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Inhibition of N-acetylglucosaminyltransferase V enhances sensitivity of radiotherapy in human prostate cancer

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ABSTRACT

The purpose of this study was to investigate the relationship between N-acetylglucosaminyltransferase V (GnT-V) and radiation sensitivity of prostate cancer (PCa) cells both in vitro and in vivo. Firstly, the GnT-V expression was studied in 84 cases of PCa tissues, in which higher level of GnT-V was detected more frequently in the advanced tumors. Secondly, the GnT-V stably suppressed cell lines PCa/1079 (Lncap/1079 and PC3/1079) were constructed from PCa cell lines (Lncap and PC3) in vitro. Attenuation of GnT-V inhibited cell proliferation, migration and increased apoptosis, which resulted in enhanced radiation sensitivity of PCa cells. The underlying mechanism may be relevant to the increasing ratio of Bax/Bcl-2, the blocking transcription of NF-κB and the reduction of cell cycle G2-M arrest. Finally, in in vivo study, compared with control groups, the irradiated PCa xenograft nude mice of PCa/1079 indicated to reduce tumor-growth rate and enhance survival time. Summary, our studies showed that inhibition of GnT-V probably improved PCa cells' radiation sensitivity.

1. Introduction

Prostate cancer (PCa) remains one of the most common cancer in males [1]. Although radiotherapy is widely used to treat PCa [2], poor rehabilitation and prognosis are often unavoidable. As the aggressiveness of PCa could be partly associate with its intrinsic factors and extrinsic resistance [3], patients with high-risk characteristics (serum PSA > 20 ng/ml, clinical > T2 and Gleason score > 7) would have more than 50% chance of biochemical and clinical relapse after radiotherapy [4]. Effectiveness of radiotherapy in higher radiation dosage (>70 Gy) could be improved, but toxicity to neighboring normal tissue was also increased [5,6]. Although biomarkers for radiation sensitivity such as Raf kinase inhibitory protein, PAK6 and DAB2IP gene had been studied [7–9], a reliable biomarker has not been identified. Thus, novel biomarkers are needed to detect radiation sensitivity of PCa.

The β1,6-branched oligosaccharides, expressing on the surface of glycoprotein, play crucial roles in carcinogenesis and participate in the regulation of the tumor biological characteristics [10]. N-acetylglucosaminyltransferase V (GnT-V), located in the Golgi apparatus, is a key enzyme for the formation of above oligosaccharides, and reported to be over-expressed in many malignant tumors, such as breast cancer and colon cancer [11,12]. A variety of studies have discovered that GnT-V is closely related to the cancer proliferation, invasion and metastasis [13]. These processes are mainly performed through the oligosaccharides modulation of the growth factor receptors [14] and cell surface receptors such as integrin [15], cadherins [16] of tumor cells. Our previous research have proved that down-regulation of GnT-V could induce apoptosis and enhance radiation sensitivity in nasopharyngeal carcinoma [17]. But the function of GnT-V in radiation sensitivity of PCa cells remains unknown.

In order to clarify the role of GnT-V in radiation sensitivity of human prostate cancer, firstly the Tissue Microarrays (TMAs) were used to explore the relationship between GnT-V and clinical pathological features in 84 cases of PCa tissues and 5 cases of prostatic hyperplasia. Secondly, GnT-V was stably inhibited in cell lines (Lncap and PC3) by a shRNA strategy and the effects of GnT-V down regulation on these cells were assessed both in vitro and in vivo. Furthermore, the possible mechanisms were also investigated.

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2. Materials and methods

2.1. Human tissue microarrays (TMAs)

TMAs were obtained from Cybrdi, Shanxi ChaoYing Biotechnology Co. Ltd. The TMAs consisted of 5 prostatic hyperplasia cases, 84 PCa cases with information of Gleason score and T stage. The expression of GnT-V in TMAs was detected by Immunohistochemistry staining and scored as previously described [18]. The scoring procedure was taken by two independent observers without any knowledge of the clinical data.

