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Original Article

Expression of prion protein in glioblastoma and related mechanisms

Weiguo Li[1], Xingang Li[1], Shujun Xu[1], Xiangyu Ma[1], Qiujie Zhang[2]

[1]Department of Neurosurgery, Qilu Hospital, Shandong University, Jinan, China; [2]Department of Health, Qilu Hospital, Shandong University, Jinan, China

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Abstract: Human glioblastoma is caused by mutation of glial cells inside brain or spinal cord tissues. As one critical pathogenic protein leading to various nervous diseases, prion protein has not been fully studied in glioblastoma cells. A rat model of brain glioma model was generated. Western blotting and RT-PCR were used to determine the correlation between prion protein level and glioma. Human glioma cell J889 was modified to over-express prion proteins, or was genetically engineered to have lower prion protein by RNA interference. MTT assay, caspase activity assay and flow cytometry were used to determine growth and apoptosis of J889 cells. The proliferation ability was further determined by clonal formation assay. Both protein and mRNA levels of prion in glioma tissues were significantly higher than control group. Targeted small interference RNA (siRNA) inhibited the growth of J889 cells, and led to cell apoptosis, lowered clonal formation ability. The over-expression of prion protein facilitated J889 cell growth, inhibited cell apoptosis and elevated clonal formation ability. Prion protein may be closely related with human glioblastoma. Inhibition of prion protein could inhibit human glioblastoma cell J889 growth and clonal formation, and lead to cell apoptosis. The elevation of prion protein facilitates cell growth and clonal formation, and inhibits cell apoptosis of human glioblastoma cells.

Keywords: Small interference RNA, human glioblastoma cell J889, cell apoptosis, cell proliferation, prion protein, clonal formation

Introduction

Human glioblastoma is one primary neural tumor that occurs in glial cells of brain and spinal cord tissues. Current opinions believe that brain glioblastoma is the result of accumulated mutation of glial cells. Occupying 60% of all intracranial tumors, brain glioblastoma has a relatively high incidence and severely affects public health [1].

Treatment for human glioblastoma consists of surgery, radiotherapy and chemotherapy. These methods, however, all have severe complications especially hematological toxicity [2, 3]. Therefore, the development of novel approaches with higher efficacy and less adverse effects is one research focus. The molecular targeted treatment based on gene engineering is one promising field [4], in which the identification of specific target site for brain glioblastoma is of critical importance.

Recent advances in molecular biology and gene engineering suggested close correlation between the occurrence of brain glioblastoma and both environmental and genetic factors. The abnormal expression of oncogenes/tumor-suppressor genes and/or gene mutation may all cause disruption of intracellular signal transduction in brain glial cells [5, 6], which are thus induced to have malignant hypertrophy and cancer occurrence. Such abnormal expression of genes and mutations may directly or indirectly regulate cell growth, cycle, proliferation and programmed death, namely, apoptosis [7]. Therefore, using those related tumor-suppressor genes or oncogenes as molecular targets may help to inhibit the growth of brain glioblastoma [8]. These genes thus may be potential drug target for molecular therapy on human glioblastoma [9, 10].

However, identified candidate genes as treatment target or molecular markers for human

brain glioblastoma are still sparse currently. Therefore the searching for novel candidate gene or molecular marker is of critical application value [10]. As one important protein leading to multiple neural diseases, prion protein's role in regulating brain glioblastoma still requires further elucidation [11]. This study thus investigated the expression of prion protein inside brain glioblastoma cells and possible regulatory mechanisms.

Materials and methods

Reagents, clinical samples and cells

Human brain glioblastoma cell J889 was purchased from American Type Culture Collection (ATCC). MTT assay kit and anti-actin and anti-prion antibody are products of Dingguo Biotech (Beijing, China). Phosphatidylserine outbreak kits, including FITC-Annexin-V and caspase-3 activity kit, RT-PCR reagents and reverse transcriptase were purchased from Baili Bio (Shanghai, China). Fetal bovine serum (FBS) and DMEM high-glucose medium were provided by Beyotime (China). Prion protein-targeted siRNA and controlled siRNA were synthesized by Gimma Biotech (Shanghai, China). Cell transfection reagent Lipofectamine 2000 was produced by Sigma (US). ImageJ 8.0 software was developed by NIH.

