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Original Article

Knockdown of long noncoding RNA UCA1 inhibits glioma cell metastasis via reduction of epithelial-mesenchymal transition

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Abstract: Urothelial carcinoma associated 1 (UCA1) is a long noncoding RNA (lncRNA) which has been identified as an oncogenic gene in multiple human tumors, but little was known about the correlation of UCA1 in glioma. This study aimed to explore the role of UCA1 in the metastasis of glioma cells. UCA1 expression was monitored by RT-PCR in glioma and adjacent tissues, as well as in normal astrocyte and glioma cell lines. U87MG and U251 cells were transfected either with siRNA against UCA1 or siRNA negative control, then cell viability, migration and invasion were respectively measured by CCK-8 and Transwell system. Western blot analysis was performed to assess the expression changes in E-cadherin, N-cadherin, vimentin, AKT and p-AKT in the transfected cells. Up-regulation was found in glioma tissues and cell lines, when compared with their corresponding controls ($P < 0.05$). Knockdown of UCA1 greatly suppressed cell viability, migration and invasion ($P < 0.05$). Besides, UCA1 knockdown up-regulated E-cadherin, while down-regulated N-cadherin and vimentin, as well as inhibited AKT phosphorylation ($P < 0.05$). In conclusion, UCA1 is high expressed in glioma and acts as an anti-proliferation and anti-metastasis factor in glioma cells. UCA1 may be a potential biomarker and therapeutic target of glioma.

Keywords: Urothelial carcinoma associated 1, glioma, metastasis, EMT, AKT

Introduction

Long non-coding RNAs (lncRNAs) are RNA molecules with greater than 200 nucleotides, which are typically transcribed by RNA polymerase II and are often multiexonic and polyadenylated [1]. To date, the exact functions of lncRNA are poorly understood, but lncRNA has been found to play key role in various biological progresses, such as imprinting control, cell differentiation, immune response and chromatin modification [2, 3]. Several lncRNAs have also been shown to be involved in carcinogenesis and cancer progression [4].

Glioma is the most common malignant brain tumor, with an incidence rate of 6.03 per 100,000 individuals each year [5, 6]. The treatment of glioma is still a major challenge due to the low sensitivity to radio-/chemo-therapeutic agents, and tumor metastasis [7]. Therefore, a better understanding of the mechanisms that involved in progression and metastasis of glioma is urgent needed to develop more effective therapies [8]. Recent studies have revealed that numerous lncRNA are functionally linked with glioma origination and progression. Based on microarray-based data, previous study shown that specific lncRNA expression patterns were associated with different histological subtypes and malignant behaviors in glioma [9]. For instance, H19, MALAT1 and POU3F3 were positively correlated with more malignant glioma phenotypes [10], and H19 also modulated glioma cells proliferation and migration [11].

Urothelial carcinoma associated 1 (UCA1) is a lncRNA which has been identified as oncogenic gene in multiple human tumors, such as non-small cell lung cancer [12], colorectal cancer [13], hepatocellular carcinoma [14], renal cell carcinoma [15], and ovarian cancer [16]. However, there is no evidence showing the correlation of UCA1 in the progression of glioma cells. Based on these above, we aimed to explore the role of UCA1 in glioma. We detected UCA1 expression in glioma tissues and cell lines, and performed siRNA transfection to alter UCA1 expression in U87MG and U251 cells. Cell viability, migration, invasion and the expression changes in epithelial-mesenchymal transition (EMT) associated factors and AKT were measured. This study may provide evidence that UCA1 has a modulatory role in glioma cells proliferation and metastasis, which may facilitate lncRNA research in glioma.

Materials and methods

Patients and clinical sample collection

The matched glioma tissues and the adjacent non-tumorous brain tissues were obtained from 20 patients with glioma from January, 2014 to February, 2016. These patients included 11 female and 9 male, with median age of 59. None of the patients received chemo- or radio-therapy before sample collection. All specimens were collected and frozen in guanidinium thiocyanate solution at -80°C until use. The project was approved by the Clinical Research Ethics Committee of our local hospital and the informed consent was provided by all the enrolled patients before the specimens were collected.

