14-3-3β regulates the proliferation of glioma cells through the GSK3β/β-catenin signaling pathway

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Abstract. We previously demonstrated that 14-3-3β is overexpressed in astrocytomas; however, the underlying mechanisms are poorly understood. Based on the reported multiple functions of 14-3-3β, we hypothesized that it interacts with glycogen synthase kinase 3 β (GSK3β), which regulates β-catenin-mediated oncogene expression and contributes to tumorigenesis and astrocytoma progression. To test these hypotheses, we used 14-3-3β overexpression vectors and small interfering RNA (siRNA) transfection in the human normal astrocyte cell line SVGp12 and the glioma cell line U87, respectively. The results showed that overexpression of 14-3-3β promoted the proliferation of SVGp12 cells, while knockdown of 14-3-3β inhibited the proliferation of U87 cells as analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and bromodeoxyuridine (BrdU) assays. In Flag-tagged 14-3-3β-overexpressing cells, GSK3β was co-immunoprecipitated with 14-3-3β using a Flag antibody. Knockdown of β-catenin by siRNA blocked cell proliferation induced by overexpression of 14-3-3β. Furthermore, overexpression of 14-3-3β suppressed the phosphorylation of β-catenin leading to its accumulation and nuclear translocation as revealed by western blot analysis. In addition, β-catenin nuclear translocation induced by overexpression of 14-3-3β activated the transcription of oncogenes including c-myc and cyclin D1. Collectively, these results revealed that 14-3-3β regulates the proliferation of astrocytes and glioma cells through the GSK3β/β-catenin signaling pathway. The delineated mechanism of 14-3-3β may be responsible for the tumorigenesis and progression of human astrocytomas. Thus, new therapeutic strategies or drugs aimed at 14-3-3β may have potential for the treatment of human astrocytomas.

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Abbreviations: GSK3β, glycogen synthase kinase 3β; siRNA, small interfering RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; BrdU, bromodeoxyuridine

Key words: astrocytoma, β-catenin, cell proliferation, 14-3-3β, GSK3β

Introduction

The highly conserved family of 14-3-3 proteins consisting of seven isoforms (β, γ, ε, η, θ, σ and ξ) has been demonstrated to bind to a wide variety of proteins and to play important roles in a variety of biological processes, including cell cycle control, cell survival and cell death through various signal transduction pathways (1-4). In normal or tumor cells and tissues, 14-3-3 proteins have been suggested to participate in a broad spectrum of human diseases such as cancer (5). However, 14-3-3 proteins exhibit isoform-specific expression in different types of cells and tissues, and the function of 14-3-3 proteins is complex and intricate owing to the high sequence homology of its isoforms (6).

The role of 14-3-3 proteins in carcinogenesis has been extensively studied. Accumulating evidence has established an association between 14-3-3 proteins and many types of cancers, including lung, breast, neck and head cancers (5,7). However, different isoforms may act as oncogenes or tumor suppressors in different types of cancers. Abundant expression of 14-3-3ξ is found in breast cancers and promotes cancer progression via the PI3K/Akt pathway (8,9). Knockdown of 14-3-3ξ was found to significantly inhibit lung cancer cell proliferation and promote lung cancer cell sensitivity to chemotherapy drugs (10,11). In contrast, 14-3-3σ is suggested to be a tumor suppressor owing to the frequent gene methylation that occurs in breast cancers (12). In addition, the β, γ and θ isoforms are also reported to be oncogenic (13-15). Thus, 14-3-3 proteins can be potential targets for cancer diagnosis and therapy.

14-3-3 proteins are a group of small and acidic proteins first identified in brain proteins that are abundant in total soluble brain extracts (3,16,17). Thus, dysregulation of 14-3-3 proteins is suggested to be related to numerous neurological diseases (18-20). Our previous studies demonstrated that 14-3-3β was highly expressed in human astrocytomas (21,22). However, the underlying mechanisms are poorly understood. Research has demonstrated that 14-3-3 proteins bind and regulate glycogen synthase kinase 3 β (GSK3β) activity in neurons (23). GSK3β is a serine-threonine kinase that regulates signaling pathways involved in cell proliferation and

the cell cycle (24,25). GSK3β was also found to contribute to cancers through Wnt/β-catenin. It was reported that GSK3β promotes the phosphorylation of β-catenin and its degradation (26). Inhibition of GSK3β by Wnt signaling leads to the accumulation and nuclear translocation of β-catenin, which results in the activation of oncogene transcription through interactions with the T-cell factor/lymphoid enhancer factor (Tcf/Lef) transcription factors (27). Loss of GSK3β was reported to be associated with prostate cancer implying dysregulation of GSK3β in cancers (28). Inhibition of GSK3β was found to induce the death of glioma cells (29). Given these findings, we hypothesized that 14-3-3β interacts with GSK3β to regulate β-catenin-mediated oncogene expression and contributes to tumorigenesis and the development of astrocytomas.