2.2. Cell culture and transfection

Lncap and PC3 cell lines were provided by the cell bank of Sun Yat-Sen University. The cells were cultured in RPMI-1640 containing 10% new bovine serum, and 1% penicillin/streptomycin at 37 °C, with 5% CO_2. The pGPU6/GFP/Neo vectors (GnT-V/NC, GnT-V/1079 and GnT-V/1564) which obtained from Shanghai GenePharma Co. Ltd. were constructed as previously described [19]. The constructed plasmids were transfected into Lncap and PC3 cells by Lipofectamine 2000™ (Invitrogen, USA). The stable transfectants were selected in RPMI-1640 containing G418, an analog of neomycin. They were named as Lncap/NC, Lncap/1079, Lncap/1564, PC3/NC, PC3/1079 and PC3/1564 respectively.

2.3. Quantitative real-time polymerase chain reaction (RT-PCR) data analysis

Total RNA was harvested from PCa cells and tumor tissues using Trizol (Invitrogen, USA). Level of GnT-V mRNA was detected by qRT-PCR as previously described [17]. The relative mRNA expression level of GnT-V was calculated by using comparative expression level $2-\Delta\Delta Ct$ method.

2.4. Western blot analysis

Cells were harvested and lysed with cold Radio Immunoprecipitation Assay (RIPA) lysis buffer. Protein concentration of the supernatant was determined by the Bicinchoninic Acid (BCA) protein assay procedure (Thermo Scientific). Immunoblotting was performed using the primary antibodies against GnT-V, GAPDH, Bax, Bcl-2 and Bcl-xl (Santa Cruz Biotechnology). Horse-radish peroxidase conjugated anti-IgG was used as the secondary antibody (Beijing Biotechnology Co. Ltd.). The membrane was stained with Enhanced Chemiluminescence (ECL) reagent. Protein bands were quantified by Quantity One.

2.5. Cell proliferation and migration assays

Cell colony forming ability was performed as previously described [17]. Cell proliferation (cell counting kit-8 assay) and migration (cell scratch-wound assay) were also tested as previously described [17], in this experiment, cells were radiated with a single dose of 6 Gy before the indicated time (0 h).

2.6. Cell apoptosis assays

Cells were seeded in 6-well plates and radiated with doses of 0 Gy and 6 Gy. Seventy-two hours after radiation, the morphological alterations of apoptotic cells were observed by fluorescence microscopy using Hoechst 33258 staining. In addition, floating and attached cells were harvested and washed with PBS, and resuspended in binding buffer containing 7-AAD for 10 min, followed by the addition of Annexin V-PE. Cell apoptosis analysis was carried out by using a flow cytometer (BD Biosciences, UK).

Caspase-3 activity was assayed using the caspase-3 colorimetric assay kit (Biovision, USA) according to the manufacturer's instructions. Cells were radiated with single dose of 6 Gy and harvested at 24 h, 48 h and 72 h post-radiation respectively. Then the protein was extracted and diluted with cell lysis buffer. The reaction buffer (80 μl) was added to each sample. The 10 μl DEVD-pNA (2 mmol/L) substrate was added to the sample and incubated at 37 °C for 2 h. Samples were read at 405 nm in a microplate reader.

2.7. Nuclear factor κB (NF-κB) luciferase assay

Cells were seeded in 24-well plates, co-transfected with pNF-κB-Luciferase plasmid and pRL-CMV plasmid (both from Peter MacCallum Cancer Centre, Australia) using lipofectin (Invitrogen) then radiated with doses of 0 Gy and 6 Gy. Six hours after radiation, Luciferase and Renilla signals were measured using the Dual Luciferase Reporter Assay kit (Promega, USA) according to manufacturer's instructions. Transfection efficiency was normalized to the control luciferase.

