or human DEPDC1 shRNA in 2 μL of Hank's balanced salt solution.

To generate the syngeneic mouse glioma model, female 6 week-old C57BL/6 J mice were stereotactically injected with 1×10^4 imBTSCs stably expressing non-silencing shRNA or mouse DEPDC1 shRNA in 2 μL of Hank's balanced salt solution as previously described [22]. All animal experiments were approved by the Institutional Animal Care and Use Committee of Keio University School of Medicine.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 software (IBM, Chicago, IL, USA). Kaplan–Meier survival analysis was performed between groups of the mouse glioma model. All other statistical analyses were performed with one-way analysis of variance (ANOVA) or Student's t test. Data are presented as the mean ± standard deviation (SD). Differences were considered statistically significant at P < 0.05.

Results

DEPDC1 expression in glioma tumor tissues, cell lines, and brain tumor initiating cells

We analyzed the expression of DEPDC1 in human malignant glioma tissues, glioma cell lines, and BTICs. RT-PCR analysis showed a higher mRNA expression of DEPDC1 in all studied glioma tissues compared with normal brain tissue (Fig. 1a). Western blot analysis also showed a higher expression of DEPDC1 in most glioma tissues, all glioma cell lines, and BTICs compared with normal brain tissue (Fig. 1b) but not in a few glioma tissues (G3-01, G3-02, and G4-01). This discrepancy might be due to degradation of DEPDC1 protein in these tissue samples. There was no apparent difference between the expression of DEPDC1 in recurrent cases and primary cases. Immunocytochemical analysis showed that DEPDC1 was localized in the nucleus of glioma cells (Fig. 1c).
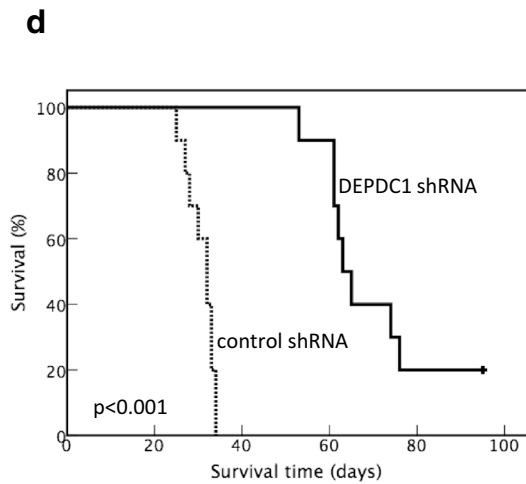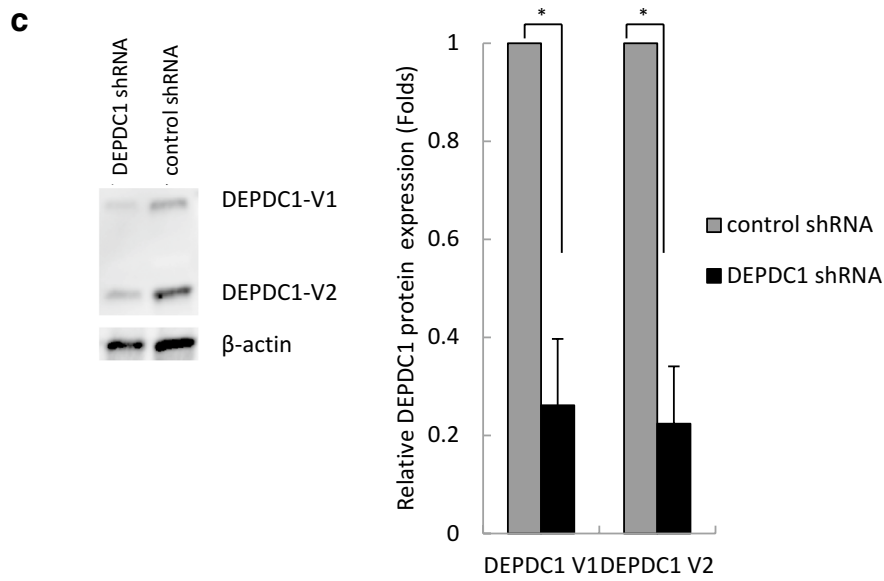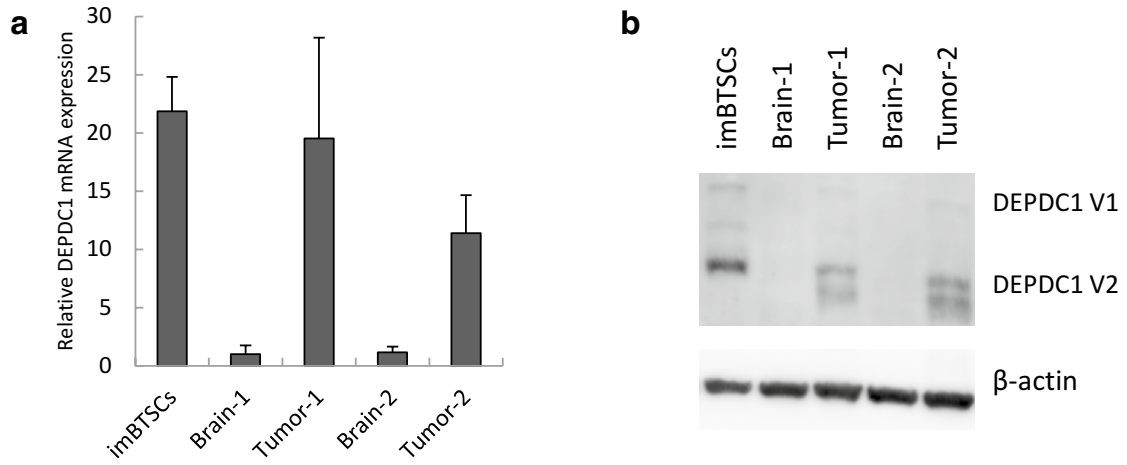
DEPDC1 has two transcriptional variants, denoted as DEPDC1 isoform a (DEPDC1-V1) and isoform b (DEPDC1-V2) [16]. The difference between the function of DEPDC1-V1 and DEPDC1-V2 is not known. In glioma, western blot analysis showed that DEPDC1-V2 was more highly expressed than DEPDC1-V1. All primers, siRNAs and shRNAs used in this study detect, or knockdown, both isoforms.

