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Down-regulation of GnT-V enhances nasopharyngeal carcinoma cell CNE-2 radiosensitivity *in vitro* and *in vivo*

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ABSTRACT

The purpose of this study was to investigate the role of GnT-V on radiosensitivity in human nasopharyngeal carcinoma (NPC) both *in vitro* and *in vivo*, and the possible mechanism. The GnT-V stably suppressed cell line CNE-2 GnT-V/2224 was constructed from CNE-2 by transfection. The radiosensitivity of the cells was studied by CCK-8 assay, flow-cytometry, caspases-3 activity analysis and tumor xenografts model. The expression of Bcl-2, Bax and Bcl-xl was analyzed with or without radiation. The results showed that down-regulation of GnT-V enhanced CNE-2 radiosensitivity. The underlying mechanisms may be link to the cell cycle G2-M arrest and the reduction of Bcl-2/Bax ratio. The results suggest that GnT-V may be a potential target for predicting NPC response to radiotherapy.

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1. Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy frequently found in China and Southeast Asia, with an incidence of 20–50 per 100,000 individuals [1–3]. Radiotherapy is the primary treatment for NPC. However, radioresistance remains a serious barrier to the successful treatment in a great many patients. Some patients with NPC occur local recurrences or distant metastases within 1.5 year after radiation treatment [4,5]. Recently, some molecules such as gp96, GDF15 and AT1R have been reported to be involved in the radiosensitivity of NPC cells [6,7].

Protein glycosylation is one of the most important post-translational modifications, which affects cell growth, differentiation and tumor metastasis [8,9]. The glycosyltransferase plays a crucial role on the protein glycosylation. Glycosyltransferase, located in the Golgi apparatus, includes at least six *N*-acetylglucosaminyltransferases (GnTs), defined as GnT-I–VI [10]. *N*-glycosyltransferase-V (GnT-V), catalyzing the formation of β1, 6 GlcNAc branching structures, is recognized as a major member of the glycosyltransferase family. Studies have revealed that the β1, 6 GlcNAc branching on *N*-glycans catalyzed by GnT-V is a key structure associated with malignant transformation by different ways, such as enhancing cell proliferation and inhibiting cell apoptosis [11–13]. It was reported that down-regulation of GnT-V facilitates all-trans retinoic acid to induce apoptosis of human hepatocarcinoma cells [14]. However, GnT-V suppression leads to a decreased cell apoptosis induced by retinoic acid in neuroblastoma cells [15]. Obviously, there are conflicts about the role of GnT-V in different tumors.

Emerging studies have revealed that GnT-V plays an important role in malignant potential of tumors. In our previous study, The GnT-V stably suppressed cell line CNE-2 GnT-V /2224 and the control for the transfected cell line CNE-2 GnT-V/NC were constructed from CNE-2, and the colony formation assay results showed that down-regulation of GnT-V inhibited NPC cell line CNE-2 clonogenic survival after radiation *in vitro* [16]. To further investigate the role of GnT-V on the radiosensitivity of NPC cells, the assays were processed both *in vitro* and *in vivo,* and its underlying mechanisms were also investigated in this study.

2. Materials and methods

2.1. Cell culture and transfection

Human poorly differentiated NPC cell line CNE-2 was provided by the cell bank of Sun Yat-Sen University. The cells were cultured at 37 °C with 5% CO_2 in RPMI-1640 medium (Life Technologies, Inc., Gibco BRL, Gaithersburg, MA) supplemented with 10% new born bovine serum (Life Technologies, Inc., Gibco BRL), 1% penicillin and streptomycin.

The NPC subline cell (CNE-2 GnT-V /2224) expressing lower GnT-V and the control for the transfected cell line (CNE-2 GnT-V/NC) were developed and confirmed as previous described [16].

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2.2. Cell counting kit-8 (CCK-8) assay

CCK-8 assay was used to determine cell survival in each radiological dose. Cells (5×10^3) were seeded onto a 96-well plate and then treated with five independent doses (2, 4, 6, 8 and 10 Gy, respectively). Ten microliter CCK-8 reagent and RPMI1640 were added to each well and incubated for 2 h. The optical density (OD) value of each sample was measured at a wavelength of 450 nm on a microplate reader (Multiskan MK3, Thermo Lab Systems). Cell survival rate = (value in the radiation group/ value in the nonradiation group) $\times$ 100%. The assay was conducted in six replicate wells for each sample and three parallel experiments were performed.

2.3. Cell apoptosis assay

Cells were harvested, washed twice in ice-cold PBS solution, and re-suspended in binding buffer containing 7-AAD (7-Amino-actinomycin D) for 10 min, followed by the addition of Annexin V-PE. Cell apoptosis analysis was carried out using a flow cytometer (BD Biosciences, Oxford, United Kingdom).

For caspase-3 activity detecting, cells were lysed with a lysis buffer (iNtron Biotech). The cell lysate was incubated with caspase substrate Ac-DEVE-pNA (caspase-3 substrate 1, Calbiochem, San Diego, CA) at 37 °C. An absorbance at 405 nm was measured in spectrophotometer. The relative increase of caspase-3 activity was determined by comparing the absorbance of pNA from CNE-2 GnT-V/2224 to the corresponding control group.

