ARTICLE
Journal of Cellular Biochemistry 115:1900–1907 (2014)

Journal of Cellular Biochemistry

Targeting SIM2-s Decreases Glioma Cell Invasion Through Mesenchymal–Epithelial Transition

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ABSTRACT

Glioma is a common primary intracranial carcinoma with high incidence, recurrence, and motility. Single minded homolog 2-short form (SIM2-s), a member of basic helix-loop-helix (bHLH) family, is reported to be expressed in glioma and might play a role in the invasion. In the present study, we investigated the importance of SIM2-s in glioma invasion and further explored the potential mechanisms. We showed that targeting SIM2-s by interference technology could decrease cell adhesion to fibronectin, induce cell aggregation and cytoskeletal changes. Furthermore, we showed that targeting SIM2-s increased the expression of epithelial markers and decreased the expression of mesenchymal markers, that is mesenchymal–epithelial transition (MET). Targeting SIM2-s decreased self-renewal of glioma stem cells by tumor sphere formation assay. Taken together, our results indicated that MET is involved in the inhibition of glioma invasion by targeting SIM2-s, and SIM2-s may be a new gene target. J. Cell. Biochem. 115: 1900–1907, 2014.

KEY WORDS: SIM2-s; GLIOMA; INVASION; MET

Glioma, the most common primary intracranial carcinoma, is a high incidence disease accounting for approximately 50% of all the primary brain tumors and is the leading cause of threatening factors to survival among adults [Iwami et al., 2011; Rainov and Heidecke, 2011; Liu et al., 2012]. Recently, advances have been made in neuroimaging, neurosurgery, radiotherapy, and chemotherapy. However, the recurrence and mortality of glioma remains high, with almost 100% postoperative recurrence rate, and a median overall survival of less than 1 year [Mikheeva et al., 2010; Kaur et al., 2012]. Invasion is responsible for the high recurrence rate, but the mechanisms of invasion remain ambiguous. Single minded homolog 2-short form (SIM2-s), a member of basic helix-loop-helix (bHLH) family, is reported to be expressed in glioma and play a role in the invasion [He et al., 2010].

SIM2-s is the short form of SIM2, which is a member of bHLH family and plays an important role in the middle cell lineage of the central nervous system [Nambu et al., 1991]. Recently, the SIM2-s gene has been reported to function on the tumor progression and metastasis. Although SIM2-s is shown to have the suppressive activity on the breast tumor [Kwak et al., 2007], the expression was found to be higher in tumors such as colon, pancreas, and prostate carcinomas compared to normal tissues [Deyoung et al., 2002]. This evidence indicated that SIM2-s plays an important role in human cancers. Previously, we showed the relationship between SIM2-s and matrix metalloproteinases (MMPs) in the invasion of glioma cell lines [He et al., 2010]. In addition, epithelial–mesenchymal transition (EMT) might occur in breast cancer cells and be relevant with overexpression of MMPs if SIM2-s are silenced [Laffin et al., 2008].

Abbreviations: SIM2-S, single minded homolog 2-short form; bHLH, basic helix-loop-helix; EMT, epithelial–mesenchymal transition; MMPs, matrix metalloproteinases; RNAi, RNA interference; siRNA, small interfering RNA; DMEM, Dulbecco's modified essential medium; HG, high glucose; E-cadherin, epithelial cadherin; N-cadherin, neural cadherin; DMSO, dimethyl sulfoxide; ZEB2, zinc finger E-box binding homeobox 2.

The authors declared that they have no potential conflicts of interest.

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Manuscript Received: 1 June 2014; Manuscript Accepted: 30 May 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 6 June 2014

DOI 10.1002/jcb.24859 • © 2014 Wiley Periodicals, Inc.

We explored the relationship between SIM2-s and EMT, which is associated with the invasion and metastasis in tumors.

Glioma is a type of tumor derived from the neural epithelium. EMT is a process featured in cell migration and reduced cellular adhesion [McConkey et al., 2009]. In general, the process of EMT is the inhibition of the epithelial marker epithelial cadherin (E-cadherin) and excitation of the mesenchymal marker neural cadherin (N-cadherin) [Mikheeva et al., 2010]. E-cadherin is a transmembrane glycoprotein which is mainly found in epithelial cells and has a molecular weight of 120 kDa. E-cadherin may play an important role in cell detachment and reattachment of tumors and further in tumor invasion through mediation of the cell and cell–matrix adhesion. N-cadherin, which is one of the calcium-dependent adhesion protein molecules and was first identified in the chicken neural retina, is almost barely in neuronal cells. The full length of N-cadherin is 130 kDa and the soluble form is 90 kDa. Contrary to E-cadherin, N-cadherin is suggested to promote invasion in some tumors such as breast tumors [Nieman et al., 1999].

In this study, we explored the expression of SIM2-s in glioma samples and glioma cell line. Then, using RNA interference (RNAi) technology, alterations in glioma cell aggregation and cytoskeleton were observed. Additionally, we further quantified the epithelial and mesenchymal markers expressed after suppressing SIM2-s and simultaneously investigated the cell proliferation and tumor sphere formation of glioma cells. These data suggest that EMT is involved in glioma cells invasion inhibition by targeting SIM2-s. Consequently, the results support SIM2-s as a potential target for controlling invasion of glioma cells.

RESULTS

SIM2-s WAS HIGHLY EXPRESSED IN HUMAN GLIOMAS

To determine the role of SIM2-s in glioma, we first measured the expression of SIM2-s in human glioma samples and glioma cell line T98G cells. By immunohistochemistry assay, we showed that SIM2-s-positive cells were detected in human glioma samples but not in normal brain cortex (Fig. 1A). Afterwards, by RT-PCR and Western blot, we found SIM2-s mRNA and protein expressed in T98G cells (Fig. 1B,C). These results demonstrated that SIM2-s was highly expressed in human glioma cells.