DEPDC1 knockdown inhibited glioma cell growth

To determine the role of DEPDC1 in glioma cell growth, we transfected two different siRNAs targeting DEPDC1 into U251 and U87 glioma cell lines. Endogenous DEPDC1 expression was continuously suppressed at day 3 and 5 after siRNA transfection (Fig. 2a). Cell viability analysis showed that suppression of DEPDC1 expression by siRNA significantly inhibited glioma cell growth (Fig. 2b).

DEPDC1–ZNF224 complex has been reported to repress A20 transactivation, resulting in activation of the antiapoptotic pathway through NFκB activation in bladder carcinoma cells [24]. Then, we investigated whether blockade of NFκB signaling is occurred through A20 expression by inhibition of DEPDC1 in glioma cells. Suppression of DEPDC1 expression upregulated the expression of A20, a negative regulator of the NFκB signaling pathway (Fig. 3a). Furthermore, DEPDC1 knockdown resulted in a decrease in phosphorylated NFκB nuclear staining (Fig. 3b). We further investigated whether DEPDC1 inhibition induces apoptosis. Annexin positive apoptotic cells were clearly increased by DEPDC1 knockdown (Fig. 3c).

Combined therapy of temozolomide and DEPDC1 knockdown

To reveal the role of the monofunctional methylating agent TMZ in DEPDC1 siRNA-transfected glioma cells, we assessed the effect of TMZ on the viability of U87 and U251 glioma cells treated with DEPDC1 siRNA. TMZ was added to the cells 24 h after siRNA transfection, and cell-viability analysis was performed 5 days after siRNA transfection. Compared with single treatment of either TMZ or DEPDC1 siRNA alone, combined treatment of DEPDC1 siRNA and TMZ significantly suppressed the cell viability of two glioma cell lines (Fig. 3d). This suggests that the effect of DEPDC1 knockdown on glioma cell growth is independent to that of TMZ.

Therapeutic effect by DEPDC1 knockdown in a mouse glioma model using human U87 glioma cells

To determine whether DEPDC1 inhibition suppress tumor progression in vivo, U87 cells transfected with shRNA were implanted into mouse brain. DEPDC1 shRNA caused a decrease in DEPDC1 expression in U87 cells (Fig. 4a). The xenogeneic mouse glioma model showed that inhibition of DEPDC1 expression prolonged overall survival.

Expression and functional analysis of DEPDC1 in induced mouse brain tumor stem cells (imBTSCs) and syngeneic mouse glioma models using imBTSCs

To analyze DEPDC1 function in BTICs, we assessed the expression of DEPDC1 in imBTSCs and a syngeneic mouse glioma model using imBTSCs. DEPDC1 mRNA and protein expression increased in imBTSCs in vitro and in vivo (Fig. 5a, b). We next examined the in vivo anti-tumor effect of DEPDC1 inhibition using a syngeneic mouse glioma model. imBTSCs were transfected with control or DEPDC1 shRNAs using the lentivirus system. Stable expression of DEPDC1 shRNA caused a decrease in DEPDC1 expression in imBTSCs (Fig. 5c). The mice implanted with imBTSCs transfected with DEPDC1 shRNA survived significantly longer than the control group (Fig. 5d). The syngeneic mouse glioma model showed hallmark features of glioblastoma including cellular and histological heterotypia, diffuse infiltration into the brain, hemorrhage, and necrosis. The tumor generated by imBTSCs expressing DEPDC1 shRNA in the mouse brain showed more necrotic and apoptotic cells than the tumor generated by imBTSCs expressing control shRNA (Supplemental Figure 1).