J889 cell culture

Human brain glioblastoma cell J889 was kept in DMEM medium containing 10% FBS in a humidified chamber with 5% CO_2 at 37°C as previously described [12].

Cell transfection

Human brain glioblastoma cell J889 were transfected using documented methods [13]. Specific siRNA targeted prion proteins (Forward, 5'-GGCAT AATCC AGGCA CCAAG GCAGG C-3'; Reverse, 5'-CAGCA CCATG CTCCA GGAAG GAGGC A-3') and controlled siRNA (Forward, 5'-GTCCG CAGCA TGGCA CCAAG AAAGG C-3'; Reverse, CACTG AGCCT CCAGG AGCAG AAGGC A-3') were used to transfect cells at a dosage of 2 μg. 12 hours after transfection, cell viability and apoptosis were tested.

MTT assay

MTT assay was used to describe activity of human brain glioblastoma J889 cells from all groups as previously reported [14] to elucidate the growth and proliferation of cells. After collected cells at log phase, 80,000 J889 cells were added into each well of the plate, which was further incubated at 37°C with 5% CO_2 for 12 hours. MTT reagent (8 mg/ml) was added into each well for another 8-hour incubation. DMSO was used to terminate reactions. Absorbance values at 490 nm were measured by a microplate reader. Parallel control group was performed using PBS instead.

Flow cytometry

Flow cytometry was performed using routine procedures [15] on human brain glioblastoma J889 cells to reveal its apoptosis, in addition to caspase activity. In brief, J889 cell from all groups were firstly centrifuged at 1000 g for 8 min. After discarding supernatant, J889 cells were re-suspended using PBS buffer. Regent and FITC-Annexin V were mixed at 50:250:1 caidaole. After dark incubation for 15 min, all sampled were loaded for flow cytometry.

Western blotting

Using routine method, we analyzed level of anti-prion protein in human brain glioblastoma J889 cells in addition to the internal reference anti-actin [16]. ImageJ 8.0 software analyzed the density of all blotting bands. The relative expression level of prion protein was presented as density of prion against actin.

RT-PCR

RT-PCR study was performed using documented methods [17] on human brain glioblastoma J889 cells to check the level of prion mRNA. ImageJ 8.0 software was used to analyze optical density of DNA bands. The relative expression level of prion mRNA was presented as optical density of prion protein gene against internal reference actin gene.

Caspase-3 activity

The activity of caspase-3 in human brain glioblastoma J889 cells was determined by assay kit following previously reported methods [18].

Clonal formation assay

Clonal formation assay was performed on human brain glioblastoma J889 cells with prion protein-targeted siRNA or over-expressed cells using previously reported methods [19].

