CEP55 promotes cell proliferation and inhibits apoptosis via the PI3K/Akt/p21 signaling pathway in human glioma U251 cells

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Abstract. Human glioma is one of the major malignancies worldwide with an increased mortality rate. Centrosomal protein of 55 kDa (CEP55) is an essential component of the CEP family and has been identified as a prognostic marker for multiple types of cancer. However, the function of CEP55 during glioma tumorigenesis remains unclear. In the present study, the data derived from the Oncomine database indicated that the expression of CEP55 is increased in glioma tissues compared with normal tissues. Furthermore, the expression of CEP55 was also increased at the level of mRNA and protein in glioma cell lines compared with normal human astrocytes. The knockdown of CEP55 expression inhibited the proliferation of glioma U251 cells, whereas overexpression of CEP55 induced the proliferation of U251 cells. Flow cytometric analysis indicated that the knockdown of CEP55 resulted in an increased number of cells arrested at G_2/M phase, and apoptosis was promoted. Further investigations revealed that the overexpression of CEP55 increased the phosphorylation of Akt and inhibited the activity of p21. By contrast, the knockdown of CEP55 resulted in the opposite effects. Taken together, the results of the present study suggested that CEP55 regulated the proliferation of glioma cells, further attributing to the carcinogenesis and progression of glioma via the PI3K/Akt/p21 signaling pathway. Therefore, CEP55 may be a novel therapeutic target for the treatment of glioma.

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Abbreviations: GBM, glioblastoma multiforme; HA, astrocyte cell; FBS, fetal bovine serum; PMSF, phenylmethanesulfonyl fluoride; PI, propidium iodide

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Key words: centrosomal protein of 55 kDa, glioma, cell proliferation, apoptosis, phosphoinositide 3-kinase/Akt/p21

Introduction

Human glioma is one of the most common and lethal primary central nervous system (CNS) tumors. The broad category of glioma represents ~27% of all CNS tumors according to the Central Brain Tumor Registry of the United States (1) and 80% of malignant tumors (1). Gliomas vary widely in histology from benign and potentially surgically curable grade I tumors (pilocytic astrocytoma) to locally aggressive grade IV tumors (particularly glioblastoma, GBM) with an increased risk of recurrence or progression (2). The morbidity and mortality rates for patients with glioma remain high, despite undergoing aggressive multimodality therapy, which consists of maximal surgical resection, adjuvant radiation and chemotherapy. Despite optimal treatment, the median survival time is only 12-15 months for patients with GBM and 2-5 years for patients with anaplastic gliomas (3). There are several risk factors for which an association with brain tumors has been established, including ionizing radiation, genetic susceptibility, and allergic aspects (2). However, the molecular pathogenesis underlying glioma tumorigenesis remains elusive. As the current understanding of the molecular pathogenesis of these tumors improves, it may be possible to select the most appropriate therapies.

Centrosomal protein of 55 kDa (CEP55), also known as FLJ10540, C10, f3 or URCC6, is an essential member of CEP family proteins, which serve a vital function in mitotic exit and cytokinesis (4). CEP55 localizes to the mitotic spindle during prometaphase and metaphase, and translocates to the spindle mid-zone and the mid-body during anaphase and cytokinesis (5). During cytokinesis, CEP55 may cooperate with members of endosomal sorting complex required for transport (ESCRT) machinery, to constrict an intracellular bridge to allow abscission (6-8). Apart from cytokinesis, CEP55 is also associated with embryonic growth (9). Additionally, CEP55 has been associated with cancer progression by activating the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway (10), and is identified as a prognostic marker for multiple types of cancer (10). A high expression of CEP55 expression occurs in a diverse range of solid tumors, including prostate cancer (11), bladder cancer (12) and epithelial ovarian carcinoma (13). Although the roles of CEP55 in different types of cancer and the regulatory mechanisms involved have been reported, the function and mechanism of CEP55 in glioma tumorigenesis has not been fully elucidated.

Materials and methods

Analysis of the Oncomine database. Oncomine (www.oncomine.org) is a cancer microarray database and web-based data-mining platform aimed at facilitating identification from genome-wide expression analyses. To evaluate the mRNA expression of CEP55 in glioma, data was extracted from the Oncomine cancer microarray database. According to the standard procedures as previously described (14), the differential gene expression of CEP55 in normal brain tissues and glioma tissues was obtained. French Brain Statistics (15), The Cancer Genome Atlas (TCGA; www.cancergenome.nih.gov), Murat Brain Statistics (16) and Sun Brain Statistics (17) were used to compare CEP55 mRNA expression levels between cancer and normal tissues.

