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Original Article

DLC2 inhibits development of glioma through regulating the expression ratio of TAp73α/TAp73β

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Abstract: To date, the anti-tumor mechanism of the deleted in liver cancer 2 (DLC2) in gliomas is still unclear. The study shows that TAp73α expression and TAp73α/TAp73β ratio are frequently high in gliomas and that TAp73α and TAp73β have opposite roles in regulating proliferation and apoptosis of glioma cells. Moreover, DLC2 is low-expressed in gliomas, which negatively correlates with TAp73α expression and TAp73α/TAp73β ratio. More importantly, DLC2 inhibits development of glioma by decreasing expression of TAp73α, which changes the expression ratio of TAp73α/TAp73β in glioma cells. Mechanically, DLC2 interacts directly with TAp73α and induces TAp73α ubiquitination and degradation, which is mediated through SAM domain of DLC2 and TAp73α. In detail, DLC2 with SAM domain deletion fails to interact with TAp73α and induce TAp73α ubiquitination and degradation, and SAM deletion decreased tumorigenesis-inhibition effect of DLC2. In conclusion, DLC2 inhibits glioma development by inducing TAp73α degradation and subsequent change of TAp73α/TAp73β expression ratio.

Keywords: Glioma, DLC2, p73, proliferation, apoptosis

Introduction

Glioblastoma (GBM) is one of most malignant primary brain tumors. Despite advances in neurosurgical techniques and radio-/chemotherapy, the treatment of GBM still remains a challenge. Upon diagnosis, GBM patients' survival time on average is no more than 15 months [1]. The reality is that the mechanism of glioma's genesis and development has not been fully explored, therefore, it is significant to identify its molecular mechanism for future therapy.

The deleted in liver cancer 2 (DLC2), one of RhoGAPs, termed also as START-GAP2 or STARD13, was identified as a tumor suppresser gene, the protein of which includes a SAM, a FAT, a START and a RhoGAP domain [2, 3]. As key regulators of diverse cellular pathways, GTPases affect many crucial processes in cells such as transcriptional regulation, cell cycle progression, apoptosis, and membrane trafficking [4]. DLC2 shows Rho GTPase activating activity for the small GTPases RhoA and Cdc42 [2]. DLC2 is low-expressed or loss in many solid tumors and associated with poor out-comes of patients [3]. The over-expression of DLC2 in cancer cells induces apoptosis, growth arrest, invasion inhibition and metastasis suppression [5-9]. However, up to now, few researches has been done on its mechanism of anti-tumor effect, besides its Rho GTPase activating activity [10]. Therefore, to identify its anti-tumor mechanism may be beneficial for cancer treatment in future.

p73, one member of p53 protein family, is an important transcription factor, which has been shown to control proliferation, apoptosis, differentiation, and angiogenesis [11]. Originally, like p53, p73 is shown to induce cell growth arrest and apoptosis by activating target genes related to cell growth arrest or apoptosis, such as $p21^{Waf1}$, mdm2, Bax, cyclin G, GADD4, IGF-BP3, and 14-3-3σ [12]. Recently, it has been found that p73 presents significant differences in

terms of regulation and function from p53. This is due to the both alternative splicing and alternative promoters of p73 gene. TAp73 and DeltaNp73 are the two main isoforms generated by the alternative promoters, while TAp73 with a TA domain and DeltaNp73 without. In addition, alternative splicing generates at least seven transcripts with different carboxyl-termini (α-η) [13]. Among these isoforms, TAp73α and TAp73β are the two main expressed isoforms in human cells [14]. TAp73α antagonizes apoptosis induced by a range of death stimuli and is over-expressed in cancers, conversely, TAp73β has the opposite effect [9, 14-16]. The counteraction of the two isoforms in the apoptotic demonstrates an equilibrium, which determines the fate of cells [14, 17, 18]. However, how to regulate the balance between these two isoforms is still unclear.

In this study, we observed that expression of TAp73α and TAp73α/TAp73β ratio are high in gliomas. TAp73α and TAp73β play opposite role in regulating proliferation and apoptosis of glioma cells. DLC2 is frequently low-expressed in glioma and negatively associated with TAp73α expression and TAp73α/TAp73β ratio. In glioma cells, DLC2 inhibits development of glioma by decreasing expression of TAp73α, which changes the expression ratio of TAp73α/TAp73β in glioma cells. Moreover, we found DLC2 could interact with TAp73α, but not TAp73β, and the SAM domain is necessary for the protein-protein interaction between DLC2 and TAp73α.

Methods

Cell culture

Human glioma cell lines (U251, Shg44, A172 and T98G) and human embryonic kidney cell line (293T) were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell lines were maintained in DMEM with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA) at 37°C in humidified air containing 5% carbon dioxide.

Clinical samples

The frozen specimens (3 normal brain tissue and 11 gliomas) were from Wuxi People's Hospital of Nanjing medical university, Changzhou Wujin people's hospital and Peace hospital of Changzhi medical college. Pathological data was from medical records. The study was in accordance with approved protocols from the ethic committee of Nanjing medical university.

Online cancer database analysis

The Cancer Genome Atlas (TCGA) GBM database and REMBRANDT glioma database was used to analyze DLC2 mRNA expression in gliomas. (http://www.betastasis.com).

Western blot assays

The proteins were extracted from cells or tissues using RIPA lysis buffer with protease inhibitor (Beyotime, Shanghai, China). The subcellular fractionation was conducted according to the manufacturer's protocol using Nuclear and Cytoplasmic Extraction Reagents (THERMO, MA, USA.). The proteins were separated via SDS-PAGE gel and then transferred to polyvinylidene fluoride membranes (PVDF, Millipore, MA, USA). After blocking with 5% skim milk, the membranes were incubated with the primary antibodies [DLC2, Bax, Caspase-3, β-actin, Ub, Lamin B1 (Abcam, Cambridge, UK); Flag, Myc, His (CST, MA, USA); p73α, p73β (Sigma-aldrich, WI, USA); GAPDH (THERMO, MA,USA)] overnight at 4°C and then with the horseradish peroxidase-conjugated secondary antibody (CWBIO, Beijing, China). Protein bands were detected with enhanced chemiluminescence system (Bio-Rad, CA, USA). p73α antibody specifically recognizes human and monkey full-length p73α (TAp73α) protein. p73β antibody recognizes p73β, but not effectively blot p73α. Considering that TAp73α and TAp73β are the two main expressed isoforms in human cells, p73β antibody is used for roughly detecting TAp73β in this study.

