

Radiosensitisation of human glioma cells by inhibition of β 1,6-GlcNAc branched N-glycans

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Received: 10 August 2015 / Accepted: 26 October 2015 / Published online: 3 November 2015
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Abstract Gliomas are the most prevalent type of primary brain tumors and are resistant to radiation therapy. β 1,6-GlcNAc branched N-glycans, which are encoded by *N*-acetylglucosaminyltransferase V (GnT-V), play important roles in glioma progression. However, the relationship between β 1,6-GlcNAc branched expression and radiosensitivity in glioma cells is still unknown. In this study, the expression of β 1,6-GlcNAc branched N-glycans in nonneoplastic brain and glioma samples was characterized by lectin histochemistry. The radiosensitivity of glioma cells was evaluated by colony formation assay. We found that β 1,6-GlcNAc branches were highly expressed in glioblastoma specimens, compared with diffuse astrocytomas and nonneoplastic brain. In addition, β 1,6-GlcNAc branched expression was negatively correlated with the radiosensitivity of glioblastoma cells. Furthermore, the inhibition of N-linked β 1,6-GlcNAc branches by GnT-V silencing in U251 cells could reduce the cell clonogenic survival after X-irradiation. Meanwhile, the G2/M checkpoint was impaired and there was an increase in the number of apoptotic cells. Tunicamycin, an inhibitor of N-glycan

biosynthesis, was also able to enhance the radiosensitivity of U251 cells. Thus, our results suggest that development of therapeutic approaches targeting N-linked β 1,6-GlcNAc branches may be a promising strategy in glioblastoma treatment.

Keywords β 1,6-GlcNAc branched N-glycans · GnT-V · Glioma · Radiosensitivity

Introduction

Gliomas are the most common and the most lethal primary brain tumors in adults [1]. According to the World Health Organization (WHO) classification, gliomas can be classified into four histopathological grades: pilocytic astrocytoma (grade I), low-grade diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (GBM, grade IV) [2]. Glioblastoma is the most aggressive form of gliomas. Both diagnostic technologies and therapeutic strategies have been greatly advanced, but prognosis of patients with glioblastomas is still very dismal. It has the worst prognosis with median survival time to be 9 to 12 months [3]. Radiotherapy is the main postoperative treatment for glioblastoma patients. However, it does not substantially improve the long-term outcomes of patients because of the intrinsic radioresistance of glioma cells [4]. Therefore, identification of a novel molecular target might help to assess more precisely the prognosis and to improve the therapeutic efficacy of radiation therapy.

Glycosylation is one of the most prevalent post-translational modifications found in most cells and tissues. Alteration of N glycosylation has been revealed to be crucial for tumor progression [5, 6] and therapeutic resistance [7, 8]. The most commonly observed aberrant N glycosylation in tumor models is β 1,6-GlcNAc branched N-glycans. *N*-

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acetylglucosaminyltransferase V (GnT-V) is responsible for the biosynthesis of β 1,6-GlcNAc branched N-glycans in median Golgi [9]. N-linked β 1,6-GlcNAc branches have been confirmed to be highly expressed on the cell surface of glioma cells [10] and involved in early gliomagenesis [11]. However, it is still unclear whether N-linked β 1,6-GlcNAc branches could regulate the radiosensitivity of glioma cells. Interestingly, high expression of GnT-V has been reported to be associated with radiosensitivity in nasopharyngeal carcinoma [12] and prostate cancer [13]. Based upon these findings, we hypothesize that β 1,6-GlcNAc branched N-glycans may be involved in the radioresistance of glioma cells to radiation therapy.

In this study, β 1,6-GlcNAc branched expression in clinical glioma samples was detected. Moreover, the effect of N-linked β 1,6-GlcNAc branches on the radiosensitivity was assessed in glioblastoma models.

Materials and methods

Clinical samples

Informed consent was obtained from all patients enrolled in this study, and the study protocol was approved by the Ethics Committee of Hubei University of Medicine. Thirty-five cases of glioblastomas (WHO grade IV), 14 cases of diffuse astrocytomas (WHO grade II), and 4 cases of non-tumoral adjacent tissues were obtained from the Taihe Hospital, Hubei University of Medicine. The pathological grade of these tumors was defined according to the 2007 WHO criteria. All tissues were formalin-fixed and paraffin-embedded.

Lectin histochemistry

The tissues were deparaffinized in xylene and rehydrated in alcohol. To block endogenous peroxidase activity, the sections were incubated in 3 % H_2O_2 for 10 min. Subsequently, sections were incubated with biotinylated PHA-L (1:200; Vector Labs, CA, USA) at 4 °C overnight. This was followed by incubation with streptavidin (Vector) conjugated to horseradish peroxidase (HRP) for 45 min at room temperature. Color development was performed using Diaminobenzidine (DAB) kit (Maixin Biol, Fu Zhou, China). For negative control, PHA-L was omitted and also replaced by PBS. All tissues were verified by two independent pathologists in a blinded fashion. The histological score adopted in this study was computed according to the previous study [14]. The score for immunoreactive extension was as follows: 0 (<5 % positive tumor cells), 1 (<6–25 % positive tumor cells), 2 (26–50 % positive tumor cells), and 3 (>50 % positive tumor cells). For intensity, the score was as follows: 0, negative; 1, weak; 2, intermediate; and 3, strong. A final immunoreactivity scores (IRS) was

obtained for each case by multiplying the percentage and the intensity score. The IRS value >4 was defined as high expression and IRS value \leq 4 as low expression.