Astrocytomas are a type of malignant cancer frequently found in the brains in both adults and children; an effective therapeutic method is still lacking to date. Therefore, it is urgent to understand the underlying mechanisms of astrocytomas (30-32). Our previous investigations demonstrated that 14-3-3β exhibited an abundant distribution in astrocytoma tissues and glioma cells, and it was closely related to the degree of malignancy (21,22). We hypothesized that 14-3-3β plays important roles in the development of human astrocytomas and interacts with GSK3β in the regulation of cell growth and proliferation. In order to test our hypotheses, gene overexpression and small RNA interference (siRNA) was performed in normal human astrocytes and glioma cells in the present study. Co-immunoprecipitation studies showed that 14-3-3β interacts with GSK3β in glioma cells. Overexpression of 14-3-3β sequestered GSK3β and enhanced its phosphorylation status, which resulted in accumulation and nuclear translocation of β-catenin. Consequently, β-catenin nuclear translocation activated oncogene transcription including c-myc and cyclin D1, which are responsible for tumorigenesis and progression. Thus, the delineated mechanism of 14-3-3β may be responsible for tumorigenesis and progression of human astrocytomas. Therefore, new therapeutic strategies or drugs aimed at 14-3-3β may have potential for the treatment of human astrocytomas.

Materials and methods

Cell lines and cell culture. The human normal astrocyte cell line SVGp12 and glioma cell line U87 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were maintained according to standard protocols. Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum to which 100 U/ml of penicillin, 100 μg/ml of streptomycin and 2 mM of L-glutamate were added. All cells were cultured at 37˚C with 5% CO_2 in an incubator (Life Technologies, Baltimore, MD, USA).

Recombinant plasmid construction and transfection. Total RNA was extracted from frozen resected tumor tissues using TRIzol (Invitrogen, Carlsbad, CA, USA). Total RNA was isolated using chloroform and precipitated with isopropanol. The resulting 1 μg of RNA was used as a template for single-stranded cDNA synthesis with 20 U avian myeloblastosis virus (AMV) reverse transcriptase (Takara Biotechnology, Dalian, China) according to the manufacturer's instructions. Primers with restriction enzyme sites *Hin*dIII and *Bam*HI were used for amplifying cDNA fragments of 14-3-3β followed by subcloning into the p3XFlag-CMV expression vector (Sigma Chemical Co., St. Louis, MO, USA). Small-interfering RNAs (siRNAs) targeting 14-3-3β (sc-29186), β-catenin (sc-270011) and control siRNA-A (sc-37007) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cells were transfected with vectors or siRNA according to the manufacturer's instructions. Briefly, cells were seeded in a 6-well culture plate ($2x10^5$ cells/well) and incubated at 37˚C with 5% CO_2 until the cells reached 80% confluence. Plasmid DNA (1 μg) or siRNA (30 pmol) were diluted in 500 μl of DMEM with 5 μl Lipofectamine (Invitrogen), mixed and incubated at room temperature for 15 min. Then, the mixtures were added to the cells with a final volume of 3 ml of medium and incubated with cells for the indicated time.

MTT assay. For the MTT assay, cells were plated in 96-well plates and cultured under regular conditions until they reached 80% confluence. Plasmid or siRNA was transfected according to standard protocols, and was continually incubated with cells at 37˚C with 5% CO_2 for 24 or 48 h. Then, the culture medium was replaced with fresh medium containing MTT (5 mg/ml in PBS, 200 μl/well) (Shanghai Sangon Biological Engineering Co., Ltd,, Shanghai, China) and incubated with the cells for an additional 4 h. Then formazan was dissolved in DMSO (150 μl/well; Sigma) for 10 min, and the absorbance at 490 nm was determined with an ELISA reader (BioTek Instruments, Winooski, VT, USA). Each cell viability assay was performed in quadruplicate and repeated three times. Data are expressed as mean ± standard error of the mean (SEM) and differences were analyzed by the Student's t-test.