2.4. Cell cycle assay

Cells were harvested, washed with phosphate buffer saline (PBS) twice and then fixed with 70% cold ethanol for 12 h. The fixed cells were spun down and re-suspended in PBS at 1×10^6 cells/ml. After incubation with ribonuclease A at a final concentration of 3000 units/ml at 37 °C for 30 min, cells were filtered through a nylon mesh (BD Biosciences, USA). The cell suspension was stained with propidium iodide before analysis on a flow cytometer (BD Biosciences). Each test was repeated in triplicate.

2.5. Tumorigenicity model and fractionated radiation

Male BALB/C nude mice aged 4–6 week-old were obtained from Animal Institute of Southern Medical University (Guangzhou, China). The mice were raised under pathogen-free conditions. Animal experiments were performed under the regulations of the institutional ethical commission (Southern Medical University).

Thirty-six nude mice were randomized into 3 groups for CNE-2, CNE-2 GnT-V /NC and CNE-2 GnT-V/2224. The cells (5×10^6) were injected subcutaneously into the left thigh of each mice to establish the tumor model. The tumor volume $V = \text{width}^2 \times \text{length}/2$. Once the tumors had reached a volume of about 300 mm^3, each group was randomly placed into the nonradiation (6 nude mice) and radiation group (6 nude mice). Tumors formed in treated group were irradiated 2 Gy once every other day with total of 10 Gy using a linear accelerator (Clinac 2300C/D; Varian, United States), while the rest of the body was shielded with lead brick. Measurements of tumor width and length were made with calipers every three days. The normalized tumor sizes = the tumor volume at day *x*/the tumor volume at day 0 in the same group. Ten days later (two day after the last radiotherapy), the mice from each group were sacrificed. Transplanted tumors were confirmed by H&E (hematoxylin and eosin stain) and the Bcl-2, Bcl-xl and Bax expression was detected by both Western blot assay and immunohistochemistry staining.

2.6. Western blot analysis

Cell proteins were extracted with RIPA (radioimmunoprecipitation assay) lysis buffer and determined by the standard BCA method (BCA™ Protein Assay Kit, Pierce, USA). Each protein sample (20 ug/well) was homogenized in the loading buffer and boiled for 5 min, then separated on 8% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Etten-leur, Netherlands) using a semi-dry transfer apparatus. The membranes were blocked with 5% non-fat dried milk for 2 h, then treated with primary antibodies (1:200- diluted antibody of Bcl-2, 1:300- diluted antibody of Bcl-xl and 1:200- diluted antibody of Bax, all from Santa Cruz Biotechnology, Inc., California, USA and 1:1000 diluted antibody of β-actin, from Abmart, Inc., China) for 12 h at 4 °C. The membranes were washed again with TBST (10 mM Tris, pH 8.0, 150 mM NaCl, and 0.1% Tween 20), followed by incubation with horseradish peroxidase-labeled secondary antibody (Beijing Biosynthesis Biotechnology Co., Ltd.) for 45 min at room temperature. Finally, after being developed with super echochemiluminescence (ECL) plus detection reagents, the protein bands of membranes were visualized by exposure to X-ray film. Protein bands were quantified by Quantity One software.

2.7. Immunohistochemistry staining

The tumor tissue was immediately fixed in formalin and embedded in paraffin. Sections were de-paraffinized by heating to 60 °C followed by xylene immersion and re-hydrated with sequential ethanol submersion. The next procedure was carried out according to the instructions for immunohistochemistry staining by streptavidin peroxidase kit (Zymed, USA). Negative controls were performed by incubation of the tissue sections with non-immune serum. Positive controls were employed according to the manufacturer's recommendations. Protein expression levels were classified semiquantitatively based on the total scores of the percent positivity of stained tumor cells and the staining intensity [17]. Namely, the percent positivity was scored as 0 if <5% (negative), 1 if 5–30% (sporadic), 2 if 30–70% (focal) and 3 if >70% (diffuse) of cells stained, whereas staining intensity was scored relative to the known positive and negative controls as 0 if no staining, 1 if weakly to moderately stained and 2 if strongly stained. The score was assessed by two reviewers independently and blindly.

2.8. Statistical analysis

Data are presented using means ± SD. Comparison of means between two samples was performed using Student's t test. Statistical comparisons of more than two groups were performed using one-way analysis of variance (ANOVA), and then least-significant difference (LSD) for multiple comparisons. Statistical significance was defined as $P < 0.05$. All the analyses were performed using SPSS13.0 software.

3. Results

3.1. Down-regulation of GnT-V enhances cell radiosensitivity in vitro

To investigate the role of GnT-V on radiosensitivity in CNE-2 cells, CCK-8 assay, flow-cytometry and caspases-3 activity analysis were used.