IP and ubiquitination assays

The cells were transfected with indicated plasmids for 48 h. Cell lysates were prepared in RIPA buffer, and IP was performed as standard protocol. In the ubiquitination assay, the transfected cells were treated with 20 μM MG132 (Abcam, Cambridge, UK) for 6 h before proteins extraction. The protein ubiquitination and degradation were determined by IP using indicated antibody followed by Western blot analysis.

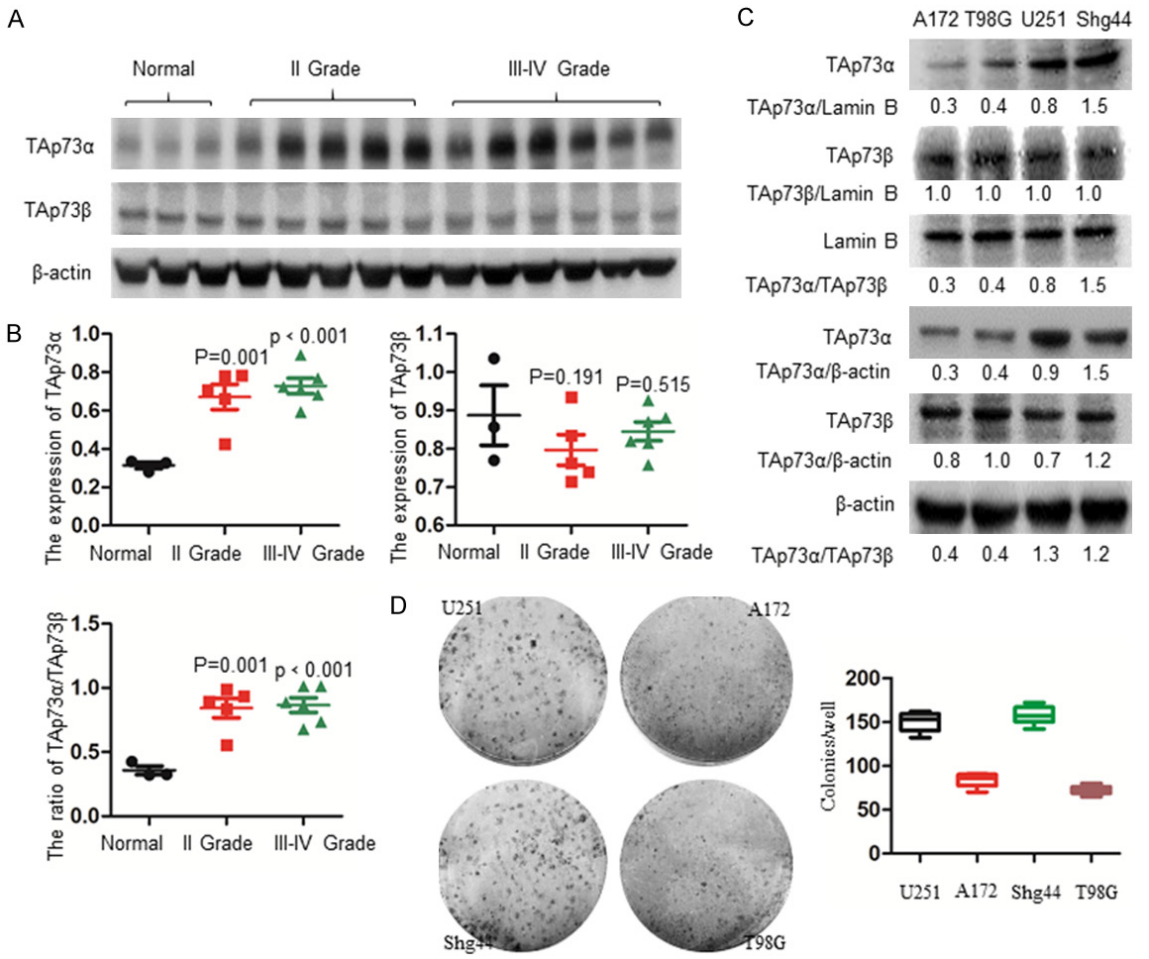

Figure 1. The expression ratio of TAp73α/TAp73β in glioma. A. Western blot (WB) analysis of TAp73α and TAp73β expression in normal and glioma tissues. B. Statistical Analysis of TAp73α, TAp73β expression and TAp73α/TAp73β expression ratio in different grade of glioma. C. The expression of TAp73α and TAp73β in glioma cell lines and the expression ratio of TAp73α/TAp73β in nucleus and cytoplasm. D. The colony formation of glioma cell lines (n=6).

RT-qPCR

The total RNA from transfected cells was extracted using the TRIzol reagent (Invitrogen, Carlsbad, USA). Then, cDNA was synthesized with the PrimeScript RT Reagent Kit according to the manufacturer's instructions (TaKaRa, Shiga, Japan). The primers specific for the TAp73α promoter was: forward CAGACAGCAC-CTACTTCGACCTT, and reverse CCGCCCACCA-CCTCATTA. The endogenous GAPDH was used as an internal control.

Clonogenic assay

A total of ~300 indicated cells were seeded into 12-well plate and cultured for 10-14 days with exchange of culture medium every 3 days. The colonies were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet solution, and then washed with PBS.

Cell apoptosis assay

Cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-annexin V antibody, which was labeled in combination with propidium iodide (PI) according to the manufacturer's instructions (CWBIO, Beijing, China).

TUNEL assay

Frozen tumor tissue sections from nude mice were processed for the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay according to the manufacturer's instructions (Beyotime, Shanghai, China).