Cell culture. The human glioma cell lines U251 and U343, normal human astrocyte cells (HA) and the lentivirus vector package cell line 293T used in the present study were obtained from Research Center for Neurobiology of Xuzhou Medical University (Xuzhou, China). All cells were cultured in Dulbecco's modified Eagle's medium/high glucose (Hyclone; GE Healthcare, Chicago, IL, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin streptomycin solution (Vicmed, Xuzhou, China). The cells were maintained at 37˚C in a humidified incubator with 5% CO_2.

Establishment of stable U251 cell lines which overexpress or downregulate CEP55. Three CEP55-targeted RNA interference (RNAi) sequences were synthesized and inserted into a GV248 plasmid [element sequence, hU6-multiple cloning site (MCS)-ubiquitin-enhanced green fluorescent protein (EGFP)-internal ribosome entry site (IRES)-puromycin] to construct the recombinant plasmids. The sequences were as follows: RNAi-1, 5'-AGC GGGAAGTCTATGTAAA-3'; RNAi-2, 5'-AGGCATGTA CTTTAGACTT-3' and RNAi-3, 5'-AAGCCTAGTAAC TCCAAAT-3'. The GV358 lentivirus vector (element sequence, Ubi-MCS-3FLAG-SV40-EGFP-IRES-puromycin; Genechem, Shanghai, China) containing scrambled RNA (sequence, 5'-TTCTCCGAACGTGTCACGT-3') was used as a negative control. The four plasmids were co-transfected into 293T cells with packaging plasmids, pHelper 1.0 and pHelper 2.0 using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. At ~48 h post-transfection, lentivirus was harvested by centrifugation at 4˚C at 2,500 x g for 10 min, to dispose of cell debris and finally collected by ultracentrifugation at 4˚C at 50,000 x g for 2 h. The transfected cells were selected by puromycin at 1.5 mg/ml for 2 weeks.

The full-length human CEP55 cDNA was cloned into the lentiviral vector GV358 to construct the recombinant plasmid. A blank GV358 lentiviral vector was used as negative control. Then, the constructs were co-transfected into 293T cells with assistant packaging plasmid using Lipofectamine® 2000, according to the manufacturer's protocol. At 48 h post-transfection, lentivirus was collected and infected into U251 cells. Transfected cells were selected by puromycin for 2 weeks, and the cell line with a stable overexpression of CEP55 was obtained. In all experiments, untransfected cells were used as controls.

Western blot analysis. U251 and U343 cells were washed three times with ice-cold PBS, and trypsinized and collected. Following lysis on ice for 30 min using radioimmunoprecipitation assay lysis buffer (Merck KGaA, Darmstadt, Germany) containing protease inhibitors and phenylmethanesulfonyl fluoride (PMSF), the lysate was centrifuged at 12,000 x g at 4˚C for 20 min. Phosphatase inhibitors were added when necessary. The concentration of the proteins was measured using a BCA protein assay kit (Beyotime Institute of Biotechnology, Haimen, China). Protein samples were loaded and separated by 12% SDS-PAGE, and transferred onto nitrocellulose membranes. The membranes were blocked in 5% non-fat milk suspended in washing buffer (100 mmol/l NaCl, 10 mmol/l Tris/HCl and 0.1% Tween-20) for 1 h at room temperature. The membranes were incubated overnight at 4˚C with primary antibodies against CEP55 (cat. no. 23891-1-AP; dilution 1:500; Proteintech Group, Inc., Chicago, IL, USA), cyclin B1 (cat. no. ab181593; dilution 1:11,000), cyclin A1 (cat. no. ab133183; dilution 1:11,000), cyclin D1 (cat. no. ab134175; dilution 1:11,000), p-Akt (cat. no. ab38449; dilution 1:11,000), Akt (cat. no. ab182729; dilution 1:11,000), p21 (cat. no. ab109199; dilution 1:11,000). GAPDH (cat. no. ab8245; dilution 1:1,000; all Abcam, Cambridge, UK) was used as a loading control. The following day, the membranes were probed using IRDye 800CW-conjugated goat anti-rabbit secondary antibody (cat. no. 926-32211; dilution 1:5,000; LI-COR Biosciences, Lincoln, NE, USA) at room temperature for 1 h. Protein bands were detected using an Odyssey infrared imaging system (LI-COR Biosciences). The intensity of the bands was quantified using ImageJ software (version 1.48; National Institutes of Health, Bethesda, MD, USA), and the level of protein expression was normalized to the expression of GAPDH.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was isolated from cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's protocol. RNA was reverse transcribed into cDNA using Vic qRT Super kit (Vicmed), according to the manufacturer's protocol. qPCR was performed using SYBR qPCR mix (Roche Diagnostics, Basel, Switzerland), run on a Light Cycler 480 Instrument II (Roche Diagnostics). The reactions were incubated in a 96-well optical plate at 95˚C for 10 min for 40 cycles of 95˚C for 10 sec, 60˚C for 20 sec and 72˚C for 30 sec. GAPDH was used as an internal control, and the relative expression levels of the gene of interest were calculated using the $2^{-\Delta\Delta Cq}$ method (18). The sequences of the primers are as follows: CEP55 forward, 5'-GAGGAT CCCCGGGTACCGGTCGCCACCATGTCTTCCAGAAGT ACCAAAG-3' and reverse, 5'-TCCTTGTAGTCCATACCC TTTGAACAGTATTCCACATGGAC-3'; GAPDH forward, 5'-CTCTCTGCTCCTCCTGTTCGAC-3' and reverse, 5'-TGA GCGATGTGGCTCGGCT-3'. All RT-qPCRs were performed in triplicate.