Cell survival was studied by CCK-8 assay in CNE-2, CNE-2 GnT-V/NC and CNE-2 GnT-V/2224. Cells were irradiated with 5 single doses (2, 4, 6, 8 and 10 Gy, respectively). Compared with the con-

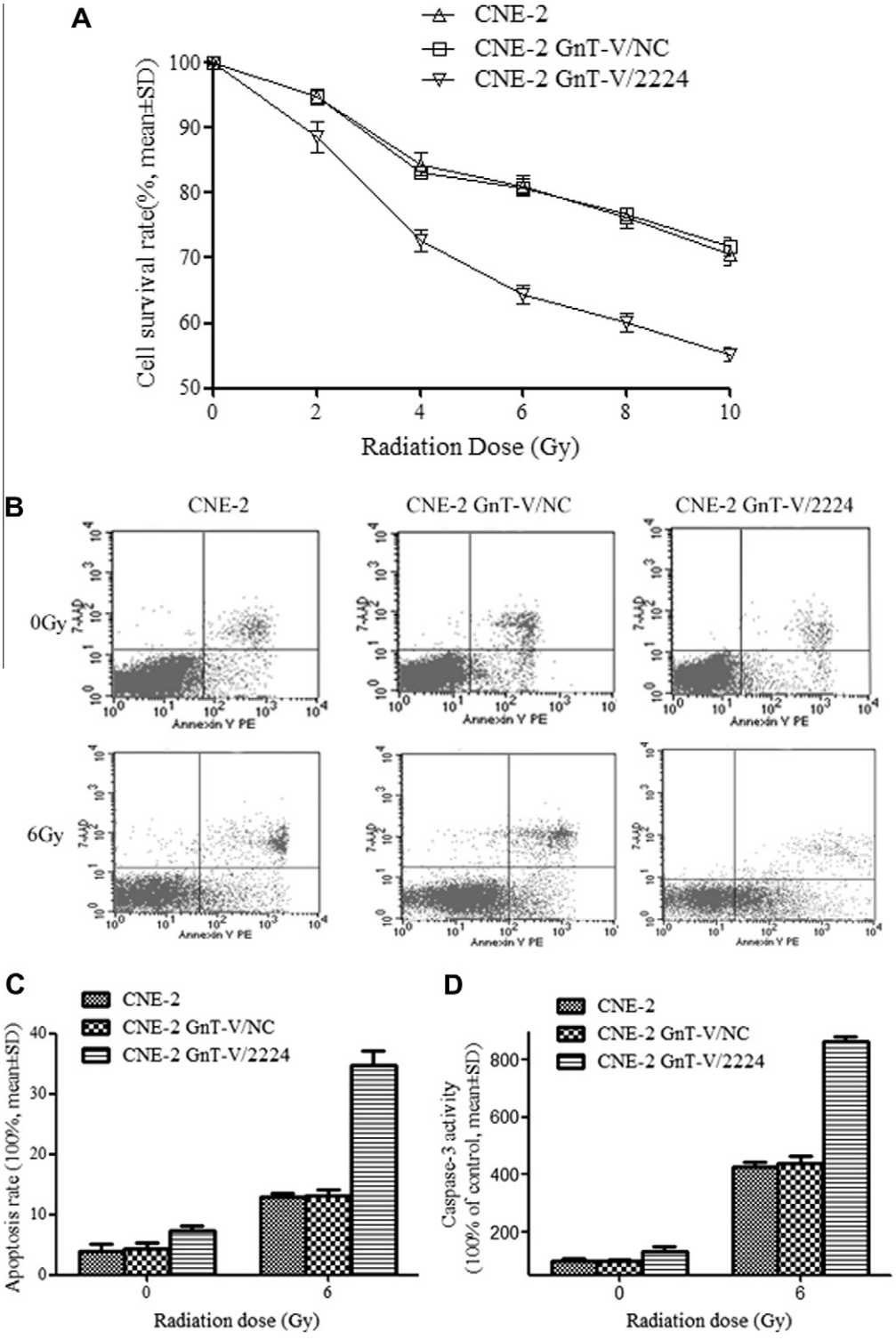

Fig. 1. (A): CCK-8 assay: cells were irradiated with 5 single radiation doses (2, 4, 6, 8 and 10 Gy, respectively) and incubated for 24 h. Then the CCK-8 solution was added to the cells and the survival rate was calculated. Cell survival rate = (A value in the radiation group/A value in the nonradiation group) × 100%. Compared with the control cells, the survival rates of CNE-2 GnT-V/2224 cell survival rates decreased significantly at any radiation dose ($P < 0.01$). Data are presented using mean ± SD. (B and C): After treated with 0 Gy, 6 Gy radiations, cells apoptosis rates in CNE-2, and CNE-2 GnT-V/2224 were detected by flow cytometer. (D) The caspases-3 activity was also analyzed. The data from flow-cytometry and caspases-3 activity analysis showed that radiation could induce cell apoptosis, the decreased extent of apoptosis rate in CNE-2 GnT-V/2224 was greater than the control groups. Data are presented using mean ± SD.

trol cells, the survival rates of CNE-2 GnT-V/2224 cell survival rates decreased significantly at any radiation dose ($P < 0.01$) (Fig. 1A).

The cell apoptosis rates were all increased in radiation groups (6 Gy) compared with the corresponding nonradiation ($P < 0.01$), what's more, the increased extent in CNE-2 GnT-V/2224 was more significant compared with CNE-2 or CNE-2 GnT-V/NC ($P < 0.01$) (Fig. 1B and C).