Cell lines

Normal human astrocyte (NHA) cells were purchased from Lonza (Basel, Switzerland), and glioma cell lines U87MG and U251 were purchased from the American Type Culture Collection (ATCC, Manassas, VA). All cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) medium with high glucose and sodium pyruvate, supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 units/mL penicillin and 100 μg/mL streptomycin (Invitrogen) at 37°C in 5% CO_2.

RT-PCR

Total RNA was isolated using Trizol reagent-phenol chloroform (Invitrogen, Carlsbad, CA, USA). cDNA syntheses were performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche, USA). Each real-time PCR was carried out on the ABI PRISM 7500 Real-time PCR System (Applied Biosystems, Foster City, CA) by using FastSTART Universal SYBR Green Master (ROX) (Roche, USA), according to the instructions of manufacture. Data were normalized with β-actin, and were calculated by $2^{-\Delta\Delta Ct}$ method. All primers were synthesized by GenePharma (Shanghai, China).

Knockdown of UCA1

siRNA targeted UCA1 (si-UCA1) and siRNA negative control (si-NC) were purchased from Invitrogen. The sequence of si-UCA1 was TGG TAA TGT ATC ATC GGC TTA GTT CAA GAG ACT AAG CCG ATG ATA CAT TAC CTT TTT TC. The sequence of si-NC was scrambled. Cells were transfected with si-UCA1 or si-NC by using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's instruction. After 48 h of transfection, the cells were harvested for RT-PCR to detect the transfection efficiency.

Cell viability assay

The transfected cells were seeded in 96-well plates at the density of 2 × 10^3 cells/well. After 24-96 h of incubation, 10 μL of Cell Counting Kit-8 (CCK-8, Beyotime, Jiangsu, China) solution was added into each well and cells were incubated for another 4 h at 37°C. Absorbance at 450 nm was recorded using a Multiskan EX (Thermo, Finland) [17].

Migration and invasion assay

Migration and invasion of the transfected cells were assayed using Transwell system (Costar, Corning, NY, USA). The upper chamber was filled with 100 μL serum-free medium with the transfected cells at a density of 5 × 10^5 cells/mL. The lower chamber was filled with complete medium as the bait. For invasion assay, the Transwell inserts were matrigel-coated with polycarbonic membrane (6.5 mm in diameter, 8 μm pore size). After 48 h of incubation at 37°C, the non-migrated and the non-invaded cells on the upper chamber were wiped off by cotton swabs. Other cells were stained with crystal violet (Beyotime, Nantong, China) and the stained cells were counted under a microscope (Olympus IX51).

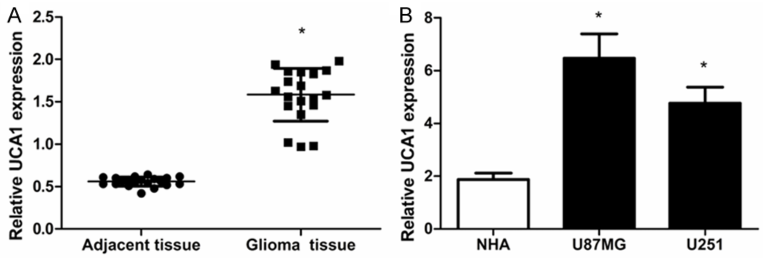

Figure 1. UCA1 was up-regulated in glioma tissues and cell lines. The expression of UCA1 in (A) glioma and adjacent tissues, as well as in (B) NHA, U87MG and U251 cell lines were monitored by RT-PCR. **P* < 0.05 when compared with control group.