TARGETING SIM2-s DECREASED CELL ADHESION TO FIBRONECTIN

Subsequently, we inhibited the expression of SIM2-s in T98G cell line using SIM2-s specific small interfering RNA (siRNA). By RT-PCR and Western blotting, We showed that after using siRNA, the

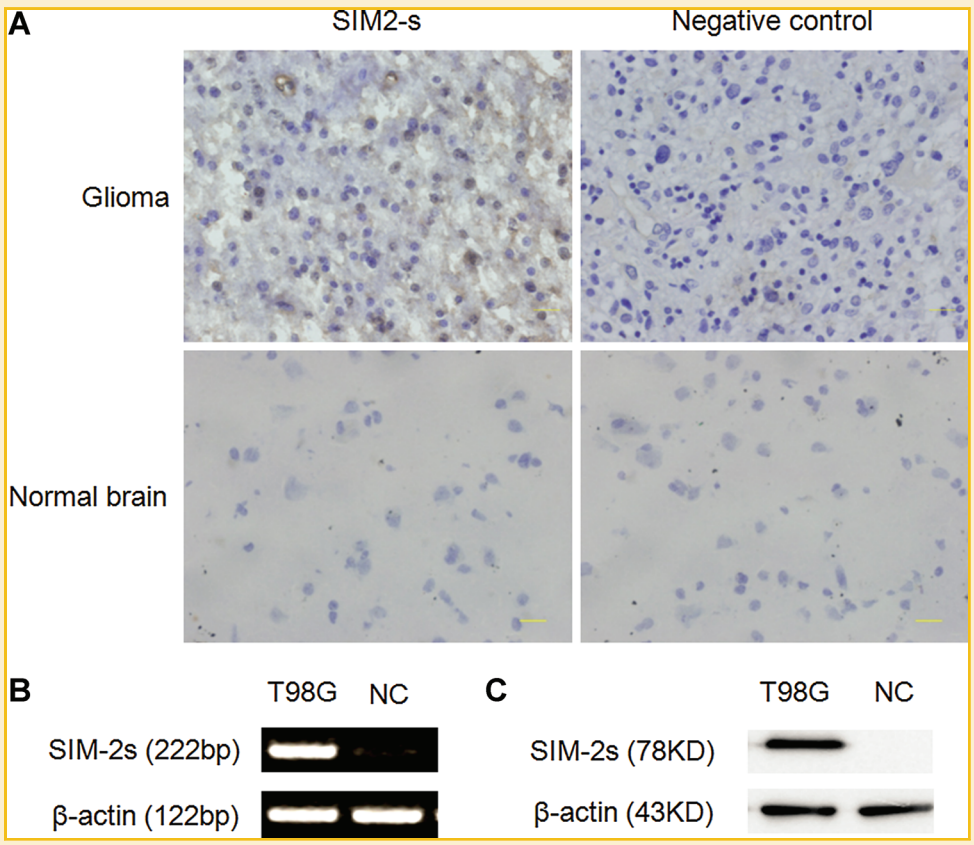

Fig. 1. Expression of SIM2-s in glioma. A: Immunohistochemistry test of SIM2-s expression in human glioma samples and normal brain tissue. SIM2-s expressed in glioma tissue but not in normal tissue. At the same time, negative control was displayed. B: RT-PCR results showed SIM2-s mRNA expressed in T98G cells. C: Western blot results showed SIM2-s protein expressed in T98G cells.

mRNA and protein levels were obviously down-regulated and the mRNA level decreased from about 100% to 25%, this was found to be significant compared to the other groups ($P < 0.01$, Fig. 2A–C). The results indicated that SIM2-s was inhibited by using SIM2-s siRNA.

We then measured the alterations of cell viability after targeting SIM2-s using siRNA in order to exclude the possible influences. Our results showed the difference of cell viability among the three groups were not significant at 24 or 48 h after the treatment (data not shown). The cell adhesion to fibronectin was evaluated. We showed 0.5 h after different treatment, the OD values of adhesion to fibronectin in three groups were about 0.097, 0.1, and 0.083, without significant differences ($P > 0.05$, Fig. 2D). At 1 h after treatment, the OD values in three groups were 0.263, 0.263, and 0.2 approximately, without significant differences ($P > 0.05$, Fig. 2D). However, the OD value in 3 h after targeting SIM2-s went from 0.473 to 0.3 ($P < 0.05$, Fig. 2D) while the OD value in 3 h after non-targeting siRNA did not change ($P > 0.05$, Fig. 2D).