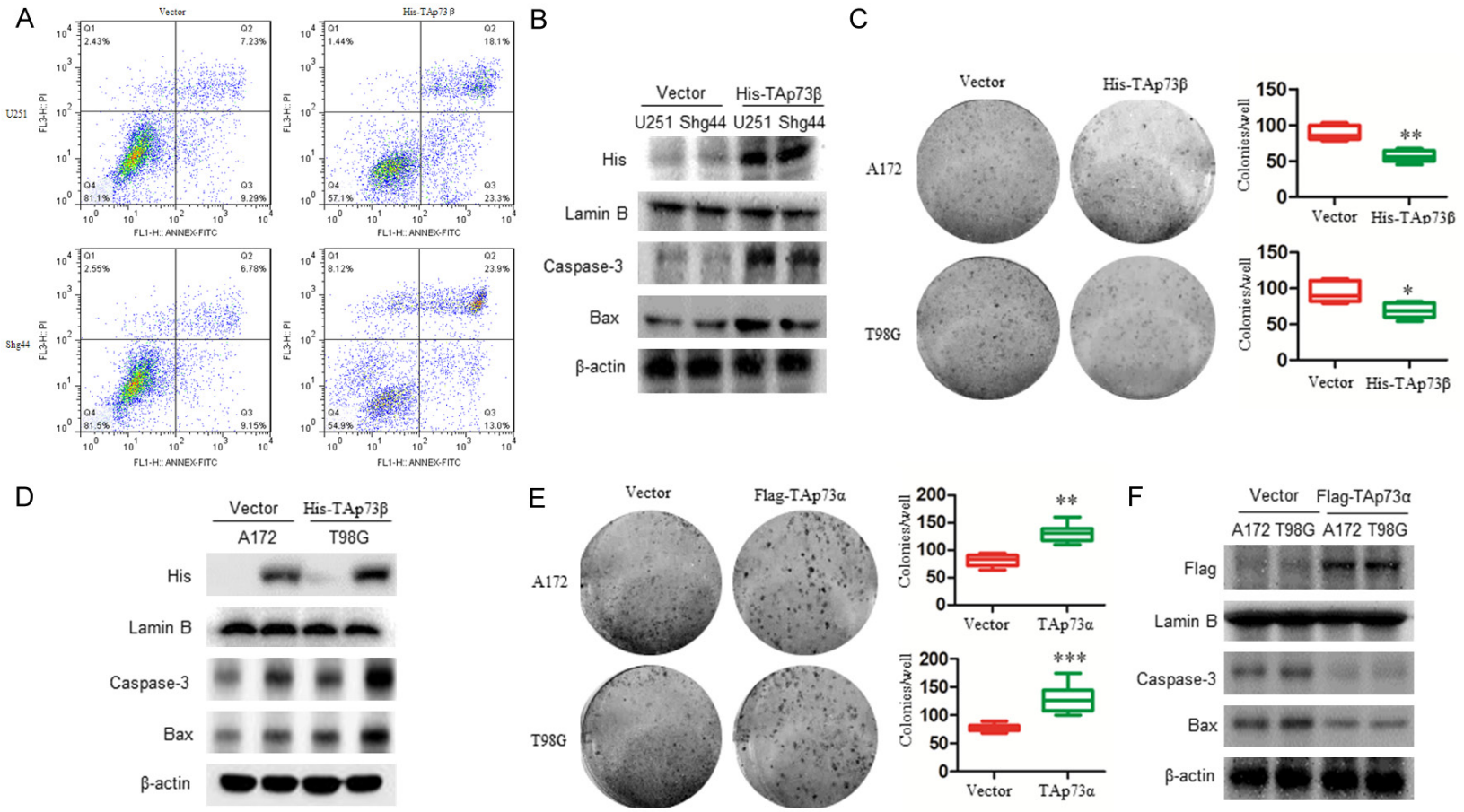

Figure 2. The opposite roles of TAp73α and TAp73β in glioma. A. TAp73β OE induced cell apoptosis in both U251 and Shg44 cells. B. Western blot analysis of caspase-3 and Bax expression in indicated cell lines after His-TAp73β overexpression. C. TAp73β OE inhibited colony formation of A172 and T98G cells. Histogram indicated the number of colonies (n=6, *P<0.05, **P<0.01). D. Western blot analysis of caspase-3 and Bax expression in A172 and T98G cell lines with His-TAp73β overexpression. E. TAp73α OE increased colony formation of A172 and T98G cells. Histogram indicated the number of colonies (n=6, **P<0.01, ***P<0.001). F. Western blot analysis of caspase-3 and Bax expression in indicated cell lines after Flag-TAp73α overexpression.

Plasmid constructing and RNA interference

The DLC2 and TAp73α cDNAs were purchased from Genechem. The cDNAS of DLC2 and its deletion mutant were amplified by PCR. The sequences were cloned into the adenoviral vector plasmid GV314 (Genechem, Shanghai, China) with a Myc-tag. Full length TAp73α cDNAs were cloned into the pcDNA3.1 plasmid (Invitrogen) with a Flag-tag to construct a TAp73α-expressing plasmid. The TAp73β plasmid was constructed based on the wild type TAp73α plasmid. DLC2 siRNA was purchased form Santa Cruz Biotechnology (Santa Cruz Biotechnology, CA, USA), and the operation was according to the manufacturer's instructions.

Tumor xenografts in nude mice

Nude mice were purchased from the Shanghai Animal Center (Chinese Academy of Sciences) and maintained in pathogen-free conditions. The flank of nude mouse was subcutaneously injected with 5×10^6 indicated cells. Every group had 6 nude mice. Tumor growth was monitored every 4 days. Volume of tumors = (length × width^2)/2. Finally, mice were euthanized and tumors were collected, weighed, and analyzed.

Statistical analysis

The statistical data were carried out using SPSS 19.0 software (SPSS, USA). The data graphs were presented with Graphpad prism 5.0 (Gra-phPad Software, CA). Pearson correlation analysis was used to determine the correlation between DLC2 and TAp73α/TAp73β expression ratio. The difference between different groups was determined by a one-way analysis of variance (ANOVA) test. Data were expressed as mean ± SD. All differences were considered significant when *P* value less than 0.05 ($P<0.05$).

Results

The expression ratio of TAp73α/TAp73β in glioma

To investigate the expression of TAp73α, TAp73β and expression ratio of TAp73α/TAp73β in primary glioma tissues, Western Blot (WB) was performed using glioma and normal brain tissues. In **Figure 1A**, we found TAp73α was high-expressed in glioma tissues. No obvious change was found in expression of TAp73β. The expression ratio of TAp73α/TAp73β was high in glioma compared with normal brain tissues. The result was confirmed by statistical analysis in **Figure 1B**. Then, we tested the TAp73α, TAp73β expression and TAp73α/TAp73β ratio in glioma cell lines and found that TAp73α expression and TAp73α/TAp73β ratio was higher in U251 and Shg44 than that in A172 and T98G (**Figure 1C**). Furthermore, the colony capacity of U251 and Shg44 was higher than A172 and T98G (**Figure 1D**).

The opposite roles of TAp73α and TAp73β in glioma

Given that TAp73α and TAp73β are the two main expressed isoforms in human cells and play opposite roles [14, 18], we first confirmed the roles of TAp73α and TAp73β in glioma. We tested the effect of TAp73α and TAp73β on proliferation of glioma cells. We found that TAp73β OE induced apoptosis of U251 and Shg44 cells and inhibited colony formation of A172 and T98G cells (**Figure 2A**, **2C**), and increased expression of caspase-3 and Bax in those glioma cells (**Figure 2B**, **2D**), whereas TAp73α OE improved the ability of colony formation and inhibited expression of caspase-3 and Bax in A172 and T98G cells (**Figure 2E**, **2F**). Those results indicate that TAp73α and TAp73β may have opposite role in apoptosis and proliferation of glioma cells.