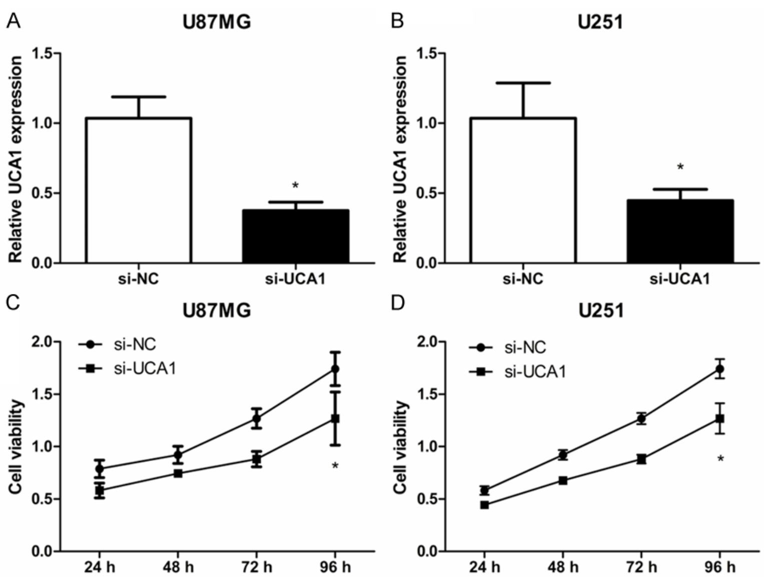

Figure 2. Knockdown of UCA1 suppressed glioma cells viability. U87MG and U251 cells were transfected with either siRNA targeted UCA1 or its negative control. A and B. The transfection efficiency was verified by detection of UCA1 expression by RT-PCR; C and D. Transfected cells viability was measured by using CCK-8. **P* < 0.05 when compared with control group.

Western blotting analysis

Cells were lysed in Radio Immunoprecipitation Assay (RIPA) lysis buffer (Beyotime, Shanghai, China). The BCA Protein Assay Kit (Beyotime, Haimen, China) was used to measure protein concentrations. Proteins were resolved over sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride (PVDF) membranes. After 1 h of incubation in 5% skim milk, the membranes were incubated with primary antibodies for 12 h at 4°C. Antibodies used in this study were purchased from Abcam: E-cadherin (ab1416), N-cadherin (ab18203), Vimentin (ab-8978), total AKT (ab8805), p-AKT (ab81283), and β-actin (ab8229). Horseradish peroxidase (HRP)-conjugated secondary antibodies were used to probe the bands at room temperature for 1 h. Band was visualized by chemiluminescence detection imaging, and its quantification was carried out by Image Lab^{TM} Software (Bio-Rad, CA, USA) [18].

Statistical analysis

Data were expressed as means ± standard derivations from three independent experiments in triplicate. Differences between groups were analyzed by SPSS 13.0 software (SPSS, Chicago, IL, USA) using a one-way analysis of variance (ANOVA). $P < 0.05$ was considered as statistical significance.

Results

UCA1 was up-regulated during glioma

RT-PCR analysis was performed to monitor the expression of UCA1 in glioma and adjacent tissues from 20 glioma patients. Results in **Figure 1A** showed that UCA1 was greatly up-regulated in glioma tissues when compared with adjacent tissues ($P < 0.05$). Additionally, the comparison of UCA1 expression between NHA and U87MG or U251 cells were also performed by RT-PCR. Coincident with the data in **Figure 1A**, significant increases of UCA1 level were found in U87MG and U251 cells when compared with NHA cells ($P < 0.05$, **Figure 1B**). These data indicated a pivotal role of UCA1 in glioma.