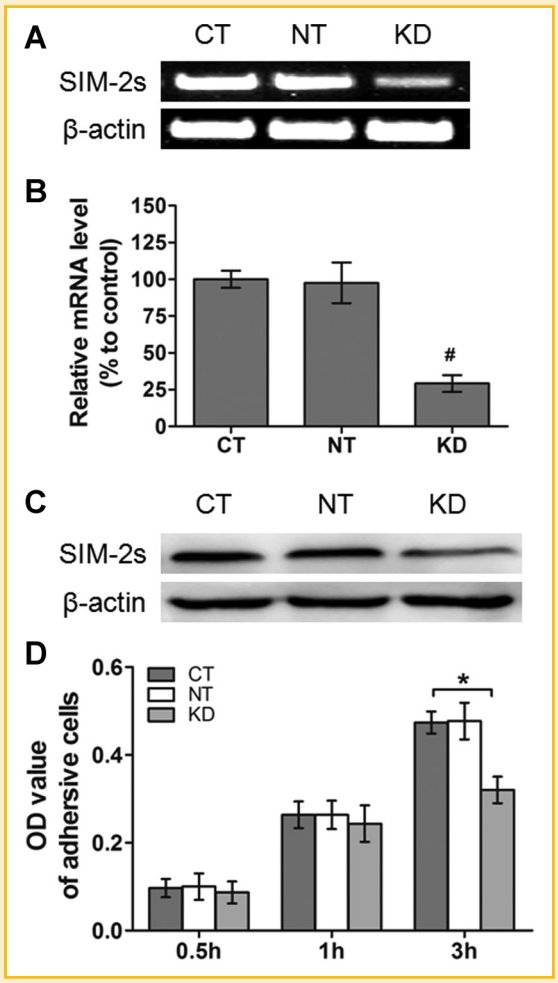

Fig. 2. Targeting SIM2-s decreased cell adhesion to fibronectin. A: RT-PCR results showed that SIM2-s mRNA expressed in control T98G cells (CT) and T98G cells treated with non-targeting siRNA (NT), but low expression in T98G cells treated with SIM2-s-targeting siRNA (KD). B: Relative mRNA levels in control T98G cells (CT) (100%), T98G cells treated with non-targeting siRNA (NT) (100%) and T98G cells treated with SIM2-s-targeting siRNA (KD) (25%). C: Western blot assay showed that SIM2-s protein expressed in control T98G cells (CT) and T98G cells treated with non-targeting siRNA (NT), but low expression in T98G cells treated with SIM2-s-targeting siRNA (KD). D: OD value of cell adhesion to adhesion in 0.5 h (about 0.1), in 1 h (about 0.3) after targeting treatment. And OD value in control T98G cells (CT) (about 0.5), T98G cells treated with non-targeting siRNA (NT) (about 0.5) was significantly different compared with T98G cells treated with SIM2-s-targeting siRNA (KD) (about 0.3). (Results are presented as mean ± SD of three independent experiments. $^*P < 0.05$, in comparison with control.)

TARGETING SIM2-s INHIBITED GLIOMA CELL INVASION

The matrigel coated transwell invasion test was performed to analyze whether SIM2-s reflected glioma cell invasion. The results showed that targeting siRNA decreased invasiveness of T98G cells. The amount of cells that invaded into the bottom went from about 67% to 33% ($P < 0.05$, Fig. 3A, B). However, non-targeting siRNA did not influence the invasiveness of T98G cells ($P > 0.05$, Fig. 3A, B).

TARGETING SIM2-s INDUCED MESENCHYMAL–EPITHELIAL TRANSFORMATION

To further explore the mechanisms of SIM2-s function on invasion of glioma cells, we investigated mesenchymal and epithelial alterations. Compared with the control, cells treated with SIM2-s-targeting siRNA show less protuberances (Fig. 4). As for cytoskeletal changes, the cells transfected with SIM2-s-targeting siRNA were changed dramatically when compared to the control. The actin in cells treated with SIM2-s was disorderly and unsystematic compared to the control. Cytoskeleton of cells transfected with non-targeting siRNA was not changed (Fig. 4).

To further explore the potential influence after silencing SIM2-s, we quantified the expression of E-cadherin and N-cadherin with real-time RT-PCR. The results showed the expression of E-cadherin dramatically increased from about 2.96×10^{-5} to 395×10^{-5} ($P < 0.05$, Fig. 4). However, the expression of N-cadherin decreased from 6.81×10^{-5} to 1.41×10^{-5} ($P < 0.05$, Fig. 4).

TARGETING SIM2-s DECREASED GLIOMA CELL PROLIFERATION AND TUMOR SPHERE FORMATION

To find out the effect of SIM2-s on glioma cells, we further observed glioma cell proliferation and tumor sphere formation after silenced SIM2-s. We showed after targeting SIM2-s, the glioma cells proliferation was significantly decreased compared to cells in the untreated group. The cell proliferation in non-targeting group did not change significantly compared with control group (Fig. 5A). Then as far as the tumor sphere formation, cells silenced SIM2-s were dramatically decreased while cells treated with non-targeting siRNA exhibited no obvious alterations compared to control (Fig. 5B).

MATERIALS AND METHODS

HUMAN SAMPLES

The human glioma and normal brain cortex samples studied here were collected from Department of Neurosurgery and Department of Emergency Surgery at Qi Lu Hospital (Jinan, China) under the