DLC2 is down-regulated and associated with TAp73α/TAp73β expression ratio in gliomas

To test the expression of DLC2 in primary glioma tissues, WB was also performed using glioma and normal brain tissues. As shown in **Figure 3A**, DLC2 protein was significantly down-regulated in gliomas compared to normal brain tissues and significantly associated with glioma grade. To further confirm these findings, we examined DLC2 expression information in a public database (http://www.betastasis.com) and showed that *DLC2* mRNA was also down-regulated in glioma tissues compared with non-tumor brain tissues (**Figure 3B**, **3C**). Collectively, these results indicate that DLC2 was frequently down-regulated in glioma tissues. In addition, correlation analysis showed that DLC2 expression levels inversely correlated with expression of TAp73α and TAp73α/TAp73β ratio (**Figure 3D**).

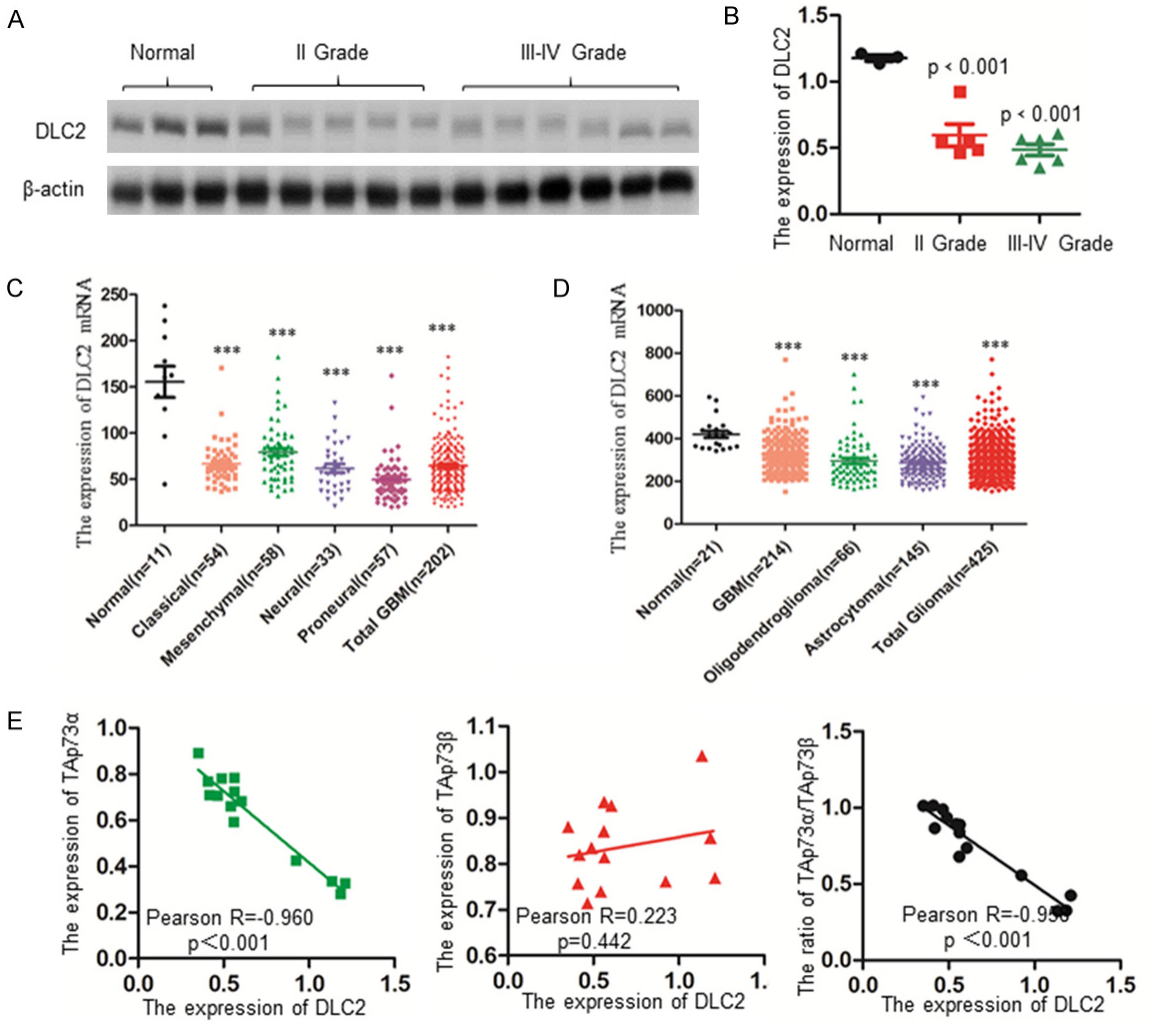

Figure 3. DLC2 is down-regulated and associated with TAp73α/TAp73β expression ratio in gliomas. A. WB for DLC2 in normal and glioma tissues. B. The expression of DLC2 in different grade of glioma. C, D. DLC2 mRNA expression in glioma and GBM based on the data of REMBRANDT and TCGA dataset. E. The expression of DLC2 negatively correlated with TAp73α expression and TAp73α/TAp73β ratio.

DLC2 inhibits colony formation, proliferation and tumorigenesis of GBM cells

To investigate the functional role of DLC2 in glioma, we first examined DLC-2 expression in GBM cell lines using Western blotting (WB). We observed that DLC2 was low expressed in U251 and Shg44 cells, but high in A172 and T98G cells (**Figure 4A**). This was inversed to TAp73α expression and TAp73α/TAp73β ratio in those cell lines. To investigate whether DLC2 influences tumorigenesis in these four cell lines, we generated DLC2 OE in U251 and Shg44 cells and found that DLC2 induced apoptosis of glioma cells (**Figure 4B**). To further verify the in vivo anti-tumor effect of DLC2, a xenograft tumor growth assay was performed in nude mice. Tumors derived from U251 and Shg44 cells with DLC2 OE grew more slowly than those derived from the control cells (**Figure 4C**). Whereas, DLC2 knock-down (KD) in A172 and T98G cells promoted colony capacity (**Figure 4D**). Taken together, these data indicate that DLC2 inhibits GBM growth and development.