UCA1 knockdown suppressed glioma cells viability

The expression of UCA1 in U87MG and U251 cells were suppressed by transfection with its targeted siRNA, and the transfection efficiency

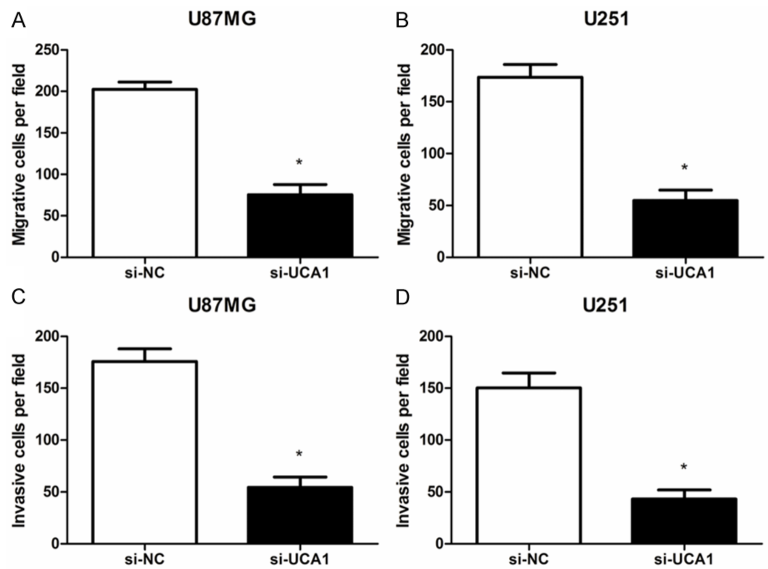

Figure 3. Knockdown of UCA1 suppressed glioma cells migration and invasion. U87MG and U251 cells were transfected with either siRNA targeted UCA1 or its negative control. The transfected cells (A and B) migration and (C and D) invasion were detected by Transwell system. **P* < 0.05 when compared with control group.

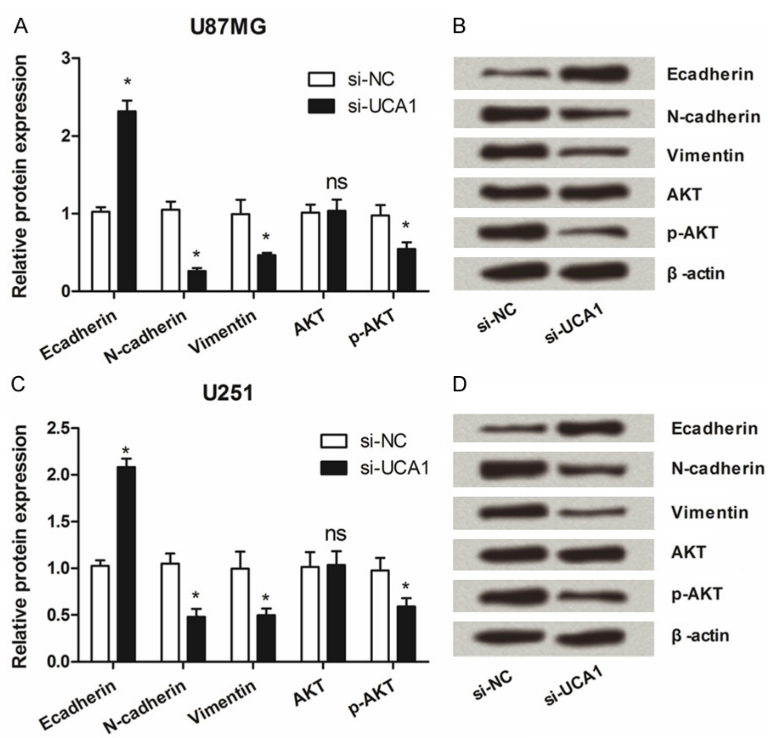

Figure 4. Knockdown of UCA1 inhibited EMT and AKT activation. U87MG and U251 cells were transfected with either siRNA targeted UCA1 or its negative control. The expression of EMT-related factors and the phosphorylation of AKT were detected in the transfected (A and B) U87MG and (C and D) U251 cells. **P* < 0.05 when compared with control group; ns, no significance.

was verified by RT-PCR. As shown in **Figure 2A** and **2B**, the expression of UCA1 in both U87MG and U251 cells were successfully down-regulated ($P < 0.05$). Then, CCK-8 assay showed that cell viability was significantly reduced in UCA1 knockdown cells when compared with control cells ($P < 0.05$, **Figure 2C** and **2D**). These data suggested knockdown of UCA1 could reduce glioma cell viability.