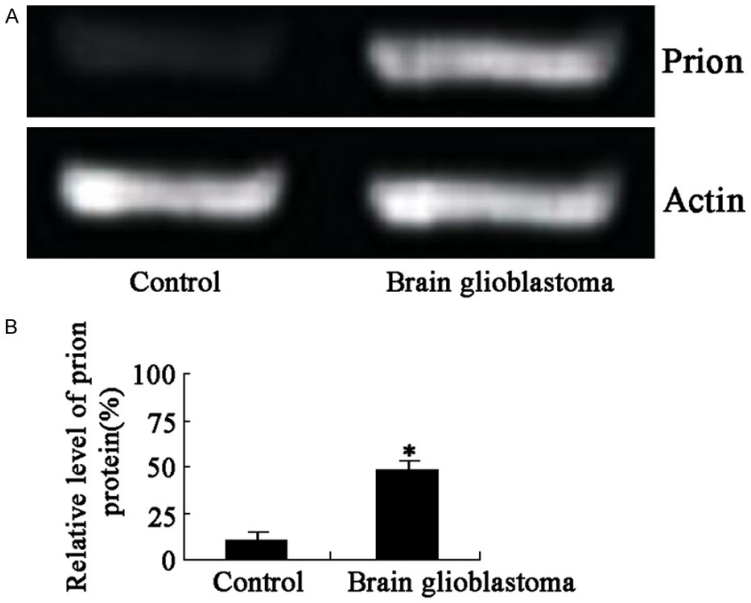

Figure 1. mRNA level of prion protein in brain glioblastoma. A. Representative results of RT-PCR of prion protein in both control (tumor adjacent) and brain glioblastoma tissues; B. Statistical results from triplicated experiments. *, P<0.05 compared to control rats.

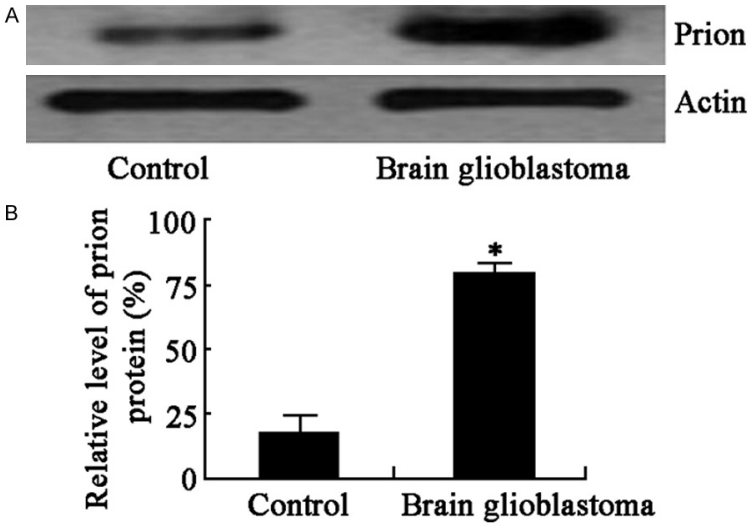

Figure 2. Protein level of prion protein in brain glioblastoma. A. Representative results of Western blotting of prion protein in both control (tumor adjacent) and brain glioblastoma tissues; B. Statistical results from triplicated experiments. *, P<0.05 compared to control rats.

Brain glioblastoma rat model

The rat model for brain glioblastoma was prepared by Dingguo Biotech (Beijing, China) using commonly accepted methods [20]. Both glioma tissues and cancer adjacent tissues were collected. Western blotting and RT-PCR were used to detect the protein and mRNA level of prion protein and its correlation with brain glioblastoma.

Statistical methods

SPSS 16.0 software was used to process all collected data, which were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare means across groups. A statistical significance was defined when $P<0.05$.

Results

Elevated prion proteins in brain glioblastoma

As shown in **Figure 1**, mRNA level of prion protein in rat brain glioblastoma tissues was significantly elevated compared to control tissues by RT-PCR ($P=0.0028$). **Figure 2** further showed higher prion protein level in brain glioblastoma cells by Western blotting than control ones ($P=0.0081$). These results collectively suggested the possible correlation between prion protein and pathogenesis or progression of human brain glioblastoma.

Prion protein-targeted siRNA inhibited J889 cell growth

As shown in **Figure 3**, those J889 cells transfected with prion protein-targeted siRNA had significantly depressed cell growth, as compared to those cells transfected with controlled scramble siRNA.