Cell lines and cell culture

The human glioblastoma cell lines U251, A172, and LN229 were obtained from the American Type Culture Collection (ATCC, MD, USA). All the cell lines were cultured in DMEM (Gibco-BRL, CA, USA) supplemented with 10 % FBS at 37 °C in 5 % CO_2 humid atmosphere.

RNA extraction and semi-quantitative RT-PCR

RNA isolation, reverse transcription, and semi-quantitative RT-PCR analysis were performed as previously described [15]. PCR amplification was performed according to the following thermal cycling parameters: 1 cycle at 94 °C for 5 min; 30 cycles of 94 °C for 45 s, 52 °C for 45 s, and 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. The primer sequences for the genes and expected product sizes were as follows: 5'-GCAATGGCTCTCTTCACTCC-3' (sense) and 5'-GCTGTCATG ACTCCAGCGTA-3' (anti-sense) for GnT-V (239 bp); 5'-CCTCTATGCCAACCA CAGTGC-3' (sense) and 5'-GTACTCCT GCTTGCTGA TCC-3' (anti-sense) for β -actin (250 bp). All expression levels were normalized to the expression levels of endogenous β -actin, which served as a control.

Protein lysate preparation and Western blot analysis

Cells were lysed in ice-cold lysis buffer, and total proteins were extracted as described previously [15]. Then proteins were separated by 10 % SDS PAGE and transferred onto PVDF membranes, followed by blocking of membranes with 5 % fat free milk for 1 h at room temperature. Membranes were incubated with specific primary antibodies followed by appropriate secondary antibodies and enhanced chemiluminescence visualization (Thermo Fisher Scientific, IL, USA). Anti- β -actin mAb (1:1000) and anti-GnT-V mAb (1:1000) as well as the second antibodies were purchased from Abcam (CA, USA).

Flow cytometry analysis for cell surface N-glycans

Cultured cells were collected by 0.25 % trypsin. After blocked with 5 % BSA in PBS, the cells were incubated with 10 $\mu\text{g}/\text{ml}$ of biotinylated PHA-L lectin in 3 % BSA at 4 °C for 1 h. Streptavidin-Phycoerythrin (Vector) of final concentration of 1 $\mu\text{g}/\text{ml}$ was used and incubated for 0.5 h at room temperature in the dark. Then cells were analyzed using a FACScan (Beckman Coulter, Brea, CA, USA). Only live cells were established as gates.

Colony formation assay

The clonogenic survival assay was carried out as described previously [16]. In brief, exponentially growing cells were plated at 500 cells/100-mm tissue culture dish in triplicate and irradiated the next day using different doses (0, 2, 4, 6, and 8 Gy). The cells were irradiated using an X-ray machine (X-RAD 320, Precision X-ray) at 320 kV, 10 mA with a 2-mm aluminum filter, and the dose rate was 2 Gy/min. These cells were incubated at 37 °C for 14 days. When most cell clones had reached >50 cells, they were stained with 0.1 % crystal violet (Sigma, MI, USA). The surviving fraction was calculated by using the equation (mean colony counts)/(cells plated) × (plating efficiency), where the plating efficiency was defined as (mean colony counts)/(cells plated for irradiated controls) [17].

Establishment of stably transfected cells

GnT-V pGPU6/GFP/Neo-shRNA and mock shRNA plasmids were purchased from the GenePharma (Shanghai, China). The target sequence for GnT-V RNAi is 5'-CCTGGAAGCTATCGCAAAT-3', and the mock sequence is 5'-GAATTACTCCTAGAACCGC-3'. For transfection studies, the cells were plated at a density of 2×10^5 cells/well in 6-well plates and incubated for 24 h. The cells were then transfected with 2–4 µg of plasmid DNA using Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Complete medium with 1 µg/ml puromycin was used as the selective medium. Transfectants were isolated and maintained in complete medium containing 0.2 µg/ml puromycin for further experiments. Untransfected cells were served as the control group.

Immunofluorescence staining

Cells were cultured in 24-well plates. After 24 h, cells were fixed by 4 % paraformaldehyde for 30 min, penetrated by 0.1 % Triton X-100 for 10 min, and blocked by 5 % BSA

for 30 min. Then, cells were incubated with 10 µg/ml of biotinylated PHA-L lectin overnight at 4 °C followed by incubating with Streptavidin-Phycoerythrin (1 µg/ml) in room temperature for 30 min. DAPI (Sigma) was added to cells, and the image was analyzed by fluorescence microscopy (Olympus, Tokyo, Japan) or laser confocal microscope (Leica LAS AF Lite, Wetzlar, Germany).