The caspases-3 activity in CNE-2 GnT-V/2224, CNE-2 and CNE-2 GnT-V/NC was (863.17 ± 20.25)%, (426.50 ± 18.94)% and (438.67 ± 24.87%) in radiation groups (6 Gy) compared with the

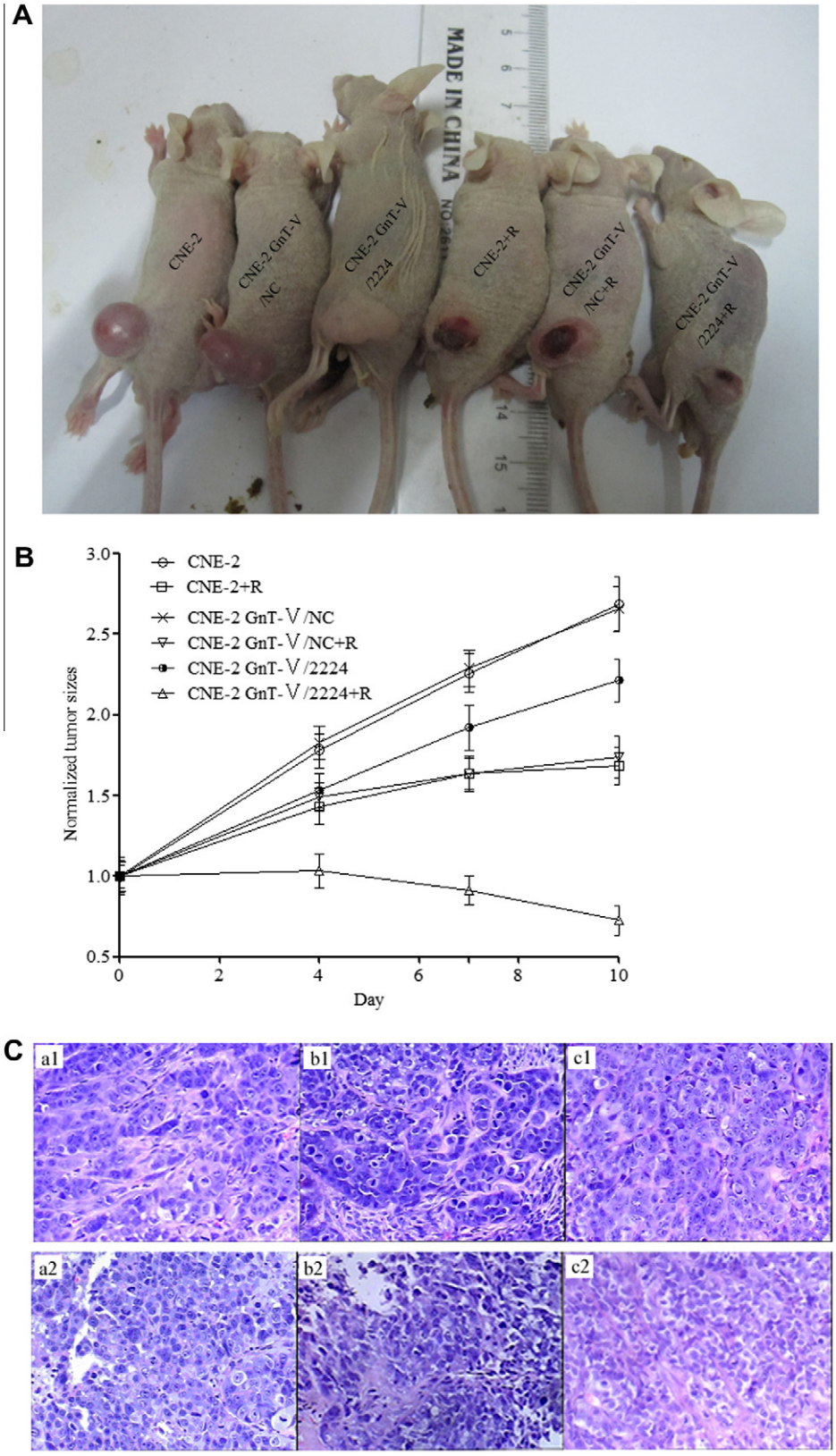

Fig. 2. (A and B) Cells were transplanted into nude mice. Radiation (2 Gy) was delivered to tumors once every other day with total of 10 Gy using a linear accelerator in radiation group when the tumor volumes reached to 300 mm^3. The tumor volumes were measured every three days with calipers. The normalized tumor sizes = the tumor volume at day *x*/the tumor volume at day 0 in the same group. Compared with corresponding nonradiation group, the normalized tumor sizes in three cell lines were all smaller than that in radiation group ($P < 0.01$), however, the decreased extent in CNE-2 GnT-V/2224 was more significant than that in CNE-2 and CNE-2 GnT-V/NC groups ($P < 0.01$) (C) Tumor tissue in each groups were confirmed using hematoxylin and eosin stain (H&E); magnification × 400. CNE-2 group (a1): nonradiation, (a2): radiation; CNE-2 GnT-V/NC group (b1): nonradiation, (b2): radiation; CNE-2 GnT-V/2224 group, (c1): nonradiation, (c2): radiation.