Bromodeoxyuridine (BrdU) assay. For the BrdU assay, a BrdU cell proliferation assay kit (Millipore, Billerica, MA, USA) was used. Transfected cells in 96-well plates were cultured for 24 or 48 h, and 10 μl of BrdU solution was added per well and incubated for 2 h. The medium was removed, and 100 μl/well of the Fixing/Denaturing solution was added and incubated at room temperature for 15 min. Then, the solution was removed, and 100 μl/well prepared detection antibody solution was added and incubated for 1 h at room temperature. After that, plates were washed three times with wash buffer followed by the addition of 100 μl/well of prepared HRP-conjugated secondary antibody solution and incubation for 30 min at room temperature. Then, the plates were washed three times with wash buffer, and 100 μl of TMB substrates was added and incubated for 30 min at room temperature. The amount of BrdU incorporated into the cells was determined at 450 nm by a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). Each cell proliferation assay was performed in quadruplicate and repeated three times. Data are expressed as mean ± SEM and differences were analyzed by the Student's t-test.

Nuclear protein extraction. Nuclear proteins were extracted using an extraction kit (Shanghai Sangon Biological Engineering) according to the manufacturer's protocol. Briefly, cells were lysed in cytoplasmic buffer containing protease

inhibitors, mixed and incubated for 15 min at 4˚C followed by centrifugation at 12,000 rpm for 20 min at 4˚C. Cell sediments were collected and resuspended in nucleus buffer for 10 min at 4˚C. Then, the sample was centrifuged at 12,000 rpm for 10 min at 4˚C. The supernatant was collected for analysis.

Co-immunoprecipitation. The transfected cells were harvested at the indicated time and lysed in RIPA buffer (Bioteke, Beijing, China) for 30 min at 4˚C followed by centrifugation at 12,000 rpm for 20 min at 4˚C. Protein A-Sepharose beads (50 μl of a 10% suspension; Amersham Biosciences AB, Uppsala, Sweden) mixed with a mouse monoclonal anti-Flag (Sigma Chemical), or mouse IgG as a control, were incubated at 4˚C in 500 μl of lysis buffer for 1 h. Cell lysates (500 μl) were added to the prepared antibody-bead mixture and incubated at 4˚C for 2 h. The bead complexes were then collected by centrifugation and washed with ice-cold lysis buffer (0.1 M Tris-HCl buffer containing 0.5 M NaCl, pH 8.0, 1 ml each time) for a total of three times. Then, the protein complex was eluted from the beads by 200 μl of 0.1 μM glycine buffer (pH 2.5). The protein complexes were then separated by SDS-PAGE for further analysis.

Western blot analysis. Proteins from cultured cells or immunoprecipitated protein complexes were collected, and a total of 20-30 μg of protein was fractionated by 12% SDS-PAGE electrophoresis and transferred to nitrocellulose membranes (Amersham, Little Chalfont, UK). The membranes were treated using the following procedure with shaking and blocking at room temperature (RT) with 2% non-fat dry milk in Tris-buffered saline (TBS) for 1 h followed by incubation in the primary antibodies (rabbit polyclonal 14-3-3β, β-catenin, GSK3β and phospho-GSK-3β; from Santa Cruz Biotechnology) diluted in blocking buffer (1:10,000) at 4˚C overnight and washed three times with TBS and Tween (TBST; 10 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.05% Tween-20) for 10 min each time at room temperature. Subsequently, the membranes were incubated in peroxidase-conjugated secondary antibody goat anti-rabbit IgG (Boster Corp., Wuhan, Hubei, China; diluted 1:3,000 in blocking buffer) for 1 h. After washing three times with TBST and once with TBS each for 10 min, 1 ml of 4-chloro-1-naphthol (4-CN) as an HRP substrate with 9 ml of TBS and 6 μl of H_2O_2 was used for visualizing the target protein in the dark for 5-30 min.