DLC2 down-regulates the expression ratio of TAp73α/TAp73β by negatively regulate TAp73α expression

To examine whether DLC2 contributes to glioma inhibition through p73 signaling pathway, we first analyzed proteins p73-downstream pro-

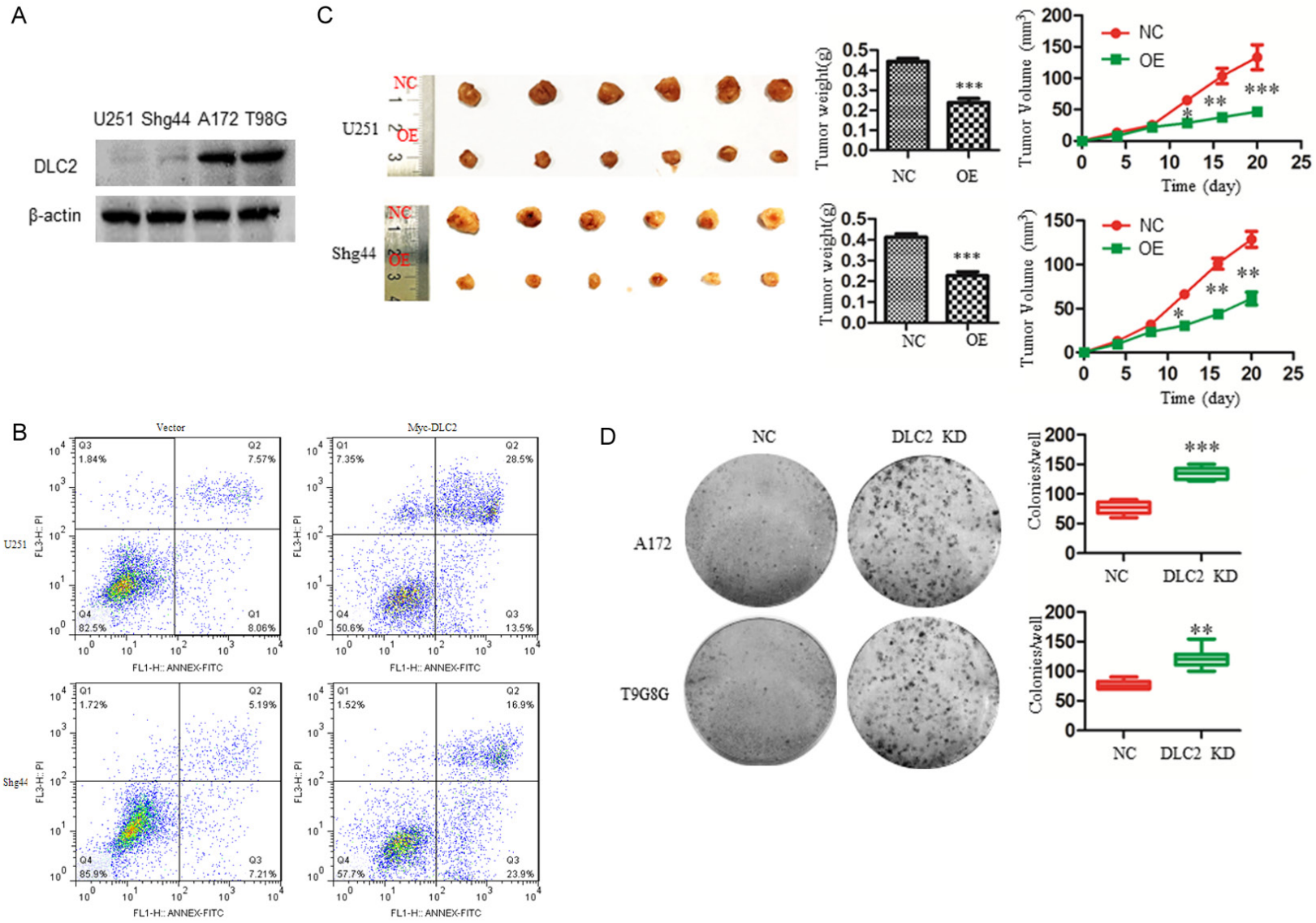

Figure 4. DLC-2 inhibits colony formation, proliferation and tumorigenesis of GBM cells. A. The expression of DLC2 in glioma cell lines. B. DLC2 OE induced apoptosis of U251 and Shg44. C. DLC2 OE inhibited tumor growth of U251 and Shg44 in nude mice. Histogram indicated the average weight of xenograft tumors (n=6, ***P<0.001). Line chart indicated growth curve of tumors (n=6, *P<0.05, **P<0.01, ***P<0.001). D. DLC2 KD increased colony formation of A172 and T98G. Histogram indicated the number of colonies (n=6, **P<0.01, ***P<0.001).