SiRNA induced human brain glioblastoma J889 cell apoptosis

As shown in **Figures 4** and **5**, prion protein-targeted siRNA induced outbreak of PS and elevated caspase-3 activity in human brain glioblastoma J889 cells, as compared to cells

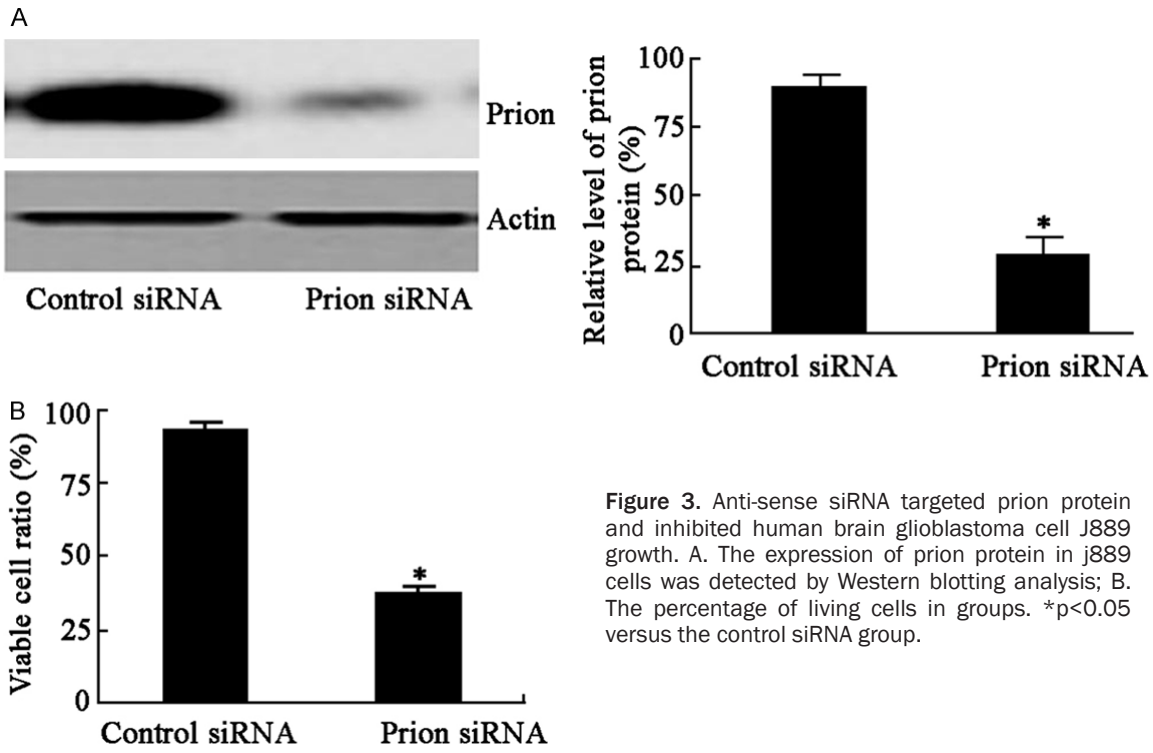

Figure 3. Anti-sense siRNA targeted prion protein and inhibited human brain glioblastoma cell J889 growth. A. The expression of prion protein in j889 cells was detected by Western blotting analysis; B. The percentage of living cells in groups. *p<0.05 versus the control siRNA group.