Cell cycle and apoptosis analysis

Cells were X-irradiated with 4 Gy. After 24 h, the cell cycle and apoptosis assay were performed as previously described [18]. Briefly, cultured cells were collected by 0.25 % trypsin and fixed in cold 80 % ethanol overnight at 4 °C. For cell cycle analysis, cells were stained with PI (Sigma). For apoptosis analysis, cells were double stained with Annexin V-FITC (Sigma) and PI. Data were acquired using flow cytometry and analyzed using FlowJo software.

Statistical analysis

Data analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL). Student's *t* test was used to test the significant differences between two groups for continuous variables and one-way analysis of variance (ANOVA) for more than two groups. All the experiments were repeated at least three times. The results were shown as means ± standard deviation (SD). Differences were considered statistically significant at $P < 0.05$.

Results

Expression of β1,6-GlcNAc branched N-glycans in glioma tissue samples

Lectin staining with PHA-L, which recognizes β1,6-GlcNAc branched N-glycans, was undertaken to identify differential expression profiles of these structures [19]. We found that PHA-L-recognizing sugars were mainly expressed in

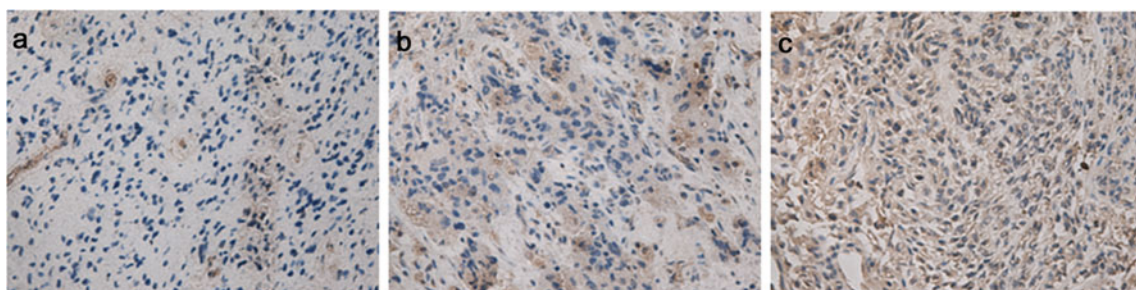


Fig. 1 Increased expression of β1, 6 GlcNAc branched N-glycans in glioblastomas. PHA-L staining was performed to identify β1,6-GlcNAc branched expression. Negative staining in nonneoplastic brain tissues (a). Low staining in diffuse astrocytomas (b). High staining in glioblastoma

tissues (c). In glioblastoma tissues, PHA-L-recognizing sugars were mainly expressed in cytoplasm and cell surface of tumor cells with brown yellow. Original magnification was ×100

Table 1 Association of β 1, 6 GlcNAc branched N-glycans expression with clinicopathological features of gliomas

Clinicopathological features	Cases	β 1, 6 GlcNAc branched N-glycans expression		P value
		High (n, %)	Low (n, %)	
Age				0.176
<55	29	18 (62.1)	11 (39.3)	
\geq 55	20	14 (70.0)	6 (30.0)	
Gender				0.158
Male	24	17 (70.1)	7 (25.9)	
Female	25	15 (60.0)	10 (40.0)	
WHO grade				0.001
II	14	6 (42.9)	8 (57.1)	
IV	35	26 (74.3)	9 (25.7)	

cytoplasm and cell surface of glioma samples (Fig. 1). In contrast, the nonneoplastic brain tissues were expressed a trace amount of β 1,6-GlcNAc branches.

Association of β 1,6-GlcNAc branches status with clinicopathological characteristics is shown in Table 1. Of the 49 patients with gliomas, the high expression of β 1,6-GlcNAc branches was detected in 65.3 % (32/49) of patients. In diffuse astrocytomas (WHO grade II), 42.9 % (6/14) of cases highly

expressed β 1,6-GlcNAc branches. However, 74.3 % (26/35) of cases highly expressed β 1,6-GlcNAc branches in glioblastomas (WHO grade IV). There was a significant increase in β 1,6-GlcNAc branched expression in glioblastomas, compared to diffuse astrocytomas and nonneoplastic brain tissues. The data also demonstrated that the expression levels of β 1,6-GlcNAc branches had no correlation with age and gender.