UCA1 knockdown suppressed glioma cells migration and invasion

Next, the function of UCA1 knockdown on U87MG and U251 cells migration and invasion were assessed by using a Transwell system. The migratory and invasive cells in UCA1 knockdown group were both less than those in control group ($P < 0.05$, **Figure 3A-D**). Based on these above, we inferred knockdown of UCA1 might be participate in the inhibition of glioma cells migration and invasion.

UCA1 reduced EMT and inactivated AKT

Further, the expression of EMT associated factors, and the phosphorylation of AKT were assessed by Western blotting. Up-regulation of E-cadherin and down-regulations of N-cadherin, Vimentin and p-AKT were found in UCA1 knockdown cells ($P < 0.05$, **Figure 4A-D**). No significance was found in AKT expression between UCA1 knockdown group and control group ($P > 0.05$). Overall, the impacts of UCA1 knockdown on glioma cells might be via inhibition of EMT and inactivation of AKT.

Discussion

Glioma is the most common malignant brain tumor and the incidence of glioma is increasing worldwide. Currently, numerous lncRNA have been found have functions on the origination and progression of glioma. In this study, we performed a preliminary study on UCA1 of its expression and roles in glioma. We found a higher expression of UCA1 in glioma tissues and cell lines than in theirs corresponding controls. Knockdown of UCA1 in both U87MG and U251 cells could suppress cell viability, migration and invasion. Besides, up-regulation of E-cadherin, down-regulations of N-cadherin and Vimentin, and inactivation of AKT were found in UCA1 knockdown cells.

Based on the results of previous studies, UCA1 is generally up-regulated in many cancer tissues and cells, such as esophageal cancer [19], pancreatic cancer [20], endometrial cancer [21] and acute myeloid leukemia [22]. In this study, UCA1 was found elevated in glioma tissues when compared with non-tumor tissues. These findings are consistence with the previous studies and evidence UCA1 as a key biomarker or regulator of tumor development in glioma.

The current study has investigated the conserved functions of UCA1 on glioma cells viability, migration and invasion. In esophageal cancer cells, UCA1 was capable of promoting cell proliferation [19]. Another study in the melanoma cells demonstrated depletion of UCA1 led to the inhibition of cell proliferation and invasion [23]. Knockdown of UCA1 in this study suppressed both U87MG and U251 cells viability, migration and invasion, which suggested the anti-proliferation and anti-metastasis roles of UCA1 in glioma. Therefore, suppression of UCA1 was evidenced as a promising therapeutic target of glioma treatment in this study.

EMT is a reversible biological process in which polarized epithelial cells are induced to undergo numerous biochemical changes, and results in a mesenchymal phenotype which is defined by an enhanced migratory capacity [24, 25]. EMT is the key mechanism in the pathogenesis of glioma [25], and PI3K/AKT is one of the cancer-related down-stream pathways of EMT [26]. In EMT, loss of E-cadherin expression with concomitant gains of N-cadherin and Vimentin are distinctive events, which are common in metastatic carcinomas [27, 28]. In this study, knockdown of UCA1 in both U87MG and U251 cells inhibited EMT and AKT activation. These data revealed modulation of EMT and AKT might be one of the functional mechanisms of UCA1 on glioma.

In conclusion, this study reveals the up-regulation of UCA1 in glioma and suggests an anti-proliferation and anti-metastasis role of UCA1 in glioma cells. UCA1 may have potentials as a therapeutic target for glioma treatment. More efforts are still needed to confirm these hypotheses.

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Disclosure of conflict of interest

None.

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