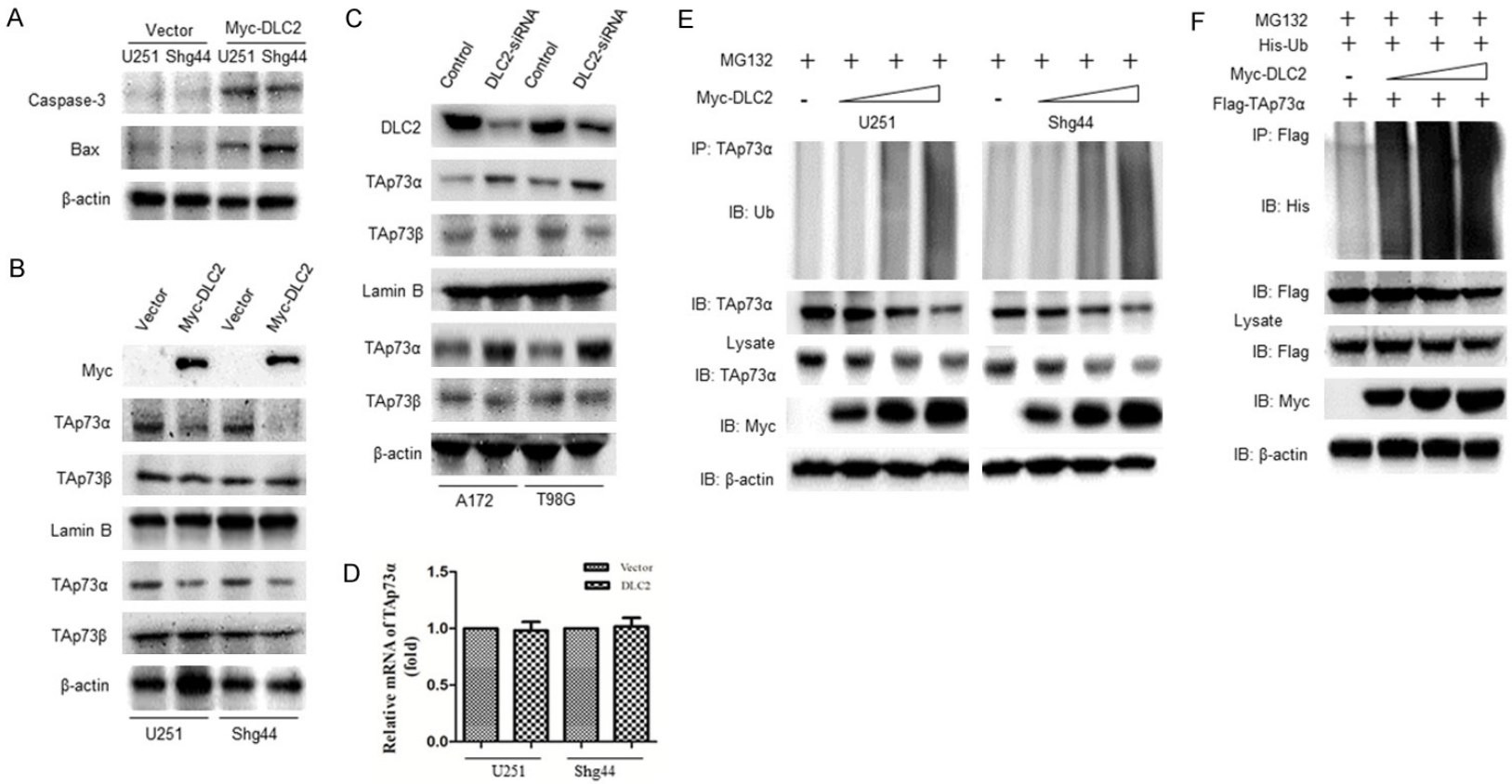

Figure 5. DLC2 down-regulates the ratio of TAp73α/TAp73β by negatively regulate TAp73α expression. A. DLC2 OE increased the expression of TP73 downstream proteins-caspase-3 and Bax in U251 and Shg44. B. DLC2 OE decreased expression of TAp73α in in U251 and Shg44. C. DLC2 KD increased expression of TAp73α in in A172 and T98G. D. DLC2 OE did not change the expression of TAp73α mRNA in U251 and Shg44. E. DLC2 OE induced TAp73α ubiquitination and degradation in U251 and Shg44 in a dose dependent manner (DLC2: 0, 0.5, 1.0, 1.5 μl). F. DLC2 induced TAp73α ubiquitination and degradation in a dose dependent manner. 293T cells were transfected with expression constructs in the indicated combinations and concentrations (DLC2: 0, 0.5, 1.0, 1.5 μg).

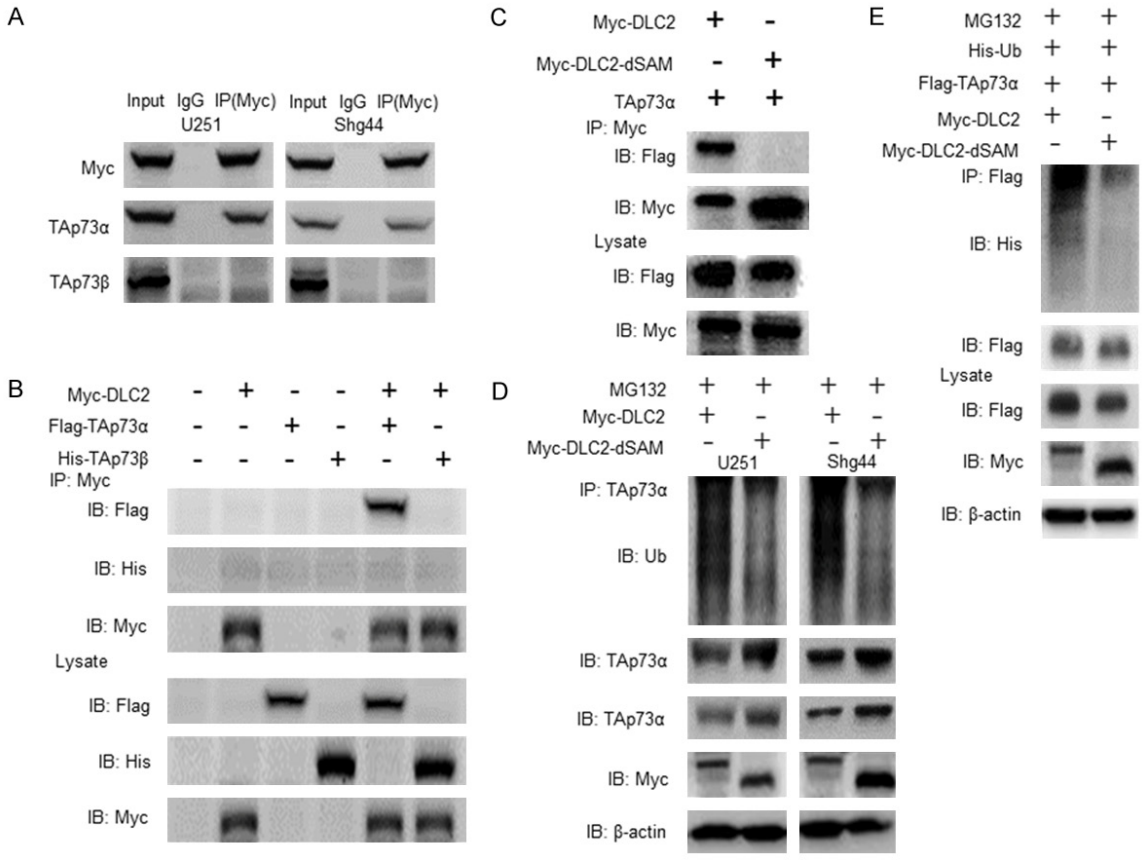

Figure 6. DLC2 interacts with TAp73α by SAM domain, but not TAp73β. A. Exogenous DLC2 interacted with endogenous TAp73α, but not TAp73β. U251 and Shg44 were transfected with DLC2 adenovirus expression constructs. B. DLC2 interacted with TAp73α, but not TAp73β. 293T cells were transfected with expression constructs in the indicated combinations. C. DLC2 SAM deletion mutant failed to interact with TAp73α in 293T transfected with expression constructs in the indicated combinations. D. SAM domain deletion impaired DLC2 induced TAp73α ubiquitination and degradation in U251 and Shg44. E. DLC2 SAM deletion mutant failed to induce TAp73α ubiquitination and degradation in 293T transfected with indicated plasmids.

teins associated with proliferation and apoptosis, including caspase-3 and Bax. The results showed that DLC2 OE resulted in increased expression of caspase-3 and Bax in U251 and Shg44 cells (**Figure 5A**). We then determined whether DLC2 results in the dysregulation of the TAp73α/TAp73β expression ratio in glioma cells. As shown in **Figure 5B**, DLC2 OE resulted in decreased expression of TAp73α and TAp73α/TAp73β ratio in U251 and Shg44 cells, whereas, DLC2 KD resulted in increased expression of TAp73α and TAp73α/TAp73β ratio in A172 and T98G cells, but no changes of TAp73β were found (**Figure 5C**). Next, we tested whether the change in TAp73α expression was at protein level or mRNA level. The results showed that DLC2 OE did not change the expression of *TAp73α* mRNA in U251 and Shg44 (**Figure 5D**). Furtherly, we found DLC2 induced degradation of TAp73α in U251 and Shg44 cells through ubiquitination pathway, and the degradation of TAp73α was in a DLC2 dose-depend manner (**Figure 5E**). We then confirmed above results by transfecting Myc-DLC2 and Flag-TAp73α into 293T cells and found DLC2 also induced TAp73α through ubiquitination pathway in a dose-depend manner (**Figure 5F**).