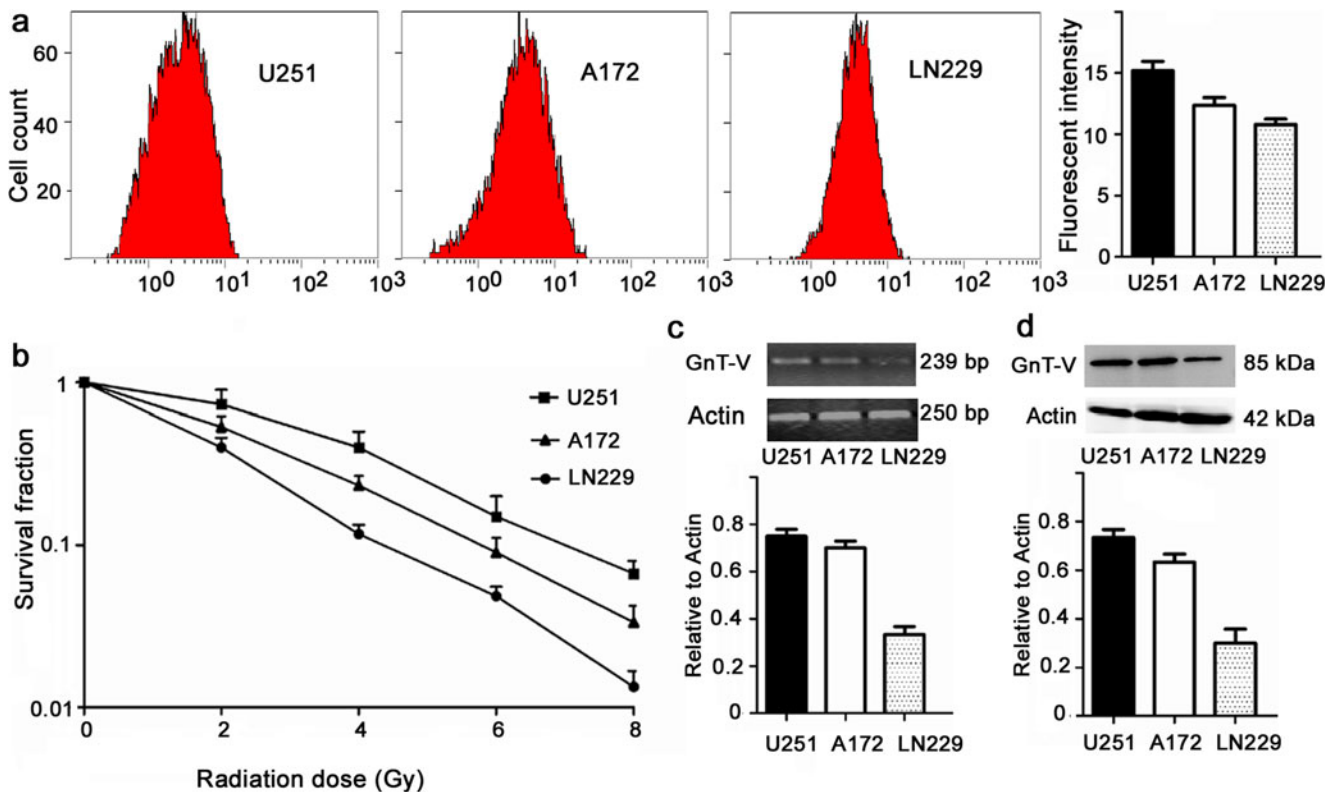


Fig. 2 Correlation between the expressions of β 1, 6 GlcNAc branched N-glycans and radiosensitivity of glioblastoma cell lines. U251 cells show the highest β 1, 6 GlcNAc branched N-glycans and GnT-V expressions among selected glioblastoma cell lines. And the radiosensitivity of U251 cells is lower than that of other cells. **a** The intensity of PHA-L staining was detected by flow cytometry. **b** The

surviving fractions were analyzed by colony formation assay. The survival curves were fitted and analyzed with Graphpad Prism4 statistical software. The GnT-V mRNA and protein expression levels were determined by **c** RT-PCR and **d** Western blotting. Results are recorded as mean \pm SD of three independent experiments

Correlation between expression of β 1,6-GlcNAc branched N-glycans and radiosensitivity of glioblastoma cells

To investigate the effects of β 1,6-GlcNAc branched N-glycans, three different glioblastoma cell lines (U251, A172, and LN229) were used in this study. Flow cytometry with PHA-L was performed to detect the expression of N-linked β 1,6-GlcNAc branches in glioma cells. Fluorescent intensity was computed as the median value of each staining. As shown in Fig. 2a, the fluorescence intensities of U251, A172, and LN229 were 15.2 ± 1.7 , 12.7 ± 1.4 , and 10.8 ± 1.6 , respectively. U251 cell line showed the highest expression of PHA-L-recognizing sugars.

We next used the colony formation assay to evaluate the radiation sensitivity of glioblastoma cell lines. The results showed that the survival fractions in U251 cells at different radiation dosages were higher than that in A172 and LN229 cells (Fig. 2b). The radiosensitivity of U251 cell line is lower than that of A172 and LN229 cell lines.

Expression of β 1-6 branched oligosaccharides is mainly dependent on the expression of GnT-V enzyme. We further examined whether the β 1,6-GlcNAc expression correlated with the expression of GnT-V by RT-PCR and Western blotting. Among the cell lines, U251 had the highest expression of GnT-V at both the mRNA and protein levels (Fig. 2c, d). Therefore, to study