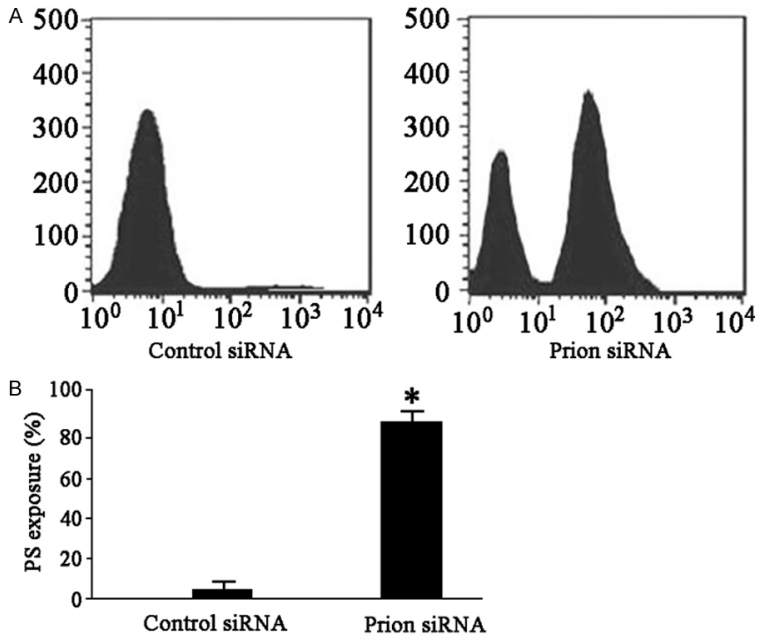

Figure 4. Anti-sense siRNA targeted prion protein and induced PS outbreak in human brain glioblastoma J889 cells. A. PS outbreak assay of J889 cells using flow cytometry; B. Statistical results from triplicated experiments. *, P<0.05 compared to control siRNA group.

transfected with control siRNA (P<0.05). These results indicated the induction of human brain glioblastoma J889 cell apoptosis by prion targeted siRNA.

Prion-targeted siRNA inhibited clonal formation of J889 cells

As shown in **Figure 6**, the transfection of prion-targeted siRNA in J889 cells caused inhibited clonal formation of cells as compared to those cells transfected with control siRNA. Results showed that prion protein silencing decreased the clonal formation ability of J889 cells.

Over-expression of prion protein facilitated human brain glioblastoma J889 cell growth without significant apoptosis

As shown in **Figure 7**, compared to those cells transfected with blank plasmid (control), those cells over-expressing prion protein facilitated J889 cell growth without significant cell death.

Discussion

This study investigated the expression and modulatory role of prion protein in rat brain glio-

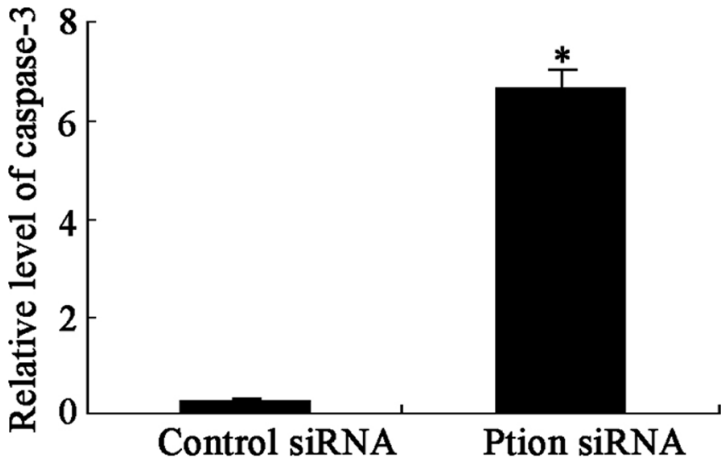

Figure 5. Caspase-3 activity of prion-targeted siRNA transfected J889 cells. *, P<0.05 compared to control siRNA group.