Cell Counting kit-8 (CCK-8) assay. Cell viability was determined by CCK-8 cell viability assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). The U251 cells were

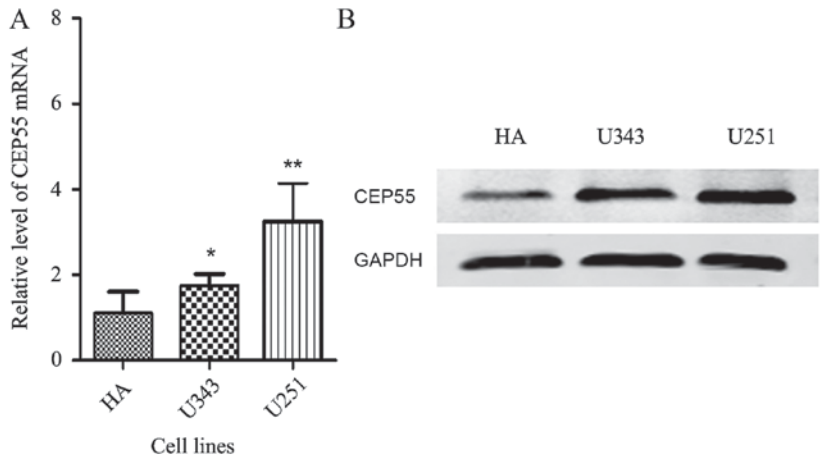

Figure 1. Upregulation of CEP55 expression in glioma cell lines. (A) RT-qPCR and (B) western blot analysis of CEP55 expression levels in normal human astrocytes (HA) and glioma cell lines. *P<0.05 and **P<0.01 vs. HA. CEP55, centrosomal protein of 55 kDa; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

seeded into 96-well plates at a density of $3x10^3$ cells/well. CCK-8 solution was added to each well at 0, 24, 48 and 72 h. Following incubation for 1.5 h at 37˚C, the optical density was measured at a wavelength of 450 nm on a microplate reader (BioTek China, Beijing, China). All experiments were performed three times.

Cell cycle distribution analysis. For analysis of the cell cycle, Cell Cycle Detection kit (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) was used according to manufacturer's protocol. The cells were trypsinized and collected. After washing twice with ice-cold PBS, the U251 cells were fixed with 70% ethanol at 4˚C overnight. Following centrifugation at 1,000 x g for 5 min, at 4˚C, the supernatant was discarded. Subsequently, the cells were resuspended in PBS with 100 μl/ml RNase A in a 37˚C water bath for 30 min. The cells were then stained using propidium iodide (400 μl/ml) and incubated for 30 min at room temperature in the dark. Finally, the cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA), and the data were analyzed using Multi Cycle software (version AV; Phoenix Flow Systems, San Diego, CA, USA).