Quantitative real-time-polymerase chain reaction (qRT-PCR) analysis. Total RNA was extracted from the cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Up to 5 μg of the total RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA). The cDNAs were used as templates for qRT-PCR. For c-myc, the sense primer was 5'-ACACATCAGCACAACTACGC-3' and the antisense primer was 5'-CCTCTTGACATTCTCCTCG GT-3'. For cyclin D1, the sense primer was 5'-GCCAACCTCC TCAACGACCGG-3' and the antisense primer was 5'-GTCC ATGTTCTGCTGGGCCTG-3'. β-actin (sense primer, 5'-CTC CATCCTGGCCTCGCTGT-3' and antisense primer, 5'-GCTG TCACCTTCACCGTTCC-3') were used as the control. The qRT-PCR mixture system contained 5 μl SsoFast™ EvaGreen Supermix (Bio-Rad), 1 μl of cDNA (diluted in 1:50) and 2 μl of each of the forward and reverse primers (1 μM) to a final volume of 10 μl. The PCR procedure was as follows: 94˚C for 4 min; 94˚C for 20 sec, 55˚C for 30 sec, and 72˚C for 20 sec; 2 sec for plate reading for 35 cycles; and melting curve from 65 to 95˚C. β-actin was used as a control for normalizing the gene expression. Three independent experiments were performed. The data obtained were calculated by $2^{-\Delta\Delta Ct}$ and treated for statistical analysis as previously described (33), followed by an unpaired sample t-test.

Statistical analysis. All experiments were performed independently at least three times. Differences between groups were analyzed by the Student's t-test. A P-value <0.01 was considered to indicate a statistically significant result.

Results

14-3-3β is involved in cell proliferation of astrocytes and glioma cells. In order to investigate the role of 14-3-3β in astrocytes, 14-3-3β was overexpressed or silenced by siRNA in the human normal astrocyte cell line SVGp12 and the glioma cell line U87, respectively. The results showed that overexpression of 14-3-3β (Fig. 1A) significantly promoted the growth and proliferation of SVGp12 cells at 48 and 72 h after transfection (Fig. 1B and C). Furthermore, 14-3-3β was significantly downregulated in U87 cells transfected with 14-3-3β siRNA (Fig. 1D), which resulted in a significant decrease in cell growth and proliferation of U87 cells at 48 h and 72 h after gene transfection (Fig. 1E and F). These results demonstrated that 14-3-3β is highly expressed in glioma cells and 14-3-3β overexpression promotes the growth and proliferation of human normal astrocytes.

14-3-3β binds to GSK3β and inhibits GSK3β activity. In order to determine whether 14-3-3β and GSK3β interact, U87 cells were transfected with Flag-tagged 14-3-3β. Flag antibody was used to bait the 14-3-3β protein complex, and the interacting proteins were analyzed by western blot analysis. The 14-3-3β protein co-immunoprecipitated with GSK3β in the U87 cell line (Fig. 2A, upper panels) and in the SVGp12 cell line (Fig. 2A, lower panels). The β-catenin protein was not detected in the protein complex. We speculated that 14-3-3β interacted with GSK3β and sequestered GSK3β, which enhanced the phosphorylation of GSK3β and disrupted its interaction with β-catenin. To test these hypotheses, the phosphorylation of GSK3β was determined by western blot analysis in transfected cells. As expected, 14-3-3β overexpression enhanced the phosphorylation of GSK3β (Fig. 2B). These results indicate that 14-3-3β may regulate cell growth and proliferation through the GSK3β/β-catenin pathway.

14-3-3β increases cell proliferation by β-catenin. To test the hypotheses that 14-3-3β regulates cell proliferation though β-catenin, we co-transfected β-catenin siRNA and 14-3-3β overexpression vectors in SVGp12 cells. Co-transfection of 14-3-3β overexpression vectors and β-catenin siRNA significantly inhibited cell proliferation induced by 14-3-3β overexpression (Fig. 3A and B). To further confirm the