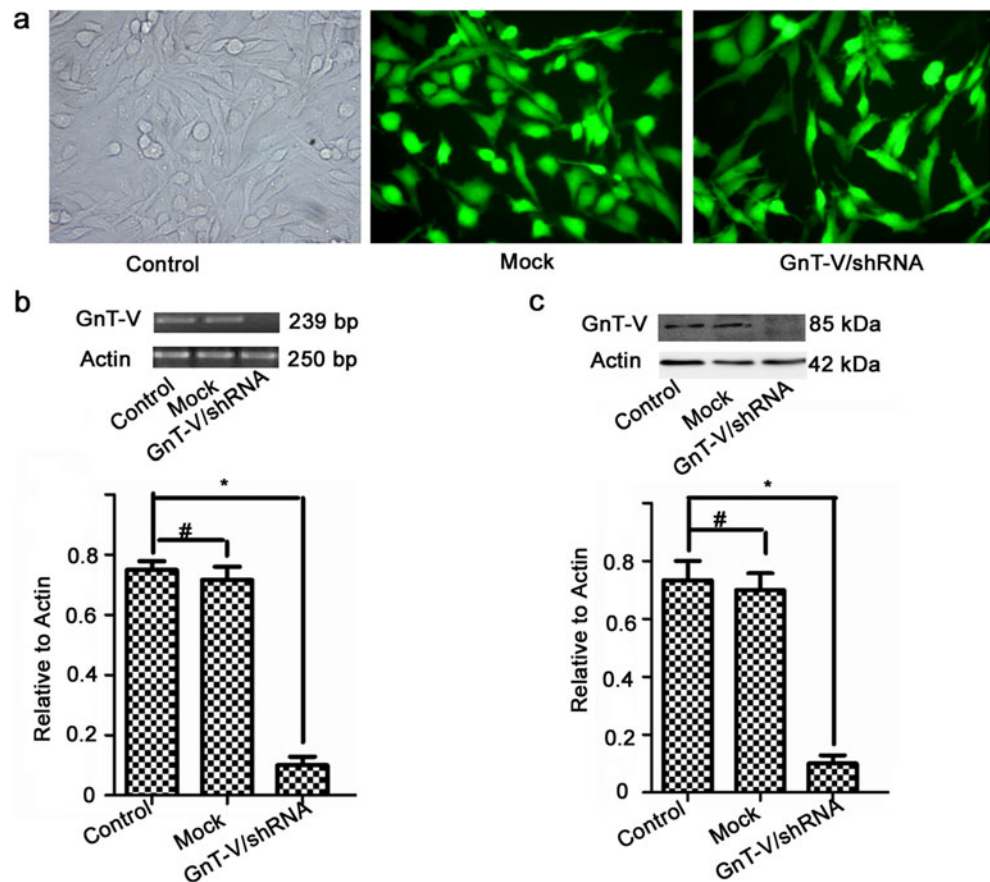
the functional significance of β 1,6-GlcNAc branches, U251 cell line was chosen as a suitable cell line for in vitro model generation. Taken together, these results indicated that β 1,6-GlcNAc branched expression was negatively correlated with the radiosensitivity of glioblastoma cell lines.

GnT-V knockdown inhibits β 1,6-GlcNAc branched N-glycans' formation in U251 cells

To inhibit the formation of β 1,6-GlcNAc branched N-glycans, U251 cells were separately transfected with GnT-V shRNA or mock shRNA plasmids. The transfected cells were observed using fluorescence microscopy (Fig. 3a). Knockdown success of more than 85 % was achieved and was confirmed at both the RNA and protein levels by RT-PCR and Western blotting, respectively (Fig. 3b, c).

Consistent with decreased GnT-V mRNA and protein levels, flow cytometry with PHA-L revealed a decrease in the expression of β 1,6-GlcNAc branching in GnT-V/shRNA cells, compared with mock and control cells. The fluorescence intensities of control, mock, and GnT-V/shRNA cells were 15.2 ± 1.7 , 13.9 ± 1.5 , and 3.2 ± 0.6 , respectively. Then the staining cells were analyzed by fluorescence microscopy or laser confocal microscope. GnT-V knockdown cells showed a dramatically reduced binding with PHA-L (Fig. 4b, c). These

Fig. 3 Construction of different ectopic expression for GnT-V in U251 cells. U251 cells were stably transfected with GnT-V/shRNA plasmid or mock shRNA plasmid. **a** GFP signals were observed using fluorescence microscopy at $\times 200$ magnification. The GnT-V mRNA and protein expression was detected by **a** RT-PCR and **b** Western blotting, respectively. Results are recorded as mean \pm SD of three independent experiments (* $P < 0.05$, # $P > 0.05$)



results suggested that the formation of β 1,6-GlcNAc branches in U251 cells could be regulated by GnT-V.

Attenuated expression of β 1,6-GlcNAc branched N-glycans enhances the radiosensitivity in U251 cells

As the β 1,6-GlcNAc expression is controlled by GnT-V, we further investigated the effects of GnT-V silencing on the radiosensitivity of U251 cells. For this purpose, we explored the consequence of GnT-V depletion on the cell cycle with/without radiation of 4Gy (Fig. 5a). In both the control and mock shRNA-transfected cells after X-irradiation, the G2/M population increased to 18.5 ± 2.1 % and 17.9 ± 2.4 %, respectively, and the G0/G1 population decreased to 30.7 ± 1.4 % and 28.1 ± 2.3 %, respectively. These findings indicated that the G2/M checkpoint response was activated after X-irradiation. Conversely, in the GnT-V shRNA-transfected cells, the G2/M population increased slightly to 6.18 ± 0.11 % and the G0/G1 population just decreased to 47.2 ± 3.8 %. These data revealed that GnT-V knockdown could inhibit X-ray-induced G2/M arrest.