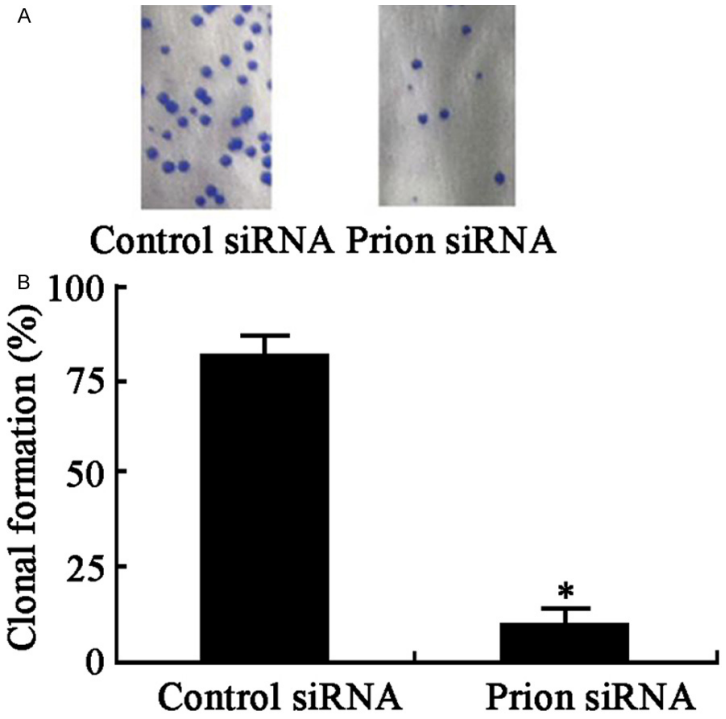

Figure 6. Prion-targeted siRNA inhibited clonal formation of human brain glioblastoma J889 cells. A. Test results in clonal formation of J889 cells; B. Statistical analysis of triplicated experiments. *, P<0.05 compared to control siRNA group.

blastoma cells. This is the first time that prion protein levels (both mRNA and protein expression) are found to be up-regulated in rat brain glioblastoma tissues compared to tumor adjacent tissues, suggesting the correlation between prion protein and pathogenesis/progression of brain glioblastoma. Moreover, we also found that the depression of human brain glioblastoma J889 cells significantly inhibited cell growth, facilitated cell apoptosis and clonal formation. The over-expression of prion protein, however, obtained opposite effects. These data suggested prion protein could regulate J889 cell growth and apoptosis at cellular level, indicating the potency of prion protein as anti-glioblastoma drugs. These results were consistent with previous finding showing the role of anti-apoptotic proteins in tumor cell apoptosis and clonal formation [20, 21].

Cell apoptosis is mainly induced by intra-mitochondrial and death receptor-induced external signal transduction pathways [22]. In this study, we found significant PS outbreak of J889 cells after transfecting with siRNA for silencing prion protein, suggesting remarkable cell apoptosis. We also explored related mechanisms underlying prion protein-induced cell apoptosis. Caspase activity assay showed no occurrence of caspase-8 activation, which represents death receptor-induced programmed cell death, accompanied with potentiated caspase-3 activity. These results thus illustrated that the prion protein-induced cell apoptosis of J889 cells was induced by mitochondria, which was inconsistent with previous study showing the major function of caspase-8 pathway in tumor cell apoptosis modulation [23]. Such inconsistency may be due to the different tumor cell lines, which may have various sensitivities to external stimuli [24]. On the other hand, we also postulated that such prion protein-induce apoptosis may directly or indirectly induce mitochondrial induced signal pathway for further cell death [25]. These models, however, need more evidences to be validated.

Our studies had certain limitations. For example, no systemic grades of human brain glio-