Discussion

DEPDC1 was first reported as a cancer/testis antigen in bladder cancer and plays an essential role in the growth of bladder cancer cells [16]. DEPDC1-derived peptide vaccination could induce peptide specific cytotoxic T lymphocytes in bladder cancer patients, identifying DEPDC1 as an oncoantigen [17]. The DEPDC1-ZNF224 complex represses transcription of the A20 gene, which inhibits IKK phosphorylation. This leads to the translocation of NFκB into the nucleus and results in the suppression of apoptosis in bladder cancer cells [24]. Recent reports have shown that DEPDC1 participates in anti-tubulin drug-induced apoptosis by promoting JNK-dependent degradation of the BCL-2 family protein MCL1 [25, 26]. Recently, DEPDC1 was reported to be overexpressed in other malignant tumor types including breast cancer, multiple myeloma, hepatocellular carcinoma, and colorectal cancer [16, 18–20]. These studies suggest that DEPDC1 plays a pivotal role in tumorigenesis, and might serve as a novel potential target in the diagnosis and / or treatment of various cancers.

In our study, DEPDC1 was highly expressed in high-grade glioma tumor tissues and cell lines. Furthermore, DEPDC1 was highly expressed in BTICs. These results suggest that DEPDC1 may be a useful target molecule for vaccine therapy of tumor cells and BTICs in the treatment of high-grade glioma. Peptide vaccine therapy against specific tumor associated antigens which are highly expressed in tumor tissues has been reported in several tumors and GBM [17, 27–30]. We are currently conducting a clinical trial of vaccine therapy for patients with recurrent malignant glioma using peptides of VEGF receptors and four oncoantigens, including DEPDC1, and DEPDC1-specific cytotoxic T lymphocytes were induced in these patients (unpublished data).

We showed that DEPDC1 suppression increased A20 expression, and induced a decrease intranuclear pNFκB and apoptosis in glioma cells. Our data suggest that DEPDC1 can inhibit apoptosis of glioma cells through the NFκB signaling pathway. While previous studies showed that DEPDC1 participates in the inhibition of apoptosis [16, 25] and cell cycle promotion [18, 31], DEPDC1 suppression did not affect cell cycle in glioma cells (data not shown).

TMZ induces the DNA methylation, thereby inducing mismatches during DNA replication and the subsequent activation of apoptotic pathways. The effect of combined DEPDC1 suppression and TMZ treatment on growth inhibition was additive, suggesting that they function through different pathways. Indeed, DEPDC1 suppression induced apoptosis through inhibition of NFκB signaling, which is a different anti-tumor mechanism than that of TMZ, indicating that DEPDC1 can be a useful target for the treatment of glioma.

DEPDC1 knockdown was associated with prolonged survival of mouse glioma models transplanted with U87 cells and imBTSCs. The antitumor effect induced by DEPDC1 knockdown in U87-bearing mice was statistically significant, but not strong, compared with the effect seen in vitro. This may be due to the difference in suppression of DEPDC1 expression by shRNA and siRNA as shown in Figs. 2a and 4a. In addition, an alternative pathway (or pathways) might be activated in DEPDC1 shRNA glioma cells during the long analysis period in vivo. In this study, in vivo analyses were performed by transplantation of glioma cells in which DEPDC1 expression was suppressed by shRNA ex vivo. The therapeutic effect should be further analyzed by in vivo DEPDC1 knockdown in an established glioma tumor in future studies.

DEPDC1 knockdown substantially extended the survival time of the syngeneic mouse glioma model using imBTSCs. It is possible that inhibition of DEPDC1 expression by shRNA was more effective in imBTSCs than in U87 cells as shown in Figs. 4a and 5c. In addition, the syngeneic imBTSC mouse glioma model is immunocompetent, and therefore some immunological reactions might have occurred. DEPDC1 may play a critical role in the proliferation and differentiation of imBTSCs through different mechanism. It would be important to investigate the effects of DEPDC1 inhibition on imBTSCs in future studies.

Conclusions

We demonstrated that DEPDC1 is highly expressed and plays a role in cell proliferation in glioma. Downregulation of DEPDC1 induced apoptosis through the NFκB signaling pathway in vitro and combination therapy, consisting of DEPDC1 downregulation with TMZ, showed a significant antitumor effect. DEPDC1 knockdown prolonged overall survival in brain tumor models using imBTSCs and human glioma cell. Although further analysis is required, these findings indicate that DEPDC1 is a promising therapeutic target for the treatment of glioma.

Compliance with ethical standards

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