Table 1
The tumor sizes of three cell lines in nude mice before and after radiation ($\overline{X}$±SD, $n = 6$).

Cell lines	Day 0	Day 4	Day 7	Day 10
CNE-2	317.5 ± 28.6(1 ± 0.09)[#]	565.9 ± 33.8 (1.78 ± 0.11)[#]	718.1 ± 37.7 (2.26 ± 0.12)[#]	854.1 ± 52.7 (2.69 ± 0.17)[#]
CNE-2 + R[*]	315.9 ± 32.3(1 ± 0.10)[#]	452.9 ± 35.7 (1.43 ± 0.11)[#]	516.4 ± 33.4 (1.63 ± 0.11)[#]	532.4 ± 38.0 (1.69 ± 0.12)[#]
CNE-2 GnT-V/NC	312.7 ± 27.6(1 ± 0.09)[#]	571.2 ± 31.8 (1.83 ± 0.10)[#]	715.9 ± 35.0 (2.29 ± 0.11)[#]	830.7 ± 44.7 (2.65 ± 0.14)[#]
CNE-2 GnT-V/NC + R[*]	308.2 ± 21.8(1 ± 0.07)[#]	461.8 ± 25.8 (1.50 ± 0.08)[#]	504.4 ± 29.4 (1.63 ± 0.10)[#]	535.8 ± 39.6 (1.73 ± 0.13)[#]
CNE-2 GnT-V/2224	300.9 ± 28.6(1 ± 0.10)[#]	461.0 ± 31.0 (1.53 ± 0.10)[#]	577.5 ± 42.5 (1.92 ± 0.14)[#]	666.3 ± 39.1 (2.21 ± 0.13)[#]
CNE-2 GnT-V/2224 + R[*]	293.3 ± 33.6 (1 ± 0.11)[#]	304.0 ± 31.0 (1.04 ± 0.11)[#]	268.5 ± 25.6 (0.92 ± 0.09)[#]	213.3 ± 27.3 (0.73 ± 0.09)[#]

[*] CNE-2, CNE-2 GnT-V/NC and CNE-2 GnT-V/2224 cells were transplanted into BALB/C nude mice. Radiotherapy was performed with a linear accelerator as described in the materials and methods, and tumor sizes were measured.
[#] The normalized tumor sizes = the tumor volume at *x* day / the tumor volume at 0 day in the same group.

corresponding nonradiation ($P < 0.01$), Apparently, the caspases-3 activity in the three cell lines were all increased in radiation group compared with the corresponding nonradiation, the increased extent in CNE-2 GnT-V/2224 was more significant compared with CNE-2 or CNE-2 GnT-V/NC groups. (Fig. 1D)

3.2. Down-regulation of GnT-V enhances cell radiosensitivity in vivo

To substantiate the *in vitro* results, the *in vivo* studies had been done by tumorigenicity model. The tumor tissues in each group were confirmed using hematoxylin and eosin stain (Fig. 2C).

In nonradiation group, the normalized tumor sizes in CNE-2 GnT-V/2224 were smaller than CNE-2 or CNE-2 GnT-V/NC ($P < 0.01$). Compared with corresponding nonradiation group, the normalized tumor sizes in CNE-2, CNE-2 GnT-V/NC and CNE-2 GnT-V/2224 were all smaller than that in radiation group ($P < 0.01$), however, the decreased extent in CNE-2 GnT-V/2224 was more significant than that in CNE-2 and CNE-2 GnT-V/NC groups ($P < 0.01$) (Fig. 2A, B and Table 1). Thus, the results suggested that down-regulation of GnT-V can inhibit CNE-2 tumor growth and enhance CNE-2 tumor radiosensitivity *in vivo*.

3.3. Inhibition of GnT-V enhanced radiation-induced G2-M phase arrest

The cell cycle analysis was performed using flow cytometry to verify the action of GnT-V on the radiation sensitivity in the CNE-2 cells (Fig. 3A and B). The G2/M phase proportion of the three groups were all enhanced in radiation group compared with corresponding nonradiation, but the increased extent in CNE-2 GnT-V/2224 was more than that in CNE-2 and CNE-2 GnT-V/NC ($P < 0.01$). This result showed that suppression of GnT-V enhanced radiation-induced G2-M phase arrest.

3.4. The change of radiosensitivity induced by GnT-V is associated with Bcl-2, Bax, but not with Bcl-xl

Bcl-2 family proteins are strongly associated with radiosensitivity in many cancers. To further investigate whether the change of radiosensitivity induced by GnT-V was caused by Bcl-2 family, Bcl-2 and its related proteins (Bcl-xl and Bax) were studied.

The Bcl-2, Bcl-xl and Bax in CNE-2 GnT-V/NC and CNE-2 GnT-V/2224 were detected by Western blot with or without radiation (6 Gy) in cytology assay (Fig. 4A) and in transplantation tumor experiment (Fig. 4B). The cellular Bcl-2/Bax ratio was lower in CNE-2 GnT-V/2224 than that in CNE-2, it much more lower in radiation group, but the Bcl-2/Bax ratio had no significant difference in CNE-2 with or without radiation (Table 2).