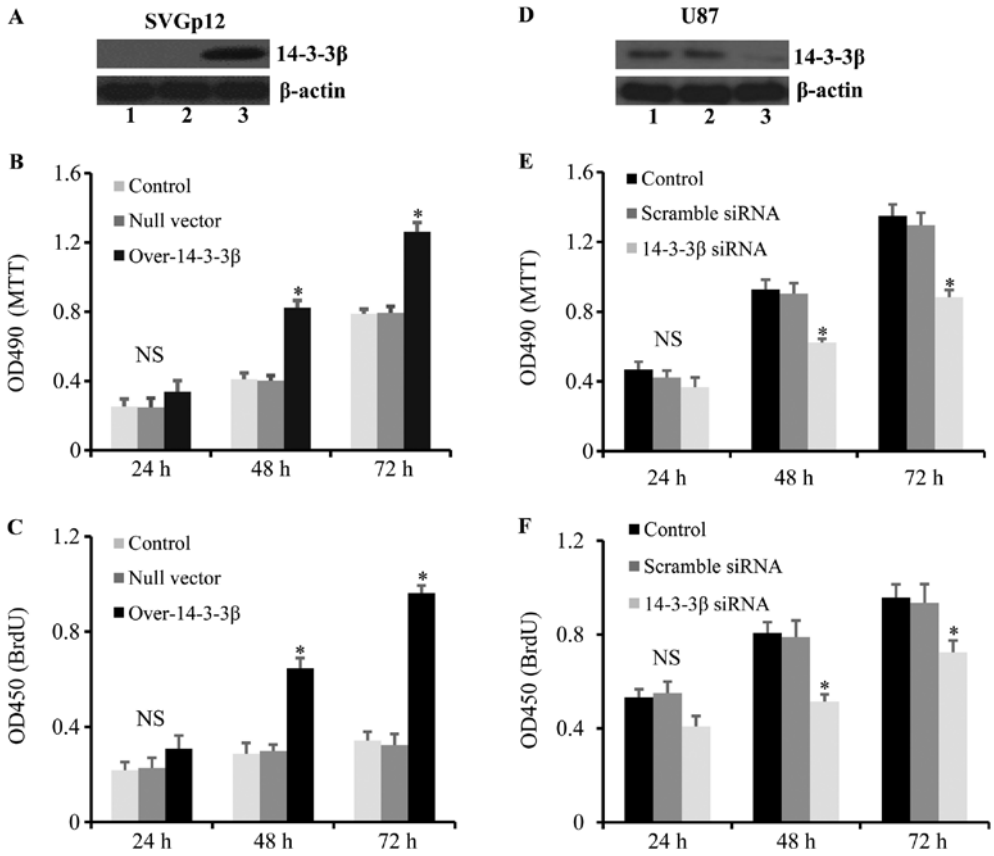

Figure 1. Analysis of the role of 14-3-3β in astrocytes and glioma cells by gene transfection. (A-C) Overexpression of 14-3-3β in the human normal astrocyte cell line SVGp12. Lane 1, non-transfected cells as a negative control; lane 2 (null vectors), null vector-transfected cells as a positive control; lane 3 (over-14-3-3β), cells transfected with expression vectors containing 14-3-3β. (A) 14-3-3β protein levels were determined by western blot analysis after 48 h of 14-3-3β expression vector transfection. (B) Cell growth and viability were analyzed by MTT assays. (C) Cell proliferation was analyzed by BrdU assays after 24, 48 and 72 h of vector transfection. (D-F) Silencing of 14-3-3β in the U87 glioma cell line. Lane 1, non-transfected cells as a negative control; lane 2 (scrambled siRNA), cells transfected with non-specific control siRNA as a positive control; lane 3 (14-3-3β siRNA), cells transfected with 14-3-3β siRNA. (D) 14-3-3β protein levels were determined by western blot analysis after 48 h of siRNA. (E) Cell growth and viability were analyzed by MTT assays. (F) Cell proliferation was analyzed by BrdU assays after 24, 48 and 72 h of siRNA. NS indicates no statistical significance, $^{*}P<0.01$ vs. negative control or positive control denotes significant differences.