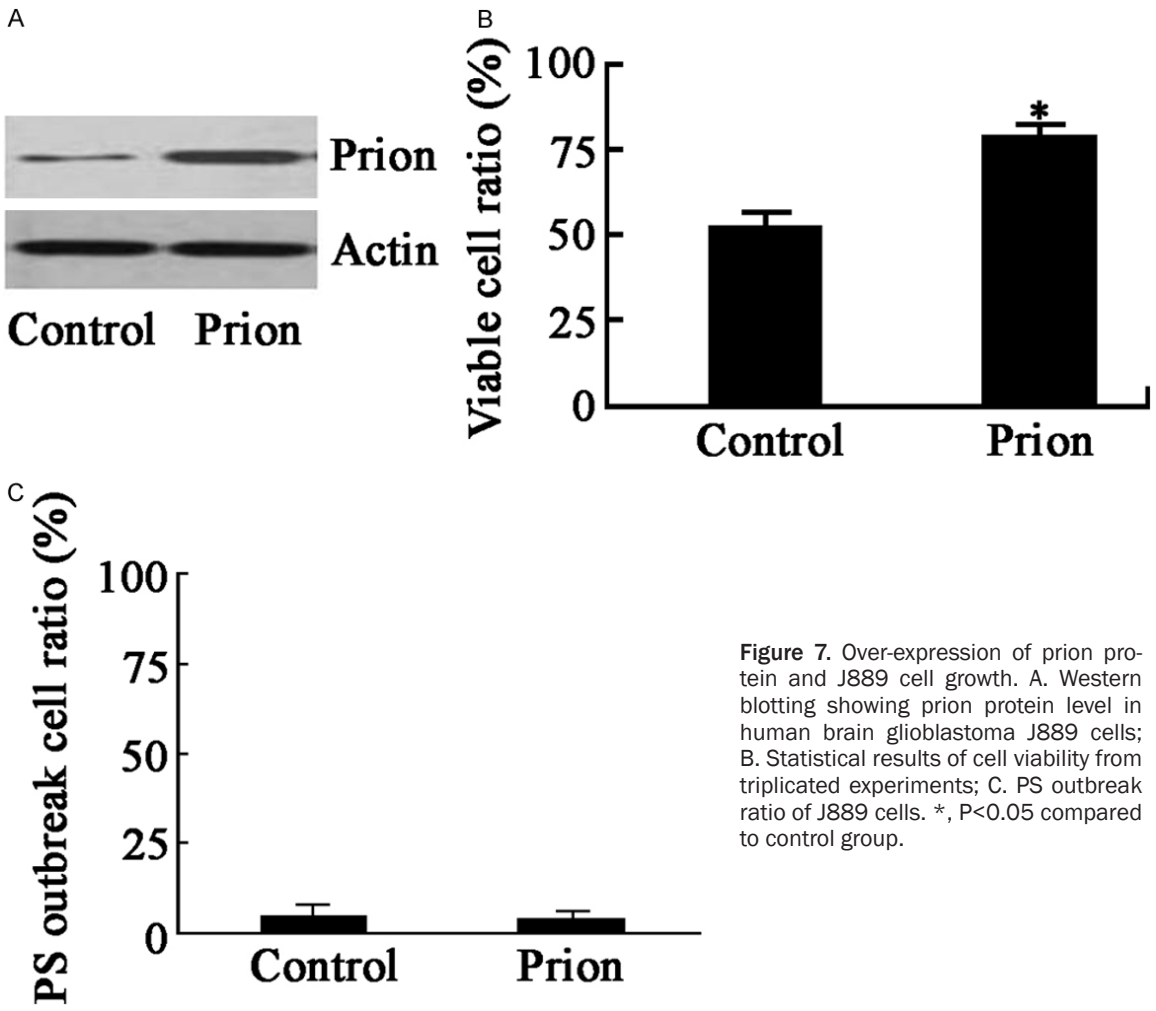

Figure 7. Over-expression of prion protein and J889 cell growth. A. Western blotting showing prion protein level in human brain glioblastoma J889 cells; B. Statistical results of cell viability from triplicated experiments; C. PS outbreak ratio of J889 cells. *, P<0.05 compared to control group.

blastoma have been performed on all samples [26], thus compromising the correlation between prion level and glioblastoma condition. We also did not perform any *in vivo* study to interfere prion protein expression for treating brain glioblastoma [27]. Thirdly, the limited sample size (N=9 each) had less test power and require larger sample studies. Moreover, the prion protein level in brain glioblastoma level should be determined in rats after chemo- or radio-therapy [28], in order to illustrate the relationship between prion protein and brain glioblastoma prognosis.

In summary, our results showed the possible correlation between prion protein level and human brain glioblastoma. The inhibition of prion protein level inhibits the growth and clonal formation of human brain glioblastoma cell J889. The elevation of prion protein expression may facilitate the growth and clonal formation of human brain glioblastoma cell J889, and inhibit their apoptosis. Results from this study thus provide new insights for developing novel molecular targeting treatment approach focusing on anti-human glioblastoma.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Weiguo Li, Department of Neurosurgery, Qilu Hospital, Shandong University, 107 Wenhua Xi Road, Jinan 250012, China. Tel: +86-531-82169114; Fax: +86-531-82169114; E-mail: weiguoliyou@126.com

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