Bcl-xl expression in CNE-2 GnT-V/2224 was reduced by (41.37 ± 3.51)% in cytology assay (Fig. 4A) and (51.65 ± 4.97)% in transplantation tumor experiment (Fig. 4B) compared with that in CNE-2 GnT-V/NC without radiation ($P < 0.01$), but either in cytology assay or in transplantation tumor experiment, there was no significant difference of Bcl-xl expression either in CNE-2 GnT-V/2224 or in CNE-2 GnT-V/NC with or without radiation ($P > 0.05$).

Accordingly, immunohistochemistry staining show that lower Bcl-2 expression was found in CNE-2 GnT-V/2224 (median, score 4) compared with CNE-2 GnT-V/NC (median, score 5), while Bax expression in CNE-2 GnT-V/2224 (median, score 3) was higher than that in CNE-2 GnT-V/NC (median, score 2) without radiation ($P < 0.01$). What's more, the Bcl-2 expression was further decreased (median, score 2) and the Bax expression was further increased (median, score 5) in CNE-2 GnT-V/2224 with radiation ($P < 0.01$). But both the expression of Bcl-2 (median, score 5) and Bax (median, score 2) in CNE-2 GnT-V/NC had no significant difference with or without radiation ($P > 0.05$).

Though the Bcl-xl expression in CNE-2 GnT-V/2224 (median, score: 4) was less than that in CNE-2 GnT-V/NC (median, score 5) without radiation ($P < 0.01$), there was no significant difference of Bcl-xl expression either in CNE-2 GnT-V/2224 or in CNE-2 GnT-V/NC with or without radiation ($P > 0.05$).

The results showed that down-regulation of GnT-V promotes CNE-2 sensitive to radiation through the suppression of Bcl-2, the enhancement of Bax and reduction of Bcl-2/Bax ratio, but no association with Bcl-xl.

4. Discussion

It has been reported that GnT-V is associated with the malignant transformation of tumor [11–18]. In human hepatocarcinoma 7721 cells, blocking of GnT-V induces cellular endoplasmic reticulum stress [13]. In breast carcinoma cell, attenuation of GnT-V resulted in the attenuation of invasiveness-related phenotypes by inhibition of epidermal growth factor-induced dephosphorylation of focal adhesion kinase [18]. However, the previous studies were scarcely associated with the role of GnT-V on radiosensitivity. In this study, the role of GnT-V on radiosensitivity in NPC was investigated both *in vitro* and *in vivo*. The CNE-2 GnT-V/2224, CNE-2 GnT-V/NC and CNE-2 cells were exposed to radiation. It was found that attenuation of GnT-V expression inhibited cell survival and enhanced cell apoptosis, resulting in elevated CNE-2 radiosensitivity *in vitro*. Moreover, *in vivo* studies verified that CNE-2 GnT-V/2224 tumors grew more slowly than CNE-2 or CNE-2 GnT-V/NC after radiation treatment. These results clarified that targeted suppression of GnT-V enhanced CNE-2 cell radiosensitivity both *in vitro* and *in vivo*.

Mitotic catastrophe causes a delayed mitosis-linked cell death and finally leads to apoptosis or necrosis [19,20]. Radiation-induced mitotic catastrophe was charactered by increased G2-M phase [21,22]. Consistent with this statement, the present study revealed that down-regulation of GnT-V increased G2-M arrest in response to radiation. Thus, targeted suppression of GnT-V may en-