2.8. Cell cycle analysis

Cells were grown in 6-well plates and radiated with four single radiation doses separately (0, 2, 6 and 10 Gy). Floating and attached cells were harvested at 24 h post-radiation, and then cells were fixed in 70% ethanol. After being washed with PBS, the cells were treated with PBS containing RNase. Next, 50 μg/ml PI was added for 10 min at 37 °C followed by flow cytometry analysis of cell cycle (BD Biosciences, UK).

2.9. In vivo tumorigenicity assays

Ninety-six male nude mice (three weeks old, weighing 18–20 g, from the Animal Institute of Southern Medical University, Guangzhou, China) were used in the following assays in vivo. Animal experiments were performed under the regulations of the institutional ethical commission.

Ninety-six nude mice were randomly divided into six groups. PCa cells (5×10^6) in RPMI1640 were subcutaneously inoculated into the legs of nude mice to establish the tumor model respectively. Tumors were measured in two dimensions with calipers and the volumes were estimated using the following calculation: $(\text{minor axis})^2 \times (\text{major axis})/2$. Radiation was delivered to tumors during consecutive 5 days (2 Gy × 5) using a linear accelerator as previously described [17] when the tumor volume reached to 200–300 mm^3. After radiation tumor growth curves were constructed. Twenty-one days later, 6 mice of each group were sacrificed and tumors were excised.

The tumors were homogenized to extract protein for detecting the expression of GnT-V, Bax, Bcl-2 and Bcl-xl by Western blot assay and immunohistochemistry. Besides, GnT-V mRNA expression was detected by qRT-PCR. The other 10 mice of each group were continually raised to record the survival time.

2.10. Statistical analysis

Data were conducted with SPSS13.0 software and were reported as means ± SD. Statistical analysis between the groups were analyzed with a Student's *t*-test, one-way ANOVA or the χ^2-test. $P < 0.05$ was considered as significant difference.

3. Results

3.1. GnT-V expression in prostate cancer tissues

Correlation of GnT-V expression with Gleason score and T grade was detected by using tissue microarrays. The TMAs results showed that GnT-V expression was not found (0/5) in hyperplasia cases, but it was widely expressed (82/84) in prostate cancer samples. Table 1 summarized the relationship between GnT-V expression and clinical characteristics in PCa patients (Fig. 1, Table 1). The results indicated that the expression of GnT-V revealed obviously correlation with the Gleason score and clinical stage ($P < 0.05$). The higher level expression of GnT-V was found more frequently in the advanced tumor with higher Gleason score and later T stage.

3.2. Down-regulation of GnT-V inhibited proliferation and migration of PCa cells

To investigate the function of GnT-V in radiation sensitivity of PCa cells, GnT-V stably down-regulated cells PCa/1079 and PCa/1564 were developed. It was subsequently confirmed that the PCa/1079 and PCa/1564 showed significantly decreased GnT-V expression at mRNA and protein level compared with PCa cells ($n = 3$, $^{**}P < 0.05$). GnT-V mRNA expression in Lncap/1079 and Lncap/1564 cells was decreased about 74.00% and 64.30% compared with that in Lncap cell, meanwhile GnT-V mRNA in PC3/1079 and PC3/1564 cells was decreased about 78.30% and 66.83% as well (Fig. 2A). Furthermore, GnT-V protein in Lncap/1079, Lncap/1564, PC3/1079 and PC3/1564 cells was decreased about 68.70%, 54.70%, 73.67% and 62.66% respectively (Fig. 2B).

In this case, colony formation assay was also performed. Comparing with the control groups, Survival fractions of PCa/1079 and PCa/1564 were notably lower after radiation ($n = 9$, $P < 0.05$) (Fig. 2C). Besides, Inhibition of GnT-V sensitizes PCa cells to radiation resulting in decreased cell proliferation. PCa/1079 and PCa/1564 showed a significant reduction of cell proliferation after radiation compared with the control groups ($n = 18$, $P < 0.05$) (Fig. 2D). We also observed that down-regulation of GnT-V decreased cell migration. The cell movement of PCa/1079 and PCa/1564 were obviously more slowly than that in the control groups after radiation. Healing rates of PCa and PCa/NC were higher than that of PCa/1079 and PCa/1564 at different time point ($n = 9$, $P < 0.05$) (Fig. 2E). Taken together, all these results suggested that inhibition of GnT-V decreased the proliferation and migration of PCa cells.