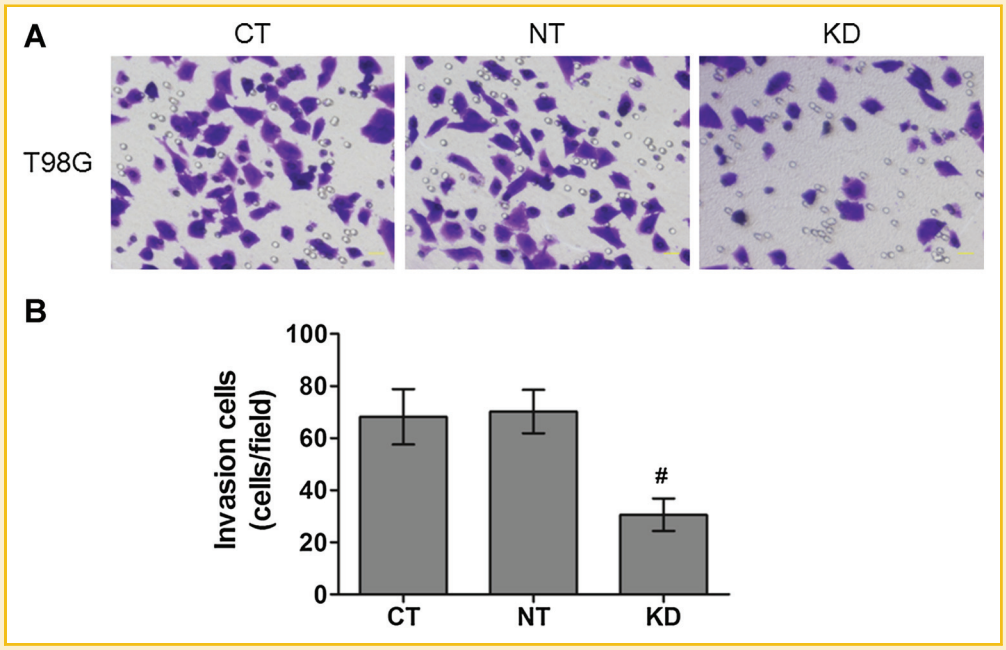

Fig. 3. Targeting SIM2-s inhibited glioma cells invasion. A: Effect of SIM2-s on cell invasiveness of glioma cells was evaluated by matrigel-coated transwell assay. Cells invaded into the lower surface of membrane were fixed and stained with 0.1% crystal violet. Photographs were taken using a microscope; scale bar represents 20 μm. B: The amount of cells invaded into the lower surface of the membrane was collected in 20 different fields. Amount of cells in control T98G (CT) was about 67%, similar to amount in T98G cells transfected with non-targeting siRNA (NT) while amount in T98G cells transfected with SIM2-s-targeting siRNA (KD) was decreased to approximately 33%. The results showed targeting SIM2-s significantly inhibited glioma cells invasiveness. (Results are presented as mean ± SD of three independent experiments. $^{\#}P < 0.01$, in comparison with control.)

National Regulation of Clinical Sampling in China. Gliomas were determined through pathological diagnosis while normal brain tissue samples were taken from surgical resection of trauma patients. Both glioma and normal tissue for further RT-PCR and western blot were frozen after surgical resection and conserved in −80 °C.

CELL CULTURE AND TREATMENT

The glioma cell line T98G cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and conserved in our library for less than 1 year. The T98G cells were maintained in a humidified incubator mixed with 5% CO_2 at 37 °C. Then cells were incubated with Dulbecco's modified essential medium (DMEM)/high glucose (HG) (Hyclone) medium added with 10% FBS.

SIRNA FOR SIM2-s TRANSFECTION

After incubation in DMEM/HG, the cells in 6-well plates were transfected with SIM2-s siRNA or non-targeting SiRNA for 24 h. The protocol for transfection was according to the description in lepoinfection 2000 (Invitrogen, Carlsbad, CA). The sequence of SIM2-s-specific siRNA was: (sense) 5′-GGCAGUAUAUGCUGGA-CAU dTdT-3′, (antisense) 3′-dTdT CCGUCAUAUACGACCUGUA-5′ and provided by Guangzhou Ribobio Co., Ltd (China). Subsequently, the three groups of cells were used. T98G cells transfected with non-targeting siRNA were used as control.

CELL ADHESION ASSAY

The cell adhesion of T98G cells treated distinctively was measured by the MTT assay. The different groups of cells were seeded into wells of 96-well plate with 5×10^3 in each well, then, incubated at 37 °C for 0.5,1, and 3 h. After washed twice with PBS, 20 μl MTT solution (5 mg/ml, Sigma-Aldrich) was added to each well and then incubated at 37 °C for 4 h. After aspirated the medium, the cells were incubated with 200 μl dimethyl sulfoxide (DMSO). We collected the absorbance value of 490 nm from a microplate reader.

THE MATRIGEL COATED TRANSWELL INVASION TEST

To observe the alterations of invasiveness of T98G cells after targeting SIM2-s, we performed the matrigel coated transwell invasion test. The filter membrane with 8-μm pores between two chambers was coated with Matrigel (BI) Biosciences. Cells were starved for 6 h and suspended in DMEM/HG at 1×10^5/ml. Two hundred microliters of supernatant for three kinds of cells were put into the upper chambers with 600 μl FBS containing medium in the lower chamber and incubated for 24 h. Afterwards, the upper chamber and the adhesive cells were removed carefully from the upper membrane. Cells on the lower surface of membrane were stained with 0.1% crystal violet in methanol for 10 min, observed with a light microscope (IX71; Olympus, Tokyo, Japan) and photographed.

COLONY FORMATION ASSAY

To measure the effects of SIM2-s to glioma cells proliferation, we observed the abilities of these single cells to grow into a colony by using colony formation assay. Cells were incubated in 6-well plates at 1,000/well 24 h after transfection and incubation with DMEM/HG