Then the Annexin V/PI staining assay was conducted to determine the apoptotic cell number induced by X-irradiation. As shown in Fig. 5b, no significant difference in the proportion of apoptotic cell was observed between the control and mock shRNA-transfected cells with X-irradiation. However, the combination of GnT-V shRNA and X-irradiation significantly increased cell apoptosis when compared with that of cells of mock and control groups ($P < 0.05$).

Colony formation assay was performed to assess the radiosensitivity of glioma cells. As shown in Fig. 5d, no differences were observed between the control and mock shRNA-transfected cells at all dose levels tested in U251 cells. However, GnT-V/shRNA cells exhibited significantly reduced clonogenic survival at all dose levels compared with control and mock shRNA-transfected cells ($P < 0.05$).

The potential effects of tunicamycin on cell apoptosis with/without radiation were also investigated. Tunicamycin stock solutions (Sigma) were diluted in DMEM. We found 90 % of cells survived at a concentration of 1 μ g/ml (data not shown). The U251 cells were pretreated with 1 μ g/ml of tunicamycin for 24 h. As shown in Fig. 5c, tunicamycin treatment resulted in a

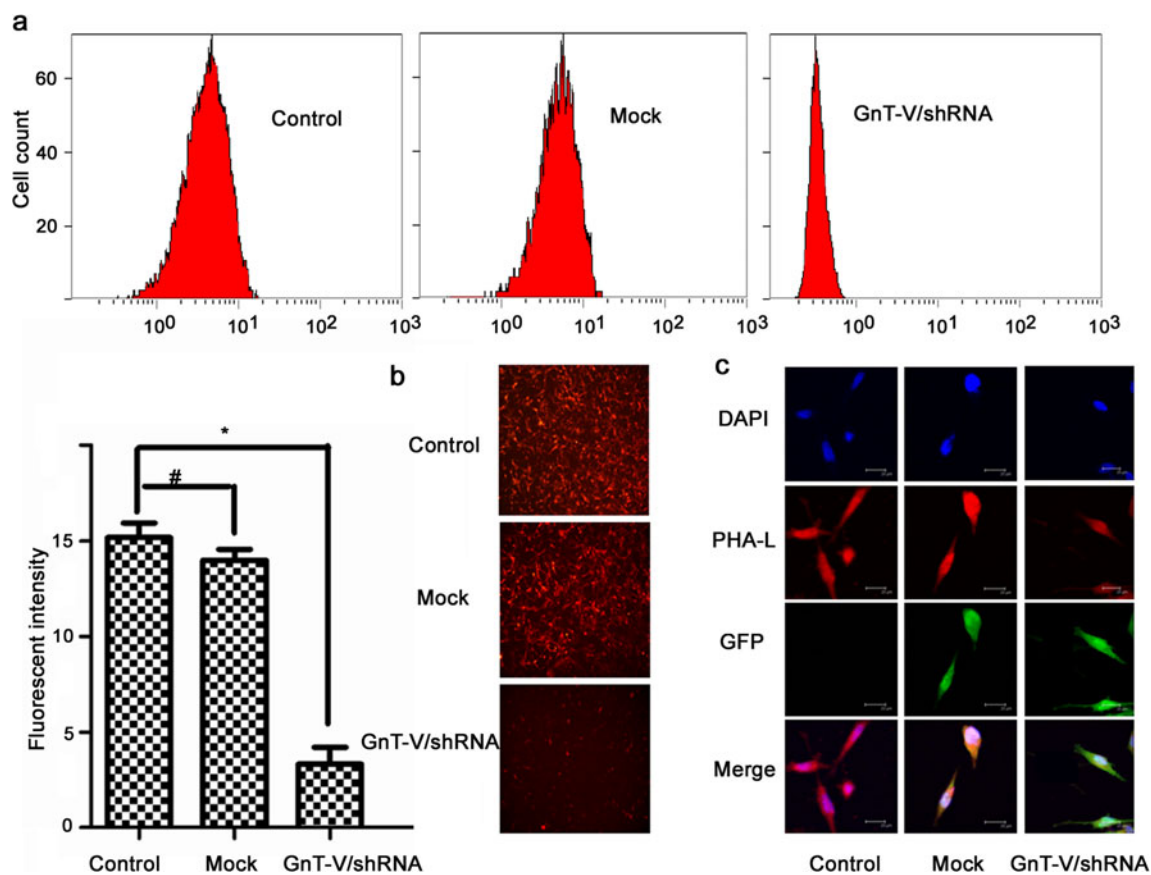


Fig. 4 Silencing of GnT-V inhibits the formation of β 1,6-GlcNAc. U251 cells were stably transfected with GnT-V/shRNA plasmid or mock shRNA plasmid. **a** The intensity of PHA-L staining was detected by flow cytometry. Immunofluorescence staining was analyzed by **b** fluorescence

microscopy at $\times 100$ magnification and **c** laser confocal microscope. Scale bar, 50 μ m. Results are recorded as mean \pm SD of three independent experiments ($*P < 0.05$, $^{\#}P > 0.05$)

dramatically reduced binding with PHA-L as determined by flow cytometry. The combination of tunicamycin and X-irradiation markedly increased cell apoptosis compared

with control cells (Fig. 5b) ($P < 0.05$). These results further confirmed that the inhibition of $\beta 1,6$ -GlcNAc branched N-glycans was able to enhance cell radiosensitivity.