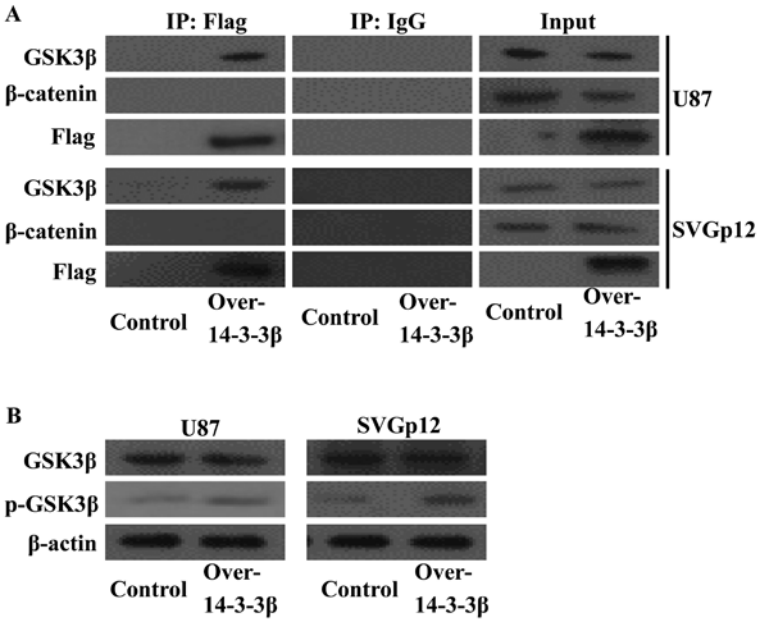

Figure 2. Examination of the interaction between 14-3-3β and GSK3β. (A) Western blot analysis of interactions in U87 and SVGp12 cells transfected with control or 14-3-3β vectors (over-14-3-3β) by immunoprecipitation (IP) with the Flag antibody. IgG was used as the control for the bait antibody. (B) Detection of total and phosphorylated protein levels of GSK-3β by western blot analysis. Phosphorylation of serine 9 of the GSK3β antibody was used to detect the phosphorylation of GSKβ. β-actin was used as a control. At least three independent experiments were performed.

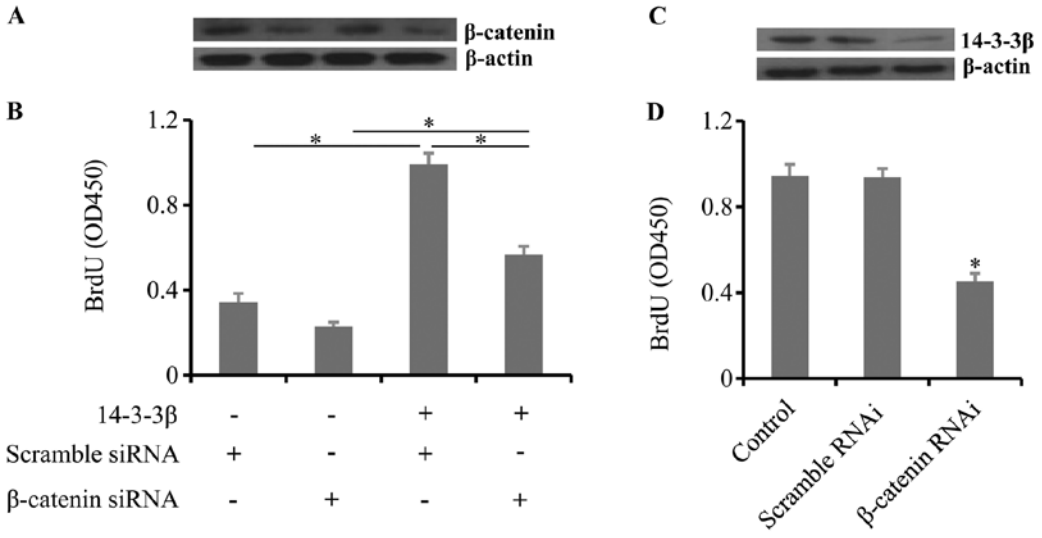

Figure 3. Effect of β-catenin siRNA on cell proliferation. (A) Western blot analysis of β-catenin protein levels in 14-3-3β overexpression and β-catenin siRNA co-transfected SVGp12 cells. (B) BrdU assays were performed to test the cell proliferation after 72 h of co-transfection in SVGp12 cells. (C) Western blot analysis of 14-3-3β protein levels in β-catenin siRNA-transfected U87 cells. (D) BrdU assays were used to detect the cell proliferation after 72 h of β-catenin siRNA transfection in U87 cells. *P<0.01 denotes a significant difference.