Cell apoptosis analysis. Cell apoptosis ratio was measured using an Annexin V-allophycocyanin (APC)/7-aminoactinomycin D (AAD) Apoptosis Detection kit (Nanjing KeyGen Biotech. Co., Ltd.), according to the manufacturer's protocol. In brief, a total of $1x10^6$ U251 cells were harvested, washed with PBS twice and resuspended in 500 μl binding buffer. Cells were subsequently dual stained with 5 μl Annexin V-APC and 5 μl 7-AAD at room temperature in the dark. Following 20 min incubation at room temperature, the cells were immediately analyzed using a FACSCalibur flow cytometer (BD Biosciences), and cell apoptosis ratio was determined using FlowJo software (version X; FlowJo LLC, Ashland, OR, USA).

Statistical analysis. Quantitative data are presented as the mean ± standard deviation of three independent experiments. SPSS software (version 16.0; SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) were used for all statistical analysis. Independent sample t-tests and paired t-tests were used within groups for repeated measures. Multiple comparisons between groups were performed using one-way analysis of variance followed by Student-Newman-Keuls test for statistical analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

CEP55 expression is upregulated in glioma tissues and cell lines. To determine the expression pattern of CEP55 in glioma, four independent microarray datasets from Oncomine database were analyzed. When compared with normal brain tissues of patients from the study of French Brain Statistics (15), The Cancer Genome Atlas (TCGA, www.cancergenome.nih.gov), Murat Brain Statistics (16) and Sun Brain Statistics (17), CEP55 expression was elevated in glioma tissues (data not shown). To validate this observation, RT-qPCR and western blotting were further performed to assess CEP55 expression levels in glioma cell lines, U343 and U251. Compared with the normal human astrocytes (HA), the mRNA (Fig. 1A) and protein levels of CEP55 were increased in these two glioma cell lines (Fig. 1B).

Successful stable RNAi knockdown and overexpression of CEP55 in U251 cell lines. To investigate the effect of CEP55 on the behavior of glioma cells, U251 cell lines with stable CEP55 knockdown and overexpression were established using lentivirus-mediated technology. Negative control and three CEP55 targeting RNA interference 1, 2 and 3 (RNAi-1, 2 and 3) were infected into U251 cells, and the effects of CEP55 knockdown in these groups were evaluated by western blot analysis. The expression of CEP55 was decreased was most marked in response to RNAi-2 treatment in U251 cells compared with that in the negative control (untreated U251 cells; Fig. 2A), therefore RNAi-2 was selected for subsequent experiments.

In addition, a CEP55-overexpressing lentivirus vector was established and stably transfected into U251 cells.

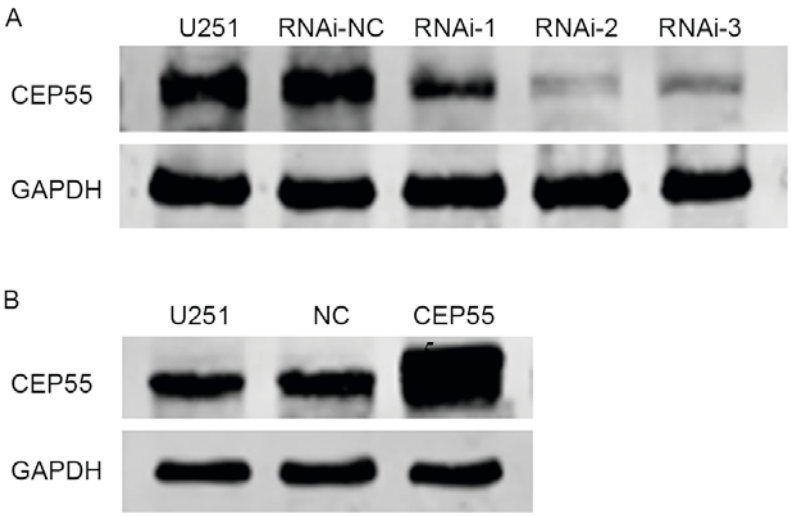

Figure 2. Stable CEP55 RNAi knockdown and overexpression in U251 cell line. Relative protein expression levels of CEP55 in U251 cells were analyzed by western blotting. (A) A reduced level of CEP55 protein was confirmed following infection with the lentivirus containing three constructs RNAi (RNAi-1, 2 and 3) or RNAi-NC. (B) An increased level of CEP55 protein was detected following infection with lentivirus overexpressing CEP55 or negative control. RNAi, RNA interference; NC, negative control. CEP55, centrosomal protein of 55 kDa; RNAi, RNA interference.