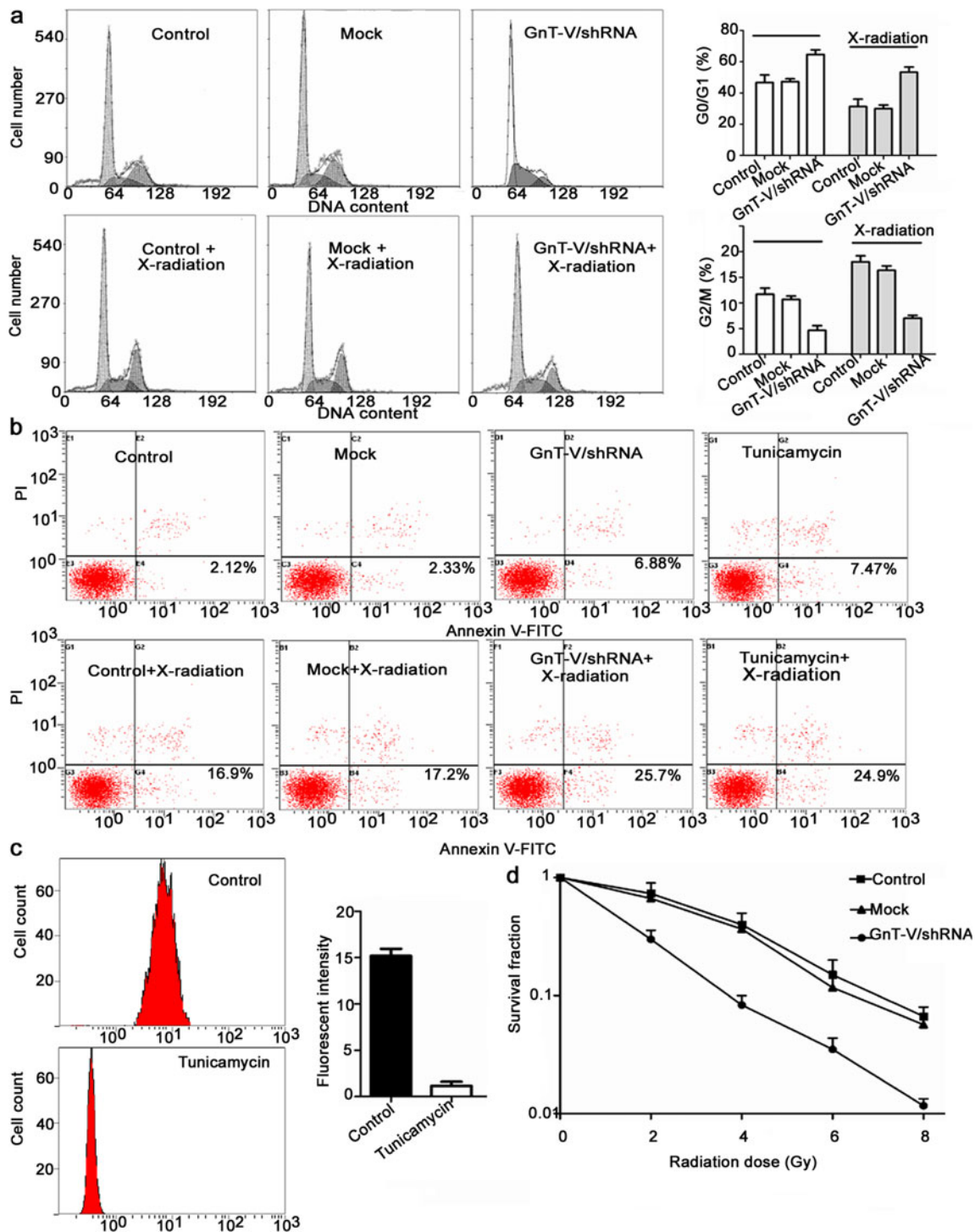


Fig. 5 Inhibition of $\beta 1,6$ -GlcNAc enhances the radiosensitivity in U251 cells. U251 cells were stably transfected with GnT-V/shRNA plasmids or mock shRNA plasmids. **a** Cell cycle and **b** apoptosis were detected by flow cytometry with/without radiation. U251 cell were pretreated with 1 μ g/ml of tunicamycin for 24 h. **b** Cell apoptosis was detected by flow

cytometry with/without radiation. **c** The intensity of PHA-L staining was detected by flow cytometry. **d** Cells were exposed to various doses of irradiation, and the surviving fractions were analyzed by colony formation assay. Results are recorded as mean \pm SD of three independent experiments

Discussion

Glioblastomas are very aggressive human neoplasms, presenting high resistance to current therapy [4]. Treatment of glioblastomas is a considerable therapeutic challenge. Thus, exploitation of new molecular targets becomes crucial in neuro-oncology. In this study, we found that the combining radiation with inhibition of N-linked β 1,6-GlcNAc branches potentiated the radiosensitivity of glioma cells. We have pointed out, for the first time, that N-linked β 1,6-GlcNAc branches have an important impact on the radiosensitivity of glioblastomas.