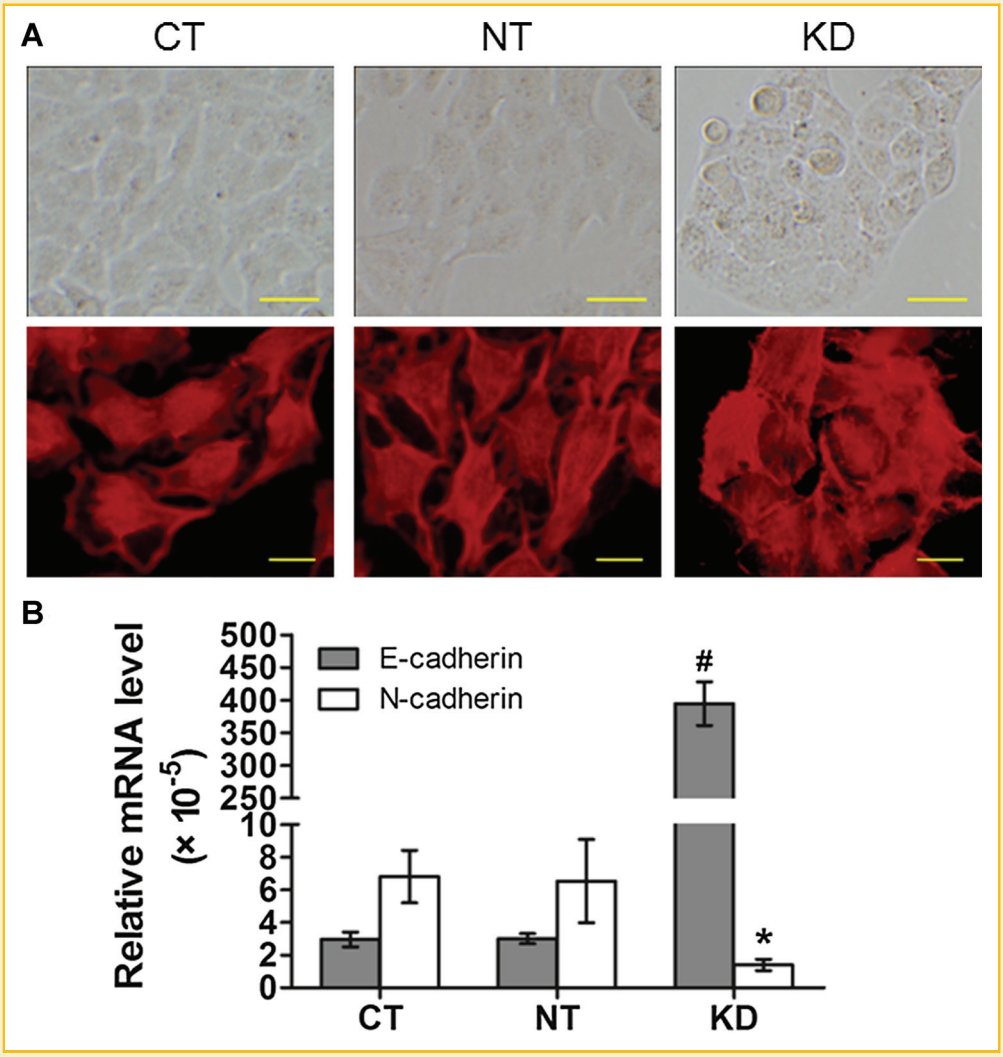

Fig. 4. Targeting siRNA induced cell morphology, cytoskeletal changes, and aberrant expression of epithelial and mesenchymal markers. A: Cell morphology and cytoskeletal changes in glioma cells with different treatment. Cells treated with SIM2-s-targeting siRNA show less protuberances and F-actin was disorderly and unsystematic compared to the control (magnification 200×). B: Relative mRNA levels of epithelial markers (E-cadherin) in control T98G cells (CT) and T98G cells treated with non-targeting siRNA (NT) were lower dramatically than T98G cells treated with SIM2-s-targeting siRNA (KD). The levels of mesenchymal markers (N-cadherin) in T98G cells treated with SIM2-s-targeting siRNA (KD) were alleviated significantly compared to control T98G cells (CT) and T98G cells treated with non-targeting siRNA (NT). (Results are presented as mean ± SD of three independent experiments. $^{*}P < 0.05$, $^{\#}P < 0.01$, in comparison with control.)

with 10% FBS for 7 days, the cells were washed with PBS and fixed with methyl hydrate for 5–10 min. Finally, the cells were stained with Giemsa for 20 min and we counted the number of colonies which contained more than 50 cells. We determined the colony formation rate using the ratio of the colony numbers and the plated cells.

TUMOR SPHERE FORMATION

The glioma T98G cells were suspended in medium containing 25 ml DMEM, 25 ml F12 medium, 10 μl 20 ng/ml of EGF, 10 μl 20 ng/ml of bFGF, and 1 ml B27 supplement and counted. Cells were seeded in 6-well plates at 5×10^3 cells/well and incubated for 5–14 days. Finally, the cells were fixed with 4% formaldehyde to observe the visible spheres formation.

IMMUNOHISTOCHEMISTRY

The sections of human glioma samples were treated as previously described [Zhang et al., 2012]. The sections were incubated with anti-SIM2-s antibody (1:100, rabbit polyclone, Santa Cruz) at 4 °C overnight. Then washed with PBST and incubated with biotinylated goat anti-rabbit IgG (ZhongShan GoldenBridge, FuZhou, China) and an avidin–biotin horseradish peroxidase solution (ZhongShan GoldenBridge) for half an hour at 37 °C. Finally, the sections were incubated with 3,3′-diaminobenzidine (DAB) diluted in 0.05 osmium tetroxide as a chromogen for 3 min and counterstained with Gills Hematoxylin. Positive and negative controls were performed at the same time for each section. The microscope (IX71, Olympus, Tokyo, Japan) with DP controller and DP manager (Olympus) was used to take the immunohistochemistry or IHC images.

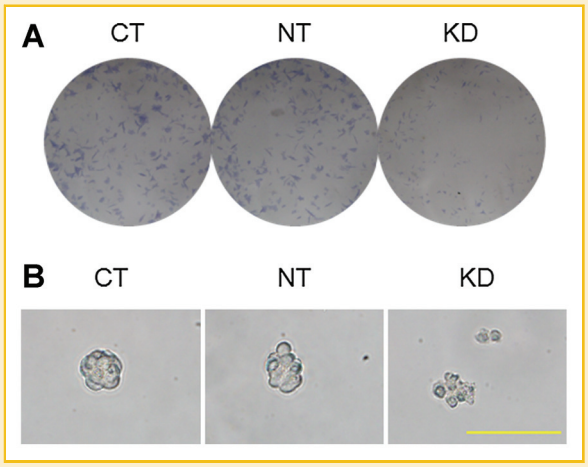

Fig. 5. Targeting SIM2-s decreased glioma cell proliferation and tumor sphere formation. A: Colony formation assay showed that T98G cells treated with SIM2-s-targeting siRNA (KD) formed fewer colonies compared to control T98G cells (CT) and T98G cells treated with non-targeting siRNA (NT). B: Tumor sphere formation assay showed that T98G cells treated with SIM2-s-targeting siRNA (KD) formed fewer tumor spheres compared to control T98G cells (CT) and T98G cells treated with non-targeting siRNA (NT).