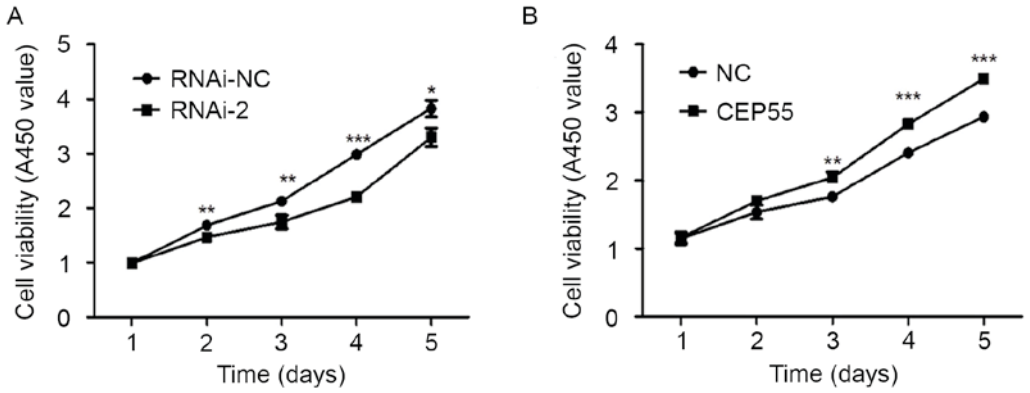

Figure 3. Effects of stable overexpression and knockdown of CEP55 expression on cell viability of U251 cells. Cell viability was evaluated using cell-counting-kit 8, and absorbance was measured at 450 nm. (A) Growth curve of CEP55 RNAi group. There were significant differences in optical density values (450 nm) on days 2-5 between the RNAi-NC and RNAi-2 groups. (B) Growth curve of CEP55 overexpression group. There were significant differences in optical density values (450 nm) on days 3-5 between U251-CEP55 and U251-NC groups. *P<0.05, **P<0.01 and ***P<0.001 vs. NC groups. CEP55, centrosomal protein of 55 kDa; NC, negative control; RNAi, RNA interference; NC, negative control.

Western blot analysis was used to evaluate the transfection efficacy (Fig. 2B). As indicated in Fig. 2B, the levels of CEP55 protein expression in CEP55-overexpressing U251 cells were increased compared with the empty vector transfected cells or untransfected cells.

CEP55 is associated with viability of U251 cells. To determine the function of CEP55 in regulating the viability of U251 cells, a CCK-8 assay was used. The results revealed that downregulating the expression of CEP55 significantly inhibited the viability of U251 cells (Fig. 3A). Additionally, overexpression of CEP55 promoted the viability of U251 cells (Fig. 3B). The results suggest that CEP55 may serve an important function in regulating the viability of U251 cells.

Knockdown of CEP55 induces cell cycle arrest and apoptosis. The regulation of cell viability may affect cell cycle progression. To further investigate the mechanism of CEP55 in proliferative ability of glioma cells, the cell cycle distribution was detected by flow cytometric analysis. As presented in Fig. 4A, knockdown of CEP55 significantly decreased the number of G_0/G_1 and S phase cells, and increased the number of G_2/M phase cells, which indicates that an increased number of cells were arrested at G_2/M phase. The cell cycle distribution is associated with the expression of cyclin-associated genes (19). Therefore, the level of cyclins was detected by western blot analysis (Fig. 4B). The level of cyclin B1, a cyclin that is important for G_2 to M transition, was significantly increased in CEP55-knockdown U251 cells compared with the RNAi-negative (NC) group (Fig. 4B). However, the levels of cyclin A1 and cyclin D1 were significantly reduced in CEP55-knockdown U251 cells compared with the RNAi-NC group (Fig. 4B). Furthermore, Annexin APC/7-AAD staining by flow cytometry was performed to evaluate the function of CEP55 in apoptosis of U251 cells. The results demonstrated that CEP55 knockdown significantly increased cell apoptotic