DLC2 interacts with p73α by SAM domain, but not p73β

These observations prompted further assessment of the relationship between DLC2 and TAp73α in human glioma. Given that SAM domain proteins could interact with each other, we speculated that DLC2 might interact with

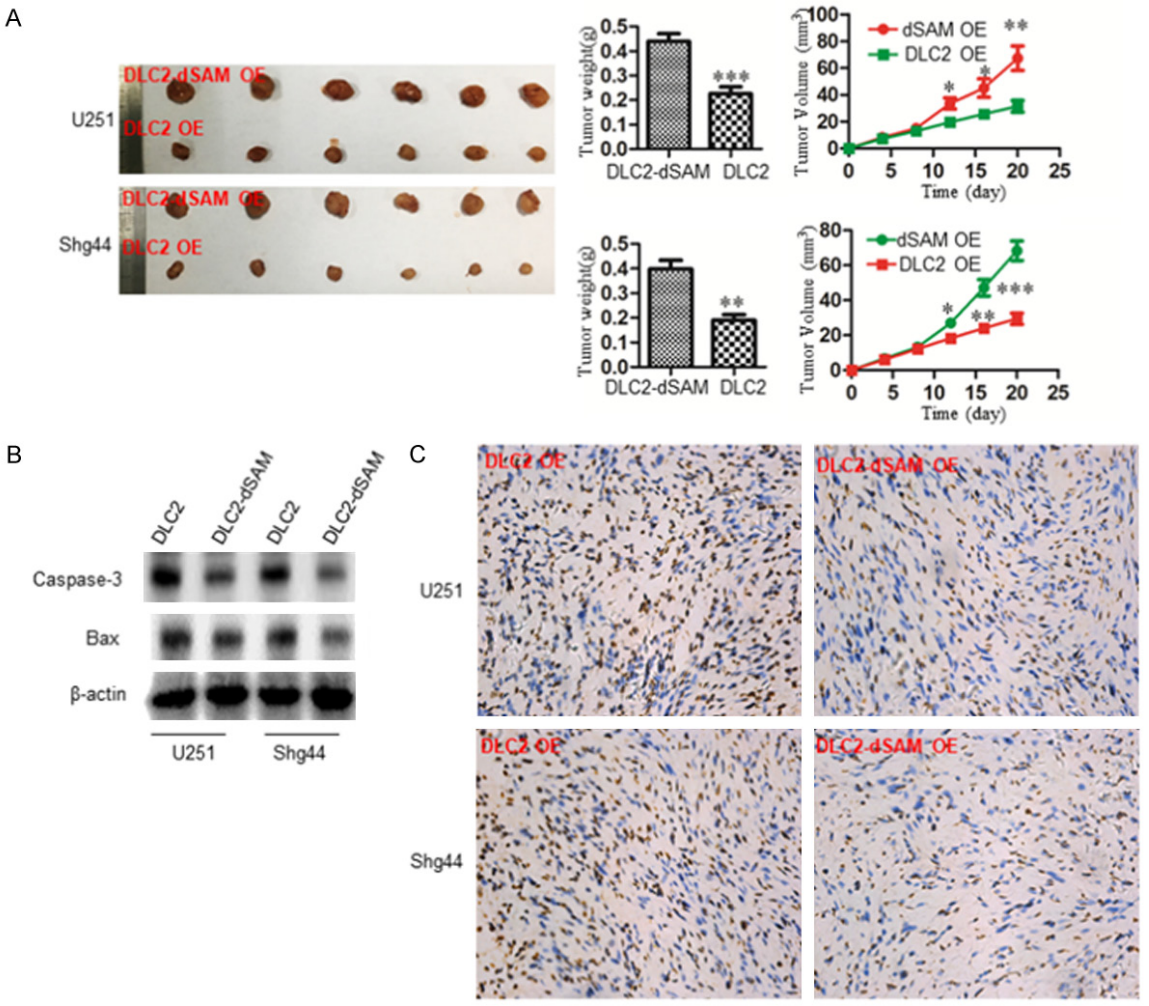

Figure 7. SAM domain is essential for DLC-2-exerted anti-tumor effect in glioma cells. A. SAM deletion impaired anti-tumor effect of DLC2 in xenograft tumors. Histogram indicated the average weight of xenograft tumors (n=6, **P<0.01, ***P<0.001). Line chart indicated growth curve of tumors (n=6, *P<0.05, **P<0.01, ***P<0.001). B. SAM deletion impaired DLC2 induced expression change of caspase-3 and Bax in xenograft tumors. C. TUNEL assay was performed to test apoptosis of glioma cells in xenograft tumors.

TAp73α as well. To address this, we firstly tested the interaction between exogenous DLC2 and endogenous TAp73α in U251 and Shg44 cells. IP assay showed the interaction between DLC2 and TAp73α, but not TAp73β (**Figure 6A**). Then, 293T cells were co-transfected with Myc-DLC2 and Flag-TAp73α or His-TAp73β expression plasmids. The result showed the interaction between DLC2 and TAp73α, but not TAp73β (**Figure 6B**). Considering that the main difference between TAp73α and TAp73β is the existence of SAM domain at carboxyl-terminal, this implies that SAM domain of TAp73α mediates the interaction with DLC2. To identify the interaction region of DLC2 with TAp73α, we performed IP experiments using truncated mutant of DLC2. Compared with full-length DLC2, DLC2 with SAM domain deletion (dSAM, residues 61-120aa) completely lost binding ability as DLC2 to TAp73α (**Figure 6C**). Next, we investigated whether DLC2 with SAM domain deletion could induce TAp73α degradation or not. We found that, compared to the full-length DLC2, the SAM-deleted DLC2 (Myc-DLC2-dSAM) failed to induce TAp73α degradation (**Figure 6D**). This was confirmed by transfecting Myc-DLC2-dSAM and Flag-TAp73α into 293T cells, and IP assay showed Myc-DLC2-dSAM failed to induce TAp73α degradation as the full-length DLC2 (**Figure 6E**).