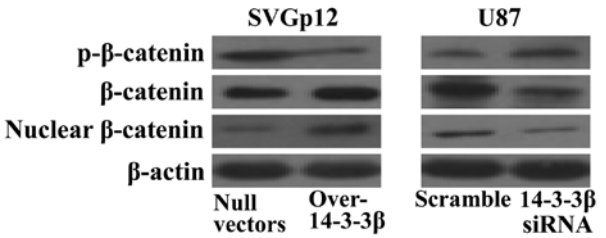

Figure 4. Levels of β-catenin protein. Western blot analysis of protein levels of p-β-catenin, total β-catenin and nuclear β-catenin in 14-3-3β-overexpressing SVGp12 cells (left panel) and in 14-3-3β siRNA-transfected U87 cells (right panel). β-actin was used as a control. At least three independent experiments were performed.

results, β-catenin was knocked down in U87 cells (Fig. 3C). Knockdown of β-catenin also resulted in a decrease in cell proliferation in U87 cells (Fig. 3D). These results suggest that 14-3-3β regulates cell proliferation through β-catenin.

14-3-3β promotes the activity of β-catenin. To further explore the underlying mechanisms of 14-3-3β and β-catenin in regulating cell proliferation, the activity of β-catenin was analyzed in transfected cells. Western blot analysis showed that phosphorylation of β-catenin was decreased after 14-3-3β overexpression in SVGp12 cells, which led to an increase in total β-catenin protein levels. In addition, there was also more β-catenin protein detected in the nucleus (Fig. 4, left panels). In contrast, knockdown of 14-3-3β in U87 cells decreased both the total protein and nuclear levels of β-catenin (Fig. 4, right panels). These results suggest that 14-3-3β may augment β-catenin stability and nuclear translocation through sequestering GSK3β.

14-3-3β increases oncogene transcription mediated by β-catenin. To further confirm that 14-3-3β augments the activity of β-catenin, the transcription levels of the c-myc oncogene and cyclin D1, which were activated by β-catenin nuclear translocation (34,35), were analyzed by qRT-PCR. Overexpression of 14-3-3β in human normal SVGp12 astrocytes increased the transcription level of c-myc and cyclin D1

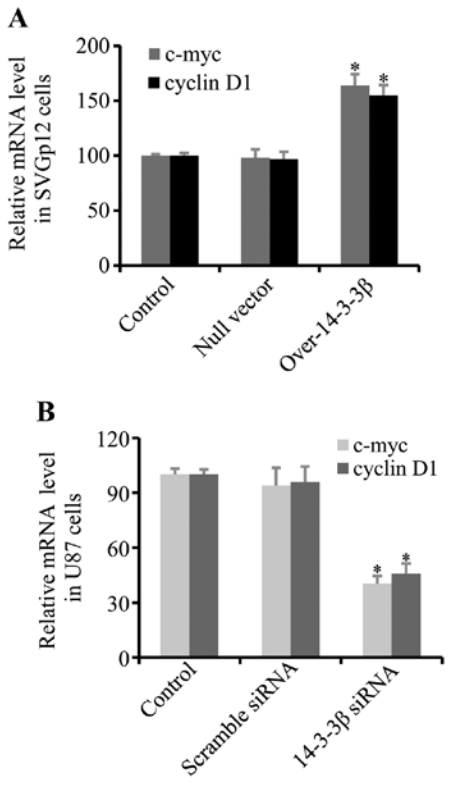

Figure 5. Transcriptional levels of oncogene c-myc and cyclin D1. Transcriptional levels of C-myc and cyclin D1 in (A) 14-3-3β-overexpressing SVGp12 cells and (B) 14-3-3β siRNA-transfected U87 cells as determined by qRT-PCR. Actin was used as a loading control. All experiments were independently performed at least three times. The data are expressed as the mean ± SEM and analyzed using the Student's t-test and *P<0.01 is considered to indicate a statistically significant result.

(Fig. 5A). In contrast, knockdown of 14-3-3β in U87 glioma cells decreased the transcription levels of c-myc and cyclin E (Fig. 5B). These results suggest that 14-3-3β promotes oncogene expression mediated by β-catenin.

Discussion

In general, we demonstrated that 14-3-3β regulated the proliferation of glioma cells through sequestering GSK3β, which augmented the nuclear translocation of β-catenin leading to an increase in oncogene expression. The present study provides a mechanism of 14-3-3β in regulating human astrocytomas. However, further investigation *in vitro* and *in vivo* of 14-3-3β in astrocytomas is required.