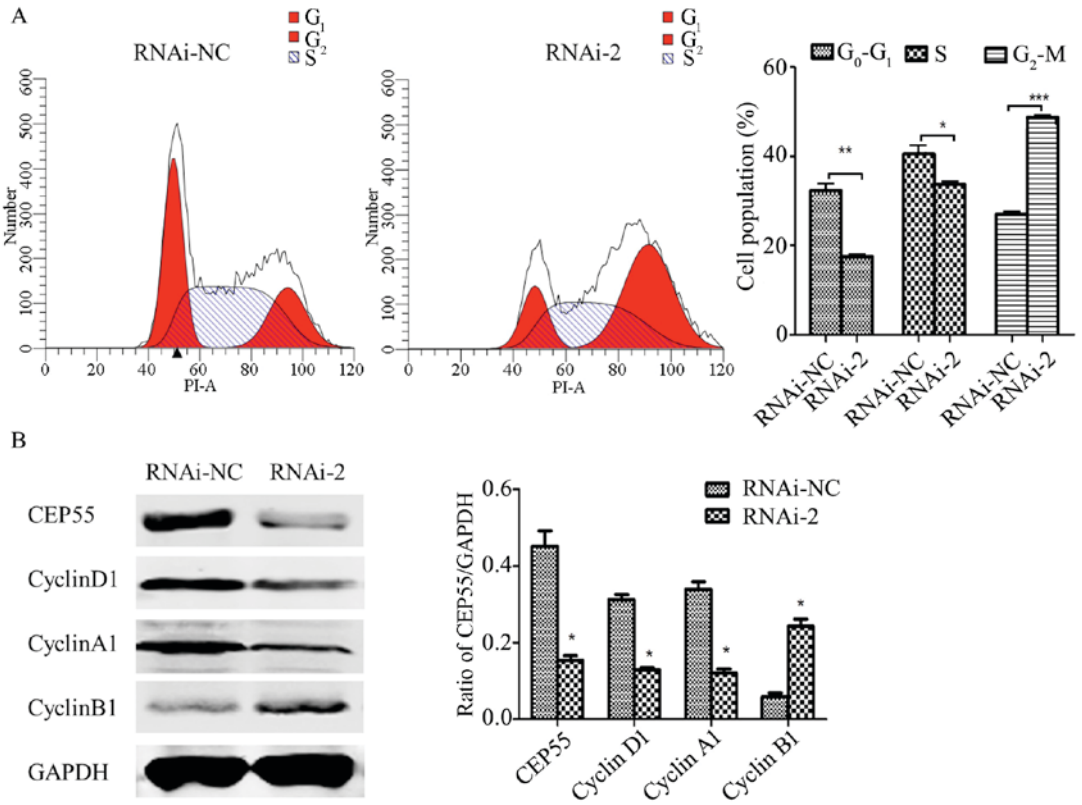

Figure 4. Knockdown of CEP55 expression in U251 cells inhibits cell cycle progression. (A) The percentage of cells in G_2/M phase was higher in U251 cells with CEP55 knockdown compared with negative control cells. (B) Protein expression levels of cyclins, which were associated with cell cycle distribution, were analyzed by western blot analysis. *P<0.05, **P<0.01 and ***P<0.001 vs. NC. CEP55, centrosomal protein of 55 kDa; RNAi, RNA interference; NC, negative control.

rates compared with the RNAi-NC group (13.67 to 22.01%; Fig. 5). Taken together, these results revealed that CEP55 may have a critical function in cell cycle progression and apoptosis of U251 cells.

CEP55 regulate cell viability via the PI3K/Akt/p21 signaling pathway. The cell cycle is regulated by a number of signaling molecules and cyclins. CEP55 has been implicated to regulate the PI3K/Akt signaling pathway via an interaction with the catalytic subunit of PI3K (9,20). Previously, several molecular targets of CEP55, including p21, cyclin-dependent kinase (CDK)4 and B-cell lymphoma (Bcl)-2, associated with the cell cycle have been assessed (21). An inhibitor of CDK, p21, was a key cell cycle regulator and also a downstream target of the PI3K/Akt signaling pathway (22). In light of previous reports, PI3K/Akt/p21 may act as a downstream target of CEP55. To investigate this hypothesis, a number of proteins associated with the PI3K/Akt/p21 signaling pathway were examined by western blot analysis. The results indicated that when CEP55 was knocked down, the level of phospho-Akt was decreased, and the level of p21 was increased (Fig. 6). Conversely, the level of phospho-Akt was increased, and the level of p21 was decreased in response to overexpression of CEP55 in the U251 cell line (Fig. 6). Collectively, the results of the present study suggested that CEP55 acts as a regulator of PI3K/Akt/p21 signaling, as overexpression of CEP55 inhibited the activation of the PI3K/Akt signaling pathway, further inhibiting cell viability.