SAM domain is essential for DLC2-exerted anti-tumor effect in glioma cells

Next, we tested whether SAM-deleted DLC2 also had the same anti-tumor effect as full length DLC2. The xenograft tumor growth assay was performed to test tumorigenesis-inhibition effect of SAM-deleted DLC2. The results showed that SAM deletion decreased tumorigenesis-inhibition effect of DLC2 (**Figure 7A**). Compared to the full-length DLC2, SAM-deleted DLC2 OE also failed to increase the expression of caspase-3 and Bax in tumor tissues (**Figure 7B**). TUNEL staining further confirmed the decreased inhibitory effect of SAM-deleted DLC2 on tumors (**Figure 7C**).

Discussion

As an important transcription factor, p73 is a major determinant of chemosensitivity in human tumors [19]. Considering its rare mutation in cancers, p73 represents an attractive alternative strategy for treatment of cancer. Unfortunately, the biology of p73 is complex for the existence of different isoforms resulted by the *p73* gene selective transcription. Due to contradictory observations, revisiting the tumor suppressing function of p73 seems necessary [20].

TAp73α is the longest form of p73 proteins and contains s sterile α motif (SAM) domain. TAp73β is a smaller polypeptide, missing the extreme carboxyl-terminal region and most of the SAM domain. Increased TAp73α expression levels has been noticed in certain cancers, such as cervical cancer, medulloblastoma, B-cell chronic lymphocytic leukaemia, ovarian carcinomas, gastric adenocarcinoma, bladder cancer and thyroid cancer. This implies that TAp73α may be involved in the genesis and development of tumors. Indeed, *in vitro* experiments show that TAp73α has anti-apoptotic effect. TAp73α inhibits drug induced apoptosis, caspase-2 induced Bax activation, loss of mitochondrial membrane potential and consequent cell death in lung cancers [14, 18]. TAp73α is also overexpressed in MB and controls proliferation of medulloblastoma (MB) cells, and is a marker of glutamine addiction in MB [21]. Hsp72 and Hypoxia improve tumorigenesis and angiogenesis depending on the existence of TAp73α [22, 23]. In addition, TAp73α promotes glioblastoma cell invasion by directly activating POSTN (periostin) expression [24], and upregulates IL-1β in cancer cells [25]. However, TAp73β has the opposite effect and contract the anti-apoptotic effect of TAp73α [14, 23]. TAp73β can induce cell apoptosis, inhibit metastasis and improve chemo-sensitivity of tumor cells [26-28]. TAp73β also can suppress invasion and migration of cancer cells by mediating actin cytoskeleton dynamics [27, 29, 30]. In our study, we found that high TAp73α expression and TAp73α/TAp73β ratio in glioma was significantly associated with glioma grade. *In vitro* experiments showed that TAp73α promoted the proliferation of glioma cells and TAp73β inversely induced apoptosis of glioma cells. They had opposite effect on the expression of caspase-3 and Bax. The opposite effect of TAp73α and TAp73β may be resulted by the difference of carboxyl-terminal region. It has been found that the carboxyl-terminus region of TAp73α contains SAM domain, which decreases the transcriptional regulation activity of TAp73α [14].

DLC2, a tumor suppressor, has been found to be downregulated in lung, breast, ovarian, uterine, renal, gastric, rectal, colon, glioma and liver tumors [10, 31]. Down-regulation of DLC2 is associated with copy number loss and promoter methylation, and predicts poor prognosis of tumors [32]. Using TCGA and REMBRANDT data from glioma and normal brain tissue samples, we evaluated the mRNA expression of DLC2 and found *DLC2* mRNA was commonly low-expressed in glioma and its subtypes. Then, in glioma WB analysis, we found glioma samples had low-expressed DLC2 protein. Moreover, the low-expression of DLC2 protein was significantly associated with glioma grade. Interestingly, the expression of DLC2 was negatively associated with TAp73α expression and TAp73αTAp73β ratio. The *in vitro* experiments have shown that the overexpression of DLC2 suppresses cell motility, proliferation, angiogenesis and anchorage-independent growth in the human cancer cell [5, 6, 8, 9, 15, 16, 33]. At present, the antitumor effect of DLC2 is mainly the existence of GAP domain, which acts by switching between an inactive GDP-bound and an active GTP-bound conformation [4]. Our results also identified DLC2 OE induced apoptosis of glioma cells, in turn, DLC2 KD promotes proliferation. We also found DLC2 OE induced the expression of caspase-3 and Bax, the down-stream genes of TP73, which is associated with cell apoptosis

and proliferation [11]. More importantly, DLC2 changed the expression of TAp73α/TAp73β ratio in glioma cells by negatively regulating expression of TAp73α through ubiquitination pathway, which inhibited the proliferation of glioma cells and promoted the apoptosis.

The protein-protein interactions play important roles in signal transduction of cell procedure [34]. The SAM domain is one of the most prominent interaction domains in animals and is present in proteins of diverse functions [35]. In this study, it was DLC2 that interacted with TAp73α not TAp73β. The difference of protein structure between TAp73α and TAp73β is in the carboxyl-terminus region. We speculated that SAM domain mediated the interaction between DLC2 and TAp73α, which was also confirmed by the absence of interaction between DLC2 deleted SAM domain and TAp73α. Further, the deletion of SAM domain inhibited the ubiquitination of TAp73α induced by DLC2. This implied that the interaction between DLC2 and TAp73α mediated by the SAM domain played an important role in ubiquitination of TAp73α induced by DLC2. The FAT domain of DLC2 directs DLC2 to localize in focal adhesion. The FAT domain contains a SIYDNV motif, which can bind to the SH2 domain of other proteins independent on tyrosine phosphorylation [36]. However, many SH2 proteins act as ubiquitin ligase [3, 37]. We speculated that DLC2 could not degrade TAp73α directly, and the degradation of TAp73α induced by DLC2 might depend on certain proteins recruited by DLC2. As for the potential mechanism, this needs further study to investigate. In addition, we also investigated the effect of SAM domain on the anti-tumor function of DLC2. We found the deletion of SAM domain impaired the anti-tumor function of DLC2.

In conclusion, our results highlight new mechanism underlying the anti-tumor effect of DLC2 in gliomas. Given that DLC2 interacts and regulates many other proteins, future studies will aim to investigate additional mechanisms involved in anti-tumorigenesis.

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Disclosure of conflict of interest

None.

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