Our previous studies showed that the mRNA and protein levels of 14-3-3β were closely related to the pathological grades of astrocytoma implying critical roles of 14-3-3β in tumorigenesis. In the present study, we found that overexpression of 14-3-3β in normal human astrocytes significantly increased cell proliferation, while silencing 14-3-3β in glioma cells inhibited cell proliferation. The function of 14-3-3β in regulating cell proliferation through various pathways is well known. It has been reported that 14-3-3β regulates the G2/M phase transition via interaction with integrin, testicular protein kinase 1 and Wee1 (36-38). 14-3-3β has been found to be oncogenic in fibroblasts, and can promote tumorigenesis in nude mice (39). Knockdown of 14-3-3β in liver cancer cells suppressed cell proliferation and decreased oncogenicity in nude mice (40). Upregulation of 14-3-3β promoted cell proliferation and tumor formation by the mitogen-activated protein kinase (MAPK)-dependent signaling pathway in NIH3T3 cells (39). Increased expression of 14-3-3β was observed in Kaposi's sarcoma and papillary thyroid carcinomas and promoted cell proliferation and tumor progression (41,42). Our previous studies demonstrated that 14-3-3β expression increased with the degree of human astrocytoma. Thus, in accordance with previous studies, our present study suggests that 14-3-3β plays important roles in glioma cells implying that targeting 14-3-3β for human astrocytoma therapy may be a promising method.

14-3-3β is expressed in tumor tissues and cell lines of many types of cancers including lung, prostate and breast cancer (42,43). However, the mechanism of 14-3-3β in the regulation of cancer cells is quite complicated. 14-3-3β is reported to be involved in cell apoptosis through interaction with apoptotic factors, such as Bcl and Bax. 14-3-3β can disturb the complex of Bax and Bcl, which promotes apoptosis upon Bax phosphorylation (39,44). In the present study, we demonstrated that 14-3-3β interacts with GSK3β in the regulation of cell proliferation. GSK3β regulates a wide range of cellular processes including cell cycle control, cell growth and cell survival via diverse signaling pathways (45). GSK3β activity depends on its phosphorylation of serine 9. Both PI3K/Akt and Wnt signaling are required for the phosphorylation of GSK3β. Activation of PI3K/Akt phosphorylates and inhibits GSK3β, which frequently occurs in cancers (25,46). Under normal conditions, GSK3β is unphosphorylated and active, and could phosphorylate and interact with β-catenin leading to β-catenin degradation (24,28). On the contrary, the accumulation of β-catenin can lead to the activation of oncogene expression. 14-3-3 proteins have the ability to bind phosphoserine-containing sequence motifs. In the present study, we found that serine-9 phosphorylation of GSK3β was enhanced in 14-3-3β-overexpressing cells implying that 14-3-3β interacted with GSK3β, which sequestered GSK3β and increased phosphorylation of GSK3β. Inhibition of GSK3β has been suggested to facilitate cancer cell proliferation (47,48).

For a long time, 14-3-3 proteins were thought to be brain-specific proteins due to their high abundance in brain tissues (49). At present, 14-3-3 proteins have been found to be expressed in all eukaryotic cells (50). The interaction of 14-3-3 proteins with GSK3β in other cell types has been demonstrated in various studies (51-53). GSK3β and β-catenin form a destruction complex along Axin and adenomatous polyposis coli. The complex is cytoplasmic and leads to β-catenin phosphorylation by GSK3β and subsequent ubiquitination and degradation. Once the destruction complex is degraded, β-catenin is stabilized leading to its accumulation in the cytoplasm, resulting in its subsequent translocation into the nucleus (54). In the present study, we demonstrated that overexpression of 14-3-3β is associated with the sequestration of GSK3β and causes β-catenin release. The nuclear translocated β-catenin along with the Tcf/Lef complex activates oncogene expression including c-myc and cyclin D1, which are highly upregulated in human tumors and induce cell proliferation (34,35). In the present study, we demonstrated that overexpression of 14-3-3β increased the transcription levels of c-myc and cyclin D1. This may be responsible for the formation and development of astrocytomas.

In conclusion, the present study revealed that 14-3-3β mediated the cell proliferation of glioma cells through GSK3β/β-catenin. Overexpression of 14-3-3β sequestered GSK3β leading to an increase in β-catenin nuclear translocation and activation of oncogene transcription. Given the high abundance of 14-3-3β in astrocytoma tissues, one can speculate that novel therapeutic strategies or drugs aimed at 14-3-3β may have potential for the treatment of human astrocytomas. However, further *in vitro* and *in vivo* studies should be conducted for verifying the precise mechanisms of 14-3-3β in astrocytomas.

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