IMMUNOFLUORESCENCE

T98G cells were seeded on coverslips in 5×10^4 cells/well, fixed with 4% paraformaldehyde (PFA) and permeabilized in 0.1% Triton X-100. Then the cells were washed and incubated with rhodamine–phalloidin (2 μg/ml) for 30 min at room temperature. Then, the cells were examined with an immunofluorescence microscope (IX-71, Olympus).

RNA EXTRACTION, SEMIQUANTITATIVE RT-PCR, AND QUANTITATIVE REAL-TIME RT-PCR

As described previously [Wang et al., 2012b], the total RNA was isolated using TRIzol reagent (Takara, Kyoto, Japan) and identical amounts of RNA (500 ng) were reverse [Wang et al., 2012a] transcribed into complement DNA (cDNA) with a commercial RT-PCR kit (Takara) to a volume of 20 μl. Quantitative real-time PCR was performed on a LightCycler 2.0 instrument (Roche Applied Science, Mannheim, Germany) using a SYBR Green I Reagent Kit (Toyobo, Tsuruga, Japan). The primers for SIM2-s were 5′-CTTC-CCTCTGGACTCTCACG-3′ (sense), 5′-AGGCTGTGCCTAGCAGTGTT-3 (antisense); the primers for E-cadherin were 5′- TGCCC-AGAAAATGAAAAAGG-3′ (sense), 5′-GTGTATGTGGCAATGCGTT-C-3′ (antisense); the primers for N-cadherin were 5′-ACAGTGGCCACCTACAAAGG-3′ (sense), 5′-CCGAGATGGGGTTGA-TAATG-3′ (antisense); and the primers for β-actin were 5′-CCAACCGCGAGAAGATGA-3′ (sense), 5′-CCAGAGGCGTACAGG-GATAG-3′ (antisense). Then, the PCR products were subjected to 1.7% agarose/TAE gels mixed with ethidium bromide and electrophoresed. Finally, the results were semiquantified with AlphaEase FC Version 4 analysis software (Alphalmager HP, Alpha Innotech).

For PCR amplification, different amounts of the synthesized complement DNA (cDNA) (diluted 1:10 in water) were analyzed to evaluate the linearity of the reaction. Then polymerase reaction was carried out in a solution contained 1.5 mM $MgCl_2$, 200 μM of each nucleotide in PCR buffer, 10 pmol of the primers, and 0.25 U platinum Taq DNA polymerase (Gibco, Invitrogen). Primers used in RT-PCR reactions are shown above. The PCR parameters included a denaturation step of 95 °C for 10 min and then followed by 28–40 cycles at 95 °C for 30 s, 58–63 °C for 30 s, and 72 °C for 30 s, with a final extension step at 72 °C for 10 min. The PCR products separated on 1.5% agarose/TAE gels were visualized by staining with ethidium bromide and semiquantified using AlphaEase FC Version4 analysis software (Alphalmager HP, Alpha Innotech, USA). The densitometric analysis of the data was normalized to the β-actin. Results were mean ± SD from three separate experiments for each group.

WESTERN BLOT ASSAY

The western blotting assay was performed as described in previous work [Wang et al., 2012a]. An equal amount of protein was loaded into a 10% gradient polyacrylamide gel, electrophoresed, transferred to polyvinylidene difluoride membrane and probed with following primary antibodies: anti-SIM2-s (1:500, rabbit polyclone, Santa Cruz); anti-E-cadherin (1:1,000, rabbit polyclone, CST); anti-N-cadherin (1:500, rabbit polyclone, Bioworlde); and anti-β-actin (1:1,000, mouse monoclone, ZhongShan GoldenBridge). Secondary antibodies were horseradish peroxidase conjugated to goat anti-rabbit/mouse IgG (1:5,000, Sigma-Aldrich). The membranes were developed using an enhanced chemiluminescence detection system (Millipore, Billerica, MA). The results were counted with β-actin used as an internal control.

DISCUSSION

In the present study, we evaluated the role of SIM2-s on the invasion of glioma. We showed the cells knocked down SIM2-s aggregated and decreased adhesion to fibronectin. We further explored the underlying mechanisms of the SIM2-s effect. The results indicated that with knocking down of SIM2-s, there is alterations in the cytoskeleton and in the expressions of mesenchymal and epithelial markers in the cell. There is up-regulation of epithelial marker and down-regulation of mesenchymal marker. Additionally, silencing SIM2-s decreased glioma cell proliferation and tumor sphere formation. In conclusion, the data suggested that SIM2-s might increase the invasion of glioma cells and the increasing effect might function through the alteration of EMT.

SIM2-s, which was first identified to have potential implication on Down syndrome [Rachidi et al., 2005], recently has been reported to be related with tumor development and progression [DeYoung et al., 2003a,b; Aleman et al., 2005]. The expression of SIM2-s were associated with the colon, pancreas, and prostate tumor tissues. Moreover, the expression even has the stage-specific characteristic in colon and pancreatic carcinomas [DeYoung et al., 2003a,b]. However, SIM2-s is shown to suppress the activity of tumor cells in breast cancer. The mice are susceptible to malignant transformation if the SIM2-s gene is knocked down [Kwak et al., 2007; Laffin et al., 2008]. In our previous study, we showed SIM2-s is expressed in some glioma cells [He et al., 2010]. And here, we further suggest SIM2-s is expressed in T98G cells involved in their invasion.