Discussion

Gliomas are the second most common brain tumors in adults, which account for ~24% of all adult brain tumors (2). Despite available therapies, including radiotherapy, chemotherapy and adjuvant therapies, the relative survival time for glioblastoma is short with only 5.1% of patients who survive five years post diagnosis (1). Therefore, there is an urgent requirement to discover more efficacious therapy methods and novel molecular biomarkers for glioma tumorigenesis. It is well known that the PI3K/Akt signaling pathway is one of the major pro-survival pathways. CEP55 has been reported to be a regulator of the PI3K/Akt signaling pathway (22-26), which may indicate that CEP55 may participate in cell viability and tumor development, by regulating this specific pathway. To date, the implication of CEP55 in cancer cell viability has been frequently demonstrated in various types of human cancer (23,25,27,28). However, the function of CEP55 in glioma tumorigenesis remains unclear.

In the present study, the expression of CEP55 in glioma was detected. According to the results from the Oncomine database, CEP55 expression was markedly increased in glioma compared with normal brain tissues. In addition, the results of the present study showed that CEP55 expression was also increased in glioma cell lines compared with normal human astrocytes. All these results indicated that CEP55 may be an interesting candidate for its involvement in glioma tumorigenesis. In order to investigate this hypothesis, the effect of

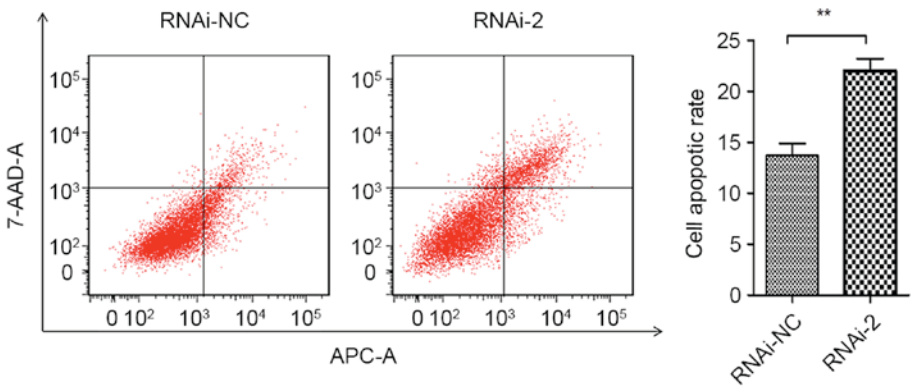

Figure 5. Effects of CEP55 knockdown by RNAi on the apoptosis of U251 cells. The percentage of apoptotic cells was significantly higher in CEP55-knockdown U251 cells compared with negative control cells. **P<0.01 vs. NC. APC, allophycocyanin; CEP55, centrosomal protein of 55 kDa; RNAi, RNA interference; NC, negative control.

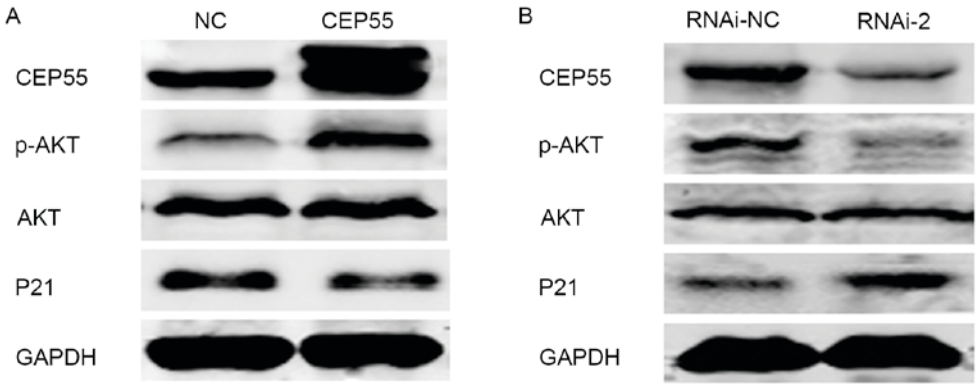

Figure 6. CEP55 regulates the PI3K/Akt/p21 signaling pathway. Protein levels of p-Akt, total Akt and p21 were determined by western blot analysis when CEP55 was overexpressed (A) or knocked down (B) in U251 cells. CEP55, centrosomal protein of 55 kDa; PI3K, phosphatidylinositol-3-kinase; NC, negative control; RNAi, RNA interference.