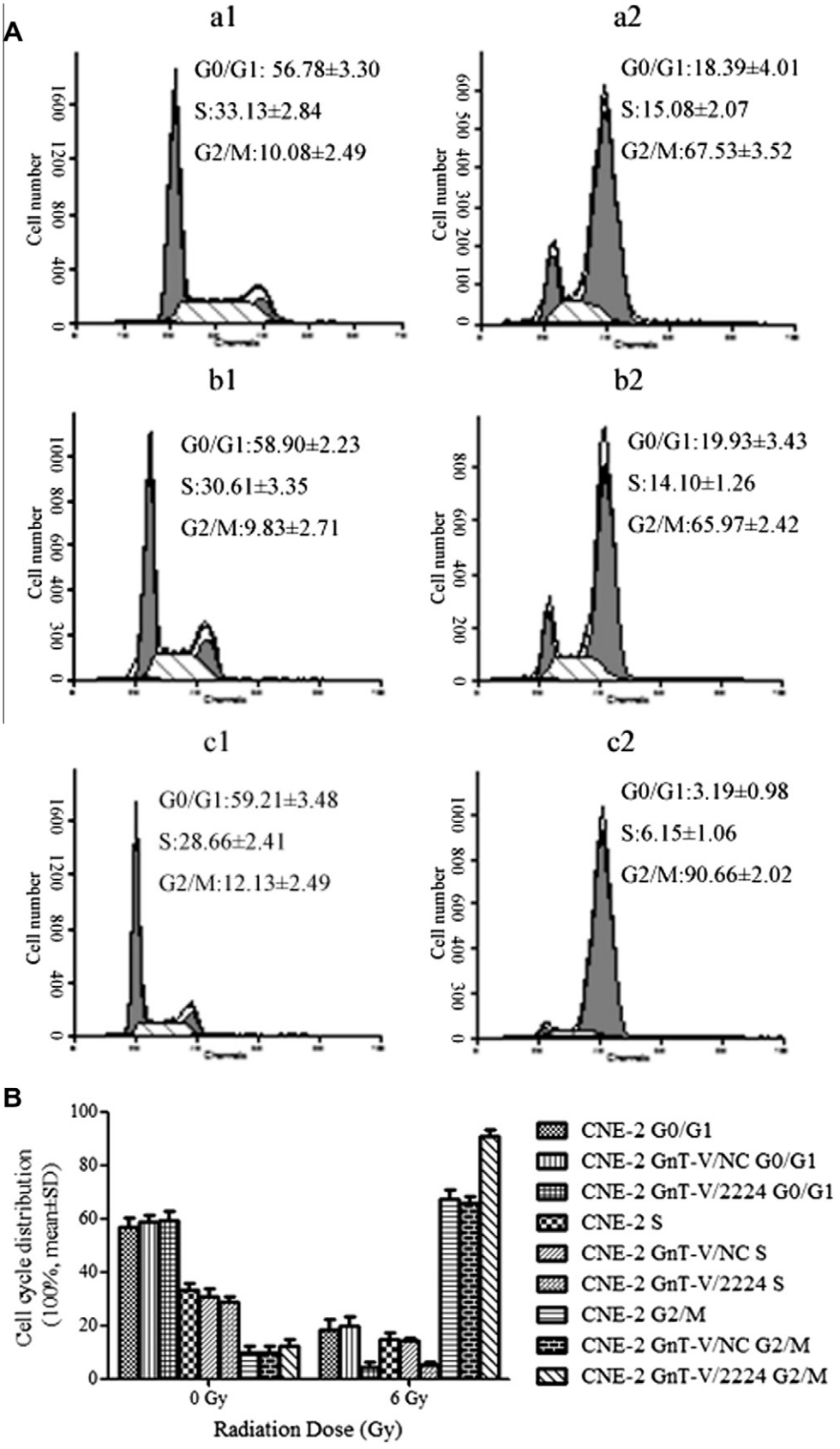

Fig. 3. (A and B) After treated with 0, 6 Gy radiations, cell cycle distribution was determined by FCM analysis. The G2/M phase proportion of the three groups were all enhanced compared with corresponding nonradiation, but the increased extent in CNE-2 GnT-V/2224 was more than that in CNE-2 and CNE-2 GnT-V/NC ($P < 0.01$) (a1): CNE-2, 0 Gy; (a2): CNE-2, 6 Gy; (b1): CNE-2 GnT-V/NC, 0 Gy; (b2): CNE-2 GnT-V/NC, 6 Gy; (c1): CNE-2 GnT-V/2224, 0 Gy; (c2): CNE-2 GnT-V/2224, 6 Gy. Data are presented by mean ± SD.