EMT is a process that leads to loss of homotypic adhesion and increased invasion and migration [McConkey et al., 2009]. EMT is essential for embryonic development, wound restoring, and recently has been connected with the invasion and metastasis of the gliomas. At the molecular level, the most widely used marker for EMT in carcinomas is loss of the epithelial marker E-cadherin while increase of the mesenchymal marker N-cadherin [Mikheeva et al., 2010]. The invasion of many tumor cells such as breast cancer [Ahmad et al., 2012], pancreatic cancer [Nakajima et al., 2004], and hepatoma [Xu et al., 2012] under EMT is dramatically increased. To determine the possible alteration of EMT and whether EMT is involved in the invasion induced by SIM2-s in glioma, we tested the expression of E-cadherin and N-cadherin. As showed in glioma cells, the expression of E-cadherin up-regulated and N-cadherin down-regulated more than in normal tissues which suggested EMT occurred in glioma T98G cells [Qi et al., 2012]. Then after knock down of SIM2-s, the expression of E-cadherin increased and N-cadherin reduced in glioma cells. The changes of E-cadherin and N-cadherin showed that EMT induced in glioma cells may be reversed by targeting SIM2-s and might subsequently lead to decreased invasion.

E-cadherin, which is a type I cadherin transmembrane glycoprotein, is a classic tumor metastasis related molecular [Cavallaro and Christofori, 2004 Nawijn et al., 2011]. It joins in the maintenance of epithelial cell morphology and structural integrity. Moreover, it can mediate the cell adhesion [Nawijn et al., 2011]. With no expression, the tumor cells may lose contact inhibition and lead to unlimited cell proliferation. In general, there is a positive relationship between the degree of differentiation and the expression of E-cadherin in carcinomas. As reported, the expression of E-cadherin was lower in liver cancer and lung cancer [Xiao and He, 2010].

N-cadherin, which is also a cadherin transmembrane glycoprotein, mediates calcium-ion-dependent adhesion [Fraiberg et al., 2010]. While E-cadherin is regarded as a tumor suppressor, N-cadherin is often seen as an activator of cancer cell migration and invasion. The past results showed the expression of N-cadherin was increased in leiomyoma, mesothelioma, and adrenal tumors. However, in osteosarcoma and ovarian cancer cells, the expression was down-regulated. In our present study, we quantified N-cadherin, and found N-cadherin overexpressed in gliomas. When targeting SIM2-s, the expression of glioma cells was down-regulated.

Tumor stem cells are important to the survival, proliferation, metastasis, and recurrence of cancer. Tumor stem cells were found to play vital roles in some cancers including breast carcinomas, lung cancer, prostate cancer, colon cancer, leukemia, and so on [Bonnet and Dick, 1997; Al-Hajj et al., 2003; Singh et al., 2004; Collins et al., 2005; Kim et al., 2005; O′Brien et al., 2007]. In ovarian cancers, nestin and Nanog were positively associated with the self-renewal ability [Hetland et al., 2012; Siu et al., 2012]. As far as glioma, tumor stem cells can form tumor sphere and were affected by Sox-2, musashi-1, Bmi-1, nestin, etc. [Hemmati et al., 2003; Park and Rich, 2009]. Tumor sphere formation was used to observe the self-renewal capability of stem cells extracted from glioma T98G cells. The results showed that after targeting SIM2-s, the stem cells from T98G cells decreased tumor sphere formation. The observations indicate that SIM2-s silencing possibly decreased the self-renewal ability of tumor stem cells from T98G cells. Accordingly, we suspect SIM2-s might further influence viability, proliferation, metastasis, and recurrence of glioma.

In conclusion, we showed that SIM2-s may take part in the invasion of cells in glioma and EMT with the loss of E-cadherin and increase of N-cadherin.

ACKNOWLEDGMENTS

This work was supported by funding from Independent Innovation Foundation of Shandong University (IIFSDU 2009TS067) and Promotive Research Fund for Excellent Young and Middle-aged Scientists of Shandong Province (BS2010YY022).

REFERENCES

Ahmad A, Sarkar SH, Bitar B, Ali S, Aboukameel A, Sethi S, Li Y, Bao B, Kong D, Banerjee S, Padhye SB, Sarkar FH. 2012. Garcinol regulates EMT and Wnt signaling pathways in vitro and in vivo leading to anticancer activity against breast cancer cells. Mol Cancer Ther 11:2193–2201.

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. 2003. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988.

Aleman MJ, DeYoung MP, Tress M, Keating P, Perry GW, Narayanan R. 2005. Inhibition of single minded 2 gene expression mediates tumor-selective apoptosis and differentiation in human colon cancer cells. Proc Natl Acad Sci USA 102:12765–12770.

Bonnet D, Dick JE. 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737.

Cavallaro U, Christofori G. 2004. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat Rev Cancer 4:118–132.

Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. 2005. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 65:10946–10951.

Deyoung MP, Scheurle D, Damania H, Zylberberg C, Narayanan R. 2002. Down′s syndrome-associated single minded gene as a novel tumor marker. Anticancer Res 22:3149–3157.

DeYoung MP, Tress M, Narayanan R. 2003a. Down′s syndrome-associated single minded 2 gene as a pancreatic cancer drug therapy target. Cancer Lett 200:25–31.

DeYoung MP, Tress M, Narayanan R. 2003b. Identification of Down′s syndrome critical locus gene SIM2-s as a drug therapy target for solid tumors. Proc Natl Acad Sci USA 100:4760–4765.

Fraiberg M, Borovok I, Weiner RM, Lamed R. 2010. Discovery and characterization of cadherin domains in Saccharophagus degradans 2–40. J Bacteriol 192:1066–1074.

He Q, Li G, Su Y, Shen J, Liu Q, Ma X, Zhao P, Zhang J. 2010. Single minded 2-s (SIM2-s) gene is expressed in human GBM cells and involved in GBM invasion. Cancer Biol Ther 9:430–436.

Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI. 2003. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci USA 100:15178–15183.