Altered N-glycans has turned out to be a universal feature of cancer cells [20]. And glioma cells frequently express N-glycans at different levels or with fundamentally different structures than those observed on normal brain cells. For example, high levels of α 2,6-sialylation of cell surface N-glycans inhibited glioma formation in vivo [21]. β 1,6-GlcNAc Man arm of the highly branched N-glycans was considered to be the characteristic of gliomas [22]. Poly lactosamine on β 1,6-GlcNAc was associated with the metastatic potential of glioma cells [23]. In the present study, we observed that β 1,6-GlcNAc branched expression increased with malignancy, with a significant increase from nonneoplastic tissue to diffuse astrocytomas and high-grade gliomas. CD147, a cell surface transmembrane glycoprotein widely expressed on many cell types, is a major carrier of β 1,6-GlcNAc on human tumor cells [24]. It has been reported that CD147 was highly expressed in glioblastomas ($n=60$) but not in low-grade gliomas ($n=4$) or nonneoplastic brain tissue ($n=1$) [25]. Our results are also in accordance with the previous study to a certain extent. Thus, it may be hypothesized that microenvironmental changes in the synthesis of β 1,6-GlcNAc greatly affect the functions of CD147 in glioblastomas.

Our work further produced evidence that β 1,6-GlcNAc branched expression was negatively correlated with the radiosensitivity of glioblastoma cells. Previous studies have shown that the alteration of β 1,6-GlcNAc branched N-glycans was involved in tumor sensitivity to chemotherapeutic drug [26, 27]. In some senses, chemotherapy and radiation use the same basic strategy of disrupting cell. Chemotherapy uses drugs to disrupt. Radiation uses high concentrated beams. Thus, N-linked β 1,6-GlcNAc branches have significant implications for the development of new treatment strategies.

Cell clonogenic survival has been discovered to be associated with response to radiotherapy. In the current study, we found that the inhibition of N-linked β 1,6-GlcNAc branches by GnT-V silencing in U251 cells significantly reduced cell clonogenic survival after X-irradiation. Irradiation results in a delay of progression through the G0/G1 and G2/M phases of the cell cycle [28]. Cell cycle arrest provides irradiated cells the time to repair DNA damage, and the G2/M transition phase is one of the main checkpoints for cell fate

determination after X-irradiation [29, 30]. We showed that the G2/M checkpoint response was activated after X-irradiation. However, G2/M arrest was not observed in GnT-V shRNA-transfected U251 cells after X-irradiation. This finding is consistent with a previous study in nasopharyngeal carcinoma cells, and the results suggest that GnT-V silencing induces impaired G2/M arrest after X-irradiation [12]. In addition, alteration of protein glycosylation was associated with DNA damage. For example, lack of GALNT3 glycosylation activity led to sharp decrease of cell proliferation and induced S-phase cell cycle arrest [31]. O-linked β -N-acetylglucosamine could regulate processes such as the cell cycle, genomic stability, and lysosomal biogenesis [32]. In summary, we are convinced that GnT-V expression partly regulates the cell cycle and is particularly useful during phase transition of G0/G1 to G2/M.

It is well known that X-irradiation causes apoptosis via multiple pathways. Then the Annexin V/PI staining assay was conducted to determine the apoptotic cell number induced by X-irradiation. We confirmed that the combination of GnT-V shRNA and X-irradiation significantly increased cell apoptosis. Tunicamycin, an inhibitor of N-glycan biosynthesis [33], was also able to increase the radiosensitivity of U251 cells as determined by flow cytometry. However, the change of cell cycle arrest after tunicamycin treatment was not observed, which indicates that other factors needs to be clarified in the further studies.

In conclusion, the inhibition of N-linked β 1,6-GlcNAc branches significantly enhanced the radiosensitivity of glioblastoma cells. β 1,6-GlcNAc branches may therefore be a useful marker for the predication of radiation response of gliomas and could be used as a novel target to increase the radiosensitivity of glioma cells. Furthermore, more glioma cell lines and glioma patients (radioresistant or non-radioresistant) should be included to confirm the roles of β 1,6-GlcNAc branched N-glycans.

Acknowledgments This study was supported by grants from the Foundation for Innovative Research Team of Hubei University of Medicine (2014CXG02), the Key Discipline Project of Hubei Province (2014XKJSXJ12), and the Scientific and Technological Project of Shiyan City of Hubei Province (15K65).

Compliance of ethical standards Informed consent was obtained from all patients enrolled in this study, and the study protocol was approved by the Ethics Committee of Hubei University of Medicine.

Conflicts of interest None

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