overexpression and knockdown of CEP55 on the cell viability of glioma U251 cells was evaluated. In the present study, it was demonstrated that the knockdown of CEP55 expression led to decreased cell viability, apoptosis induction and cell cycle arrest as well as the inactivation of the PI3K/Akt/p21 signaling pathway. By contrast, the overexpression of CEP55 promoted cell viability and the activation of the PI3K/Akt/p21 signaling pathway in the U251 glioma cell line. Therefore, it was hypothesized that CEP55 is likely to regulate cell viability via the PI3K/Akt/p21 signaling pathway in the glioma cell line.

The deregulation of cell viability is a key characteristic of cancer cells, which is associated with cell cycle regulation (29,30). CEP55 exerts a pivotal role in cell cycle progression (31). It was indicated in the present study that an increased number of cells accumulated in G_2/M phase following CEP55 knockdown. This result was consistent with previous research on hepatocellular and gastric carcinoma (25). In addition, cyclin D1 (32), cyclin A (33) and cyclin B1 (34) are the major cyclins that control the G_0/G_1, S and G_2/M phases of the cell cycle, respectively. In the present study, it was demonstrated that the knockdown of CEP55 expression promoted cyclin B1 expression and inhibited the expression of cyclin D1 and cyclin A1, which further supported the results indicating G_2/M phase arrest. However, some other studies have data which differed from the current results, where the knockdown of CEP55 induced G_0-G_1 phase arrest in lung cancer cells (21) and breast cancer (27). These differences in the results may be due to the different mechanisms of cancer occurrence. Furthermore, the potential mechanism underlying CEP55-associated tumor phenotypes has not been elicited, and this maybe a key area for future study.

The PI3K/Akt signaling pathway is tightly controlled via a multistep process (20). Activated PI3K/Akt signaling mediates numerous cellular functions, including angiogenesis, metabolism, growth, cell viability, survival, protein synthesis, transcription and apoptosis (20). Akt has diverse roles in cell survival, cell cycle, angiogenesis, protein synthesis and metabolism (35). Increased PI3K activation results in an increased pool of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) and a subsequent increase in Akt activation as marked by Ser^{473} phosphorylation (10). Whereas mammalian target of rapamycin (mTOR) and p21 are substrates for Akt, those two proteins have been identified to exhibit opposite functions. The present study identified an increase in those proteins in the U251 cell line consistent with previous studies (10,32-35). CEP55 is confirmed to be overexpressed in a wide range of solid tumors, including lung adenocarcinoma (23), hepatocellular carcinoma (26), glioma (24) and gastric carcinoma (25), where the overexpression of CEP55 promotes cell metastasis, invasion

and cell viability via upregulation of the PI3K/Akt signaling pathway. CEP55 directly interacts with the PI3K catalytic subunit, PIK3CA (also known as p110), which promotes its stability and activation (28). Consistent with previously reported studies, the results of the present study demonstrated that the overexpression of CEP55 resulted in the upregulation of the PI3K/Akt signaling pathway, leading to dysregulated cell cycle via inhibition of p21. However, Wang *et al* (24) implicated that CEP55 regulates glucose, metabolism, cell viability and apoptosis of glioma cells via the Akt/mTOR signaling pathway. Taken together, all these studies demonstrate that CEP55 may promote tumor cell viability through activation of the PI3K/Akt/p21 signaling pathway in glioma. It is premature to draw any conclusions from the present studies with CEP55, as several important questions remain unanswered, including the underlying molecular signaling pathways of CEP55 in glioma. Additional *in vivo* studies are required to confirm the conclusions of the present study.

In conclusion, the results of the present study suggested that CEP55 has important roles in regulating various cellular processes, including cell viability, cell cycle and apoptosis, by mediating PI3K/Akt/p21 signaling in glioma cell lines. Combined with previous studies, the present study indicates that CEP55 may be a potential therapeutic target for glioma. Additional studies investigating the regulation and function of CEP55 during cancer development and reoccurrence are required to design therapeutic strategies for various human malignancies with CEP55 overexpression.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FL and DJ contributed equally to the present study by designing and conducting experiments, analyzing data and writing the paper. FL, DJ and CXT conducted experiments and collected data. DSG conceived of the project and experiments and analyzed data. All authors critically revised the manuscript and provided final approval.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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