Hetland TE, Holth A, Kaern J, Florenes VA, Trope CG, Davidson E. 2012. HMGA2 protein expression in ovarian serous carcinoma effusions, primary tumors, and solid metastases. Virchows Arch 460:505–513.

Iwami K, Natsume A, Wakabayashi T. 2011. Cytokine networks in glioma. Neurosurg Rev 34:253–263;discussion 263–254.

Kaur H, Phillips-Mason PJ, Burden-Gulley SM, Kerstetter-Fogle AE, Basilion JP, Sloan AE, Brady-Kalnay SM. 2012. Cadherin-11, a marker of the

mesenchymal phenotype, regulates glioblastoma cell migration and survival in vivo. Mol Cancer Res 10:293–304.

Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. 2005. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 121:823–835.

Kwak HI, Gustafson T, Metz RP, Laffin B, Schedin P, Porter WW. 2007. Inhibition of breast cancer growth and invasion by single-minded 2s. Carcinogenesis 28:259–266.

Laffin B, Wellberg E, Kwak HI, Burghardt RC, Metz RP, Gustafson T, Schedin P, Porter WW. 2008. Loss of singleminded-2s in the mouse mammary gland induces an epithelial-mesenchymal transition associated with up-regulation of slug and matrix metalloprotease 2. Mol Cell Biol 28:1936–1946.

Liu B, Wang L, Shen LL, Shen MZ, Guo XD, Wang T, Liang QC, Wang C, Zheng J, Li Y, Jia LT, Zhang H, Gao GD. 2012. RNAi-mediated inhibition of presenilin 2 inhibits glioma cell growth and invasion and is involved in the regulation of Nrg1/ErbB signaling. Neuro Oncol 14:994–1006.

McConkey DJ, Choi W, Marquis L, Martin F, Williams MB, Shah J, Svatek R, Das A, Adam L, Kamat A, Siefker-Radtke A, Dinney C. 2009. Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer. Cancer Metastasis Rev 28:335–344.

Mikheeva SA, Mikheev AM, Petit A, Beyer R, Oxford RG, Khorasani L, Maxwell JP, Glackin CA, Wakimoto H, Gonzalez-Herrero I, Sanchez-Garcia I, Silber JR, Horner PJ, Rostomily RC. 2010. TWIST1 promotes invasion through mesenchymal change in human glioblastoma. Mol Cancer 9:194.

Nakajima S, Doi R, Toyoda E, Tsuji S, Wada M, Koizumi M, Tulachan SS, Ito D, Kami K, Mori T, Kawaguchi Y, Fujimoto K, Hosotani R, Imamura M. 2004. N-cadherin expression and epithelial-mesenchymal transition in pancreatic carcinoma. Clin Cancer Res 10:4125–4133.

Nambu JR, Lewis JO, Wharton KA, Jr., Crews ST. 1991. The Drosophila single-minded gene encodes a helix-loop-helix protein that acts as a master regulator of CNS midline development. Cell 67:1157–1167.

Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH. 2011. E-cadherin: Gatekeeper of airway mucosa and allergic sensitization. Trends Immunol 32:248–255.

Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ. 1999. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. J Cell Biol 147:631–644.

O'Brien CA, Pollett A, Gallinger S, Dick JE. 2007. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445:106–110.

Park DM, Rich JN. 2009. Biology of glioma cancer stem cells. Mol Cells 28:7–12.

Qi S, Song Y, Peng Y, Wang H, Long H, Yu X, Li Z, Fang L, Wu A, Luo W, Zhen Y, Zhou Y, Chen Y, Mai C, Liu Z, Fang W. 2012. ZEB2 mediates multiple pathways regulating cell proliferation, migration, invasion, and apoptosis in glioma. PLoS ONE 7:e38842.

Rachidi M, Lopes C, Charron G, Delezoide AL, Paly E, Bloch B, Delabar JM. 2005. Spatial and temporal localization during embryonic and fetal human development of the transcription factor SIM2 in brain regions altered in Down syndrome. Int J Dev Neurosci 23:475–484.

Rainov NG, Heidecke V. 2011. Clinical development of experimental therapies for malignant glioma. Sultan Qaboos Univ Med J 11:5–28.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. 2004. Identification of human brain tumour initiating cells. Nature 432:396–401.

Siu MK, Wong ES, Kong DS, Chan HY, Jiang L, Wong OG, Lam EW, Chan KK, Ngan HY, Le XF, Cheung AN. 2012. Stem cell transcription factor NANOG controls cell migration and invasion via dysregulation of E-cadherin and FoxJ1 and contributes to adverse clinical outcome in ovarian cancers. Oncogene 32:3500–3509.

Wang J, Hao H, Yao L, Zhang X, Zhao S, Ling EA, Hao A, Li G. 2012a. Melatonin suppresses migration and invasion via inhibition of oxidative stress pathway in glioma cells. J Pineal Res 53:180–187.

Wang J, Yao L, Zhao S, Zhang X, Yin J, Zhang Y, Chen X, Gao M, Ling EA, Hao A, Li G. 2012b. Granulocyte-colony stimulating factor promotes proliferation, migration and invasion in glioma cells. Cancer Biol Ther 13:389–400.

Xiao D, He J. 2010. Epithelial mesenchymal transition and lung cancer. J Thorac Dis 2:154–159.

Xu XM, Yuan GJ, Li QW, Shan SL, Jiang S. 2012. Hyperthermia inhibits transforming growth factor beta-induced epithelial-mesenchymal transition (EMT) in HepG2 hepatocellular carcinoma cells. Hepatogastroenterology 59:2059–2063.

Zhang X, Wang J, Xing Y, Gong L, Li H, Wu Z, Li Y, Wang Y, Dong L, Li S. 2012. Effects of ginsenoside Rg1 or 17beta-estradiol on a cognitively impaired, ovariectomized rat model of Alzheimer's disease. Neuroscience 220:191–200.