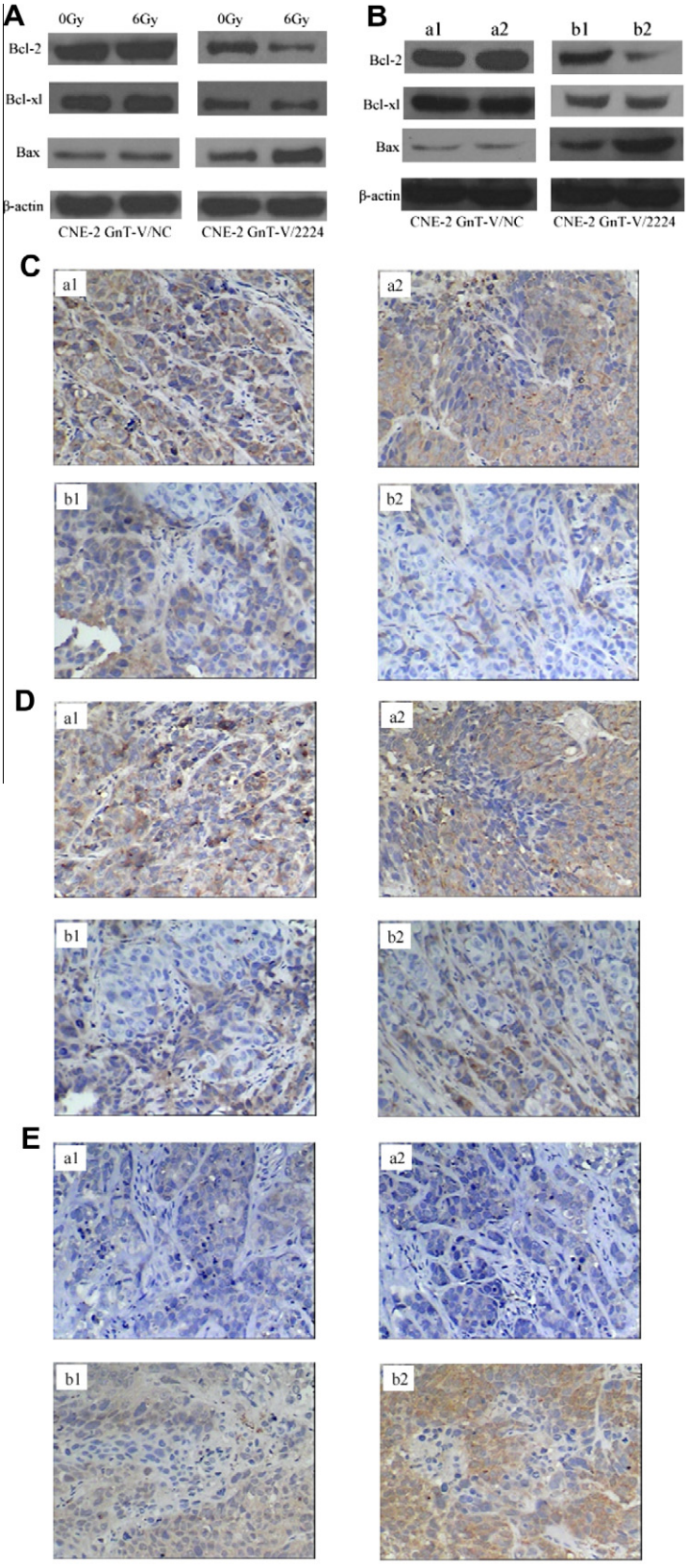

Fig. 4. The Bcl-2, Bcl-xl and Bax in CNE-2 GnT-V/NC cell and CNE-2 GnT-V/2224 cell were detected by Western blot with and without radiation (6 Gy) in cells (A) and tumor xenografts (B). The expression of Bcl-2 (C), Bcl-xl (D) and Bax (E) in xenograft tumors was studied by immunohistochemistry staining. Magnification × 400. CNE-2 GnT-V/NC group, (a1): nonradiation, (a2): radiation; CNE-2 GnT-V/2224 group, (b1): nonradiation, (b2): radiation.

Table 2
The Bcl-2/Bax ratio in the cell lines before and after radiation ($(\overline{X} \pm SD)$).

Cell lines	In cytology assay			In transplantation tumor experiment		
	Before radiation	After radiation	*P*	Before radiation	After radiation	*P*
CNE-2 GnT-V/NC	2.29 ± 0.27	2.10 ± 0.16	0.34	2.94 ± 0.30	2.66 ± 0.20	0.25
CNE-2 GnT-V/2224	1.21 ± 0.08[a]	0.36 ± 0.05	0.00	1.22 ± 0.09[b]	0.27 ± 0.05	0.00

[a] vs Bcl-2/Bax ratio of CNE-2 GnT-V/NC before radiation in *vitro* ($P < 0.01$).
[b] vs Bcl-2/Bax ratio of CNE-2 GnT-V/NC before radiation in *vivo* ($P < 0.01$).

hance radiosensitivity in NPC by aggravating radiation-induced mitotic catastrophe.

GnT-V has been found to have both anti-apoptotic and pro-apoptotic building upon the cell contexts. Repression of GnT-V facilitates all-trans retinoic acid to induce apoptosis of human hepatocarcinoma cells by enhancing ER stress-mediated apoptosis [14,23]. However, human glioma cell line U-373 cell, which are typically highly resistant to cell death induced by chemotherapeutic agents, become more sensitive to apoptosis after up-regulation of GnT-V [24]. Obviously, the role of GnT-V on apoptosis in various tumors was different. This study showed that CNE-2 GnT-V/2224 apoptosis rate was increased more greatly than control groups after radiation. Thus, down-regulation of GnT-V enhanced radiation-induced apoptosis in NPC.

Radiation-induced apoptosis is considered to be one of the main mechanisms in tumor radiosensitivity [25–27]. Bcl-2 family, which comprise of both anti-apoptotic (Bcl-2, Bcl-xl, Mcl-1, etc.) and pro-apoptotic members (Bax, Bak, etc.), was the main controller and mediator of cell apoptosis.[28,29] Particularly, the high Bcl-2/Bax ratio is considered as a crucial factor of cell resistance to apoptosis [30,31]. To investigate the role of GnT-V in radiation-induced apoptosis pathway in NPC, Bcl-2 family proteins were analyzed in our study. The results indicated that Bcl-2 was decreased, Bax was increased and the Bcl-2/Bax ratio was lower in CNE-2 GnT-V/2224 with radiation compared with that in CNE-2 GnT-V/2224 without radiation. And there was no statistically significant difference in CNE-2 GnT-V/NC with or without radiation. Bcl-xl expression had no statistically significant difference either in CNE-2 GnT-V/2224 or in CNE-2 GnT-V/NC with or without radiation. Thus, suppression of GnT-V promotes the radiation-induced apoptosis through the Bcl-2 and Bax, but not the Bcl-xl, eventually leading to the enhanced radiosensitivity.

In summary, down-regulation of GnT-V in NPC enhances radiosensitivity *in vitro* and *in vivo*. The underlying mechanisms may be link to the cell cycle G2-M arrest and the reduction of Bcl-2/Bax ratio, eventually leading to the enhanced radiosensitivity. These findings suggest that GnT-V may be a potential target for predicting NPC response to radiotherapy.

Acknowledgments

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