As it was discovered that pGPU6/GFP/Neo GnT-V/1079 plasmid reduced the GnT-V mRNA and protein expression more efficiently than pGPU6/GFP/Neo GnT-V/1564 plasmid, thus PCa/1079 (Lncap/1079 and PC3/1079) cells were chosen for further assays.

3.3. Inhibition of GnT-V increased radiation-induced apoptosis

Apoptotic morphology was characterized as nuclear condensation and fragmentation by Hoechst 33258 staining (Fig. 3A). It was observed in PCa/1079 cells, while negligible apoptosis in the control groups before radiation, the apoptosis rates in PCa/1079 group were higher than that in the control groups after radiation ($n = 9$, $P < 0.05$) (Fig. 3B). The apoptosis rate in Lncap/NC and Lncap/1079 was about 1.30% and 3.15% before radiation, about 9.66% and 12.38% at 72 h after radiation. Those in PC3/NC and PC3/1079 were about 1.88% and 9.23% before radiation, 8.74% and 20.00% at 72 h after radiation. Though there was only about 1.85% difference in apoptosis rate between Lncap/NC and Lncap/1079 before radiation, the difference was amplified after radiation. It was the same case in PC3/NC and PC3/1079. These data indicated that suppression of GnT-V increased radiation-induced apoptosis in vitro.

Bcl-2 family was the crucial controller and mediator of cell apoptosis. The Bax protein expression in PCa/1079 was markedly higher than that in the control groups whether before or after radiation by Western blot ($n = 3$, $P < 0.05$) (Fig. 3C). The expression of Bax protein was increased about 44.8% and 62.5% in Lncap/1079 and PC3/1079 after radiation compared with that before radiation. The results indicated that inhibition of GnT-V might directly induce the expression of Bax protein and radiation could result in enhancement of the induction. Besides, the Bcl-2 protein expression in PCa/1079 was lower than that in the control groups whether before or after radiation ($n = 3$, $P < 0.05$) (Fig. 3C). It was further decreased about 53.8% and 50.3% in Lncap/1079 and PC3/1079 after radiation compared with that before. These data suggested that radiation could regulate Bcl-2 expression in PCa cell lines and attenuation of GnT-V might inhibit radiation-induced Bcl-2 expression. Neither radiation nor suppression of GnT-V have any effects on Bcl-xl ($n = 3$, $P > 0.05$) (Fig. 3C).

Caspase-3 activity which was associated with apoptosis was further investigated. Caspase-3 activity of PCa/1079 was higher than that in the control groups after radiation ($n = 9$, $P < 0.05$) (Fig. 3D). It was confirmed that down-regulation of GnT-V might increase caspase-3 activity, which was related to PCa radiation sensitivity.

3.4. Suppression of GnT-V decreased radiation-induced NF-κB transcription

NF-κB transcriptional activity in Lncap/1079 was lower than that in the control groups before radiation, and this phenomenon was magnified after radiation. The similar results could be found in PC3/1079 and its control groups ($n = 9$, $P < 0.05$) (Fig. 3E). All these results indicated that inhibition of GnT-V sensitized PCa cells to radiation via blocking NF-κB pathway.

Table 1
Correlation of GnT-V expression with Gleason score and T grade in prostate cancer.

GnT-V	Gleason score			*P*	T grade				*P*
	2–4	5–7	8–10		T1	T2	T3	T4	
No (%) −	2 (14.3)	0 (0.0)	0 (0.0)	<0.05	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	<0.05
Low (%) +	10 (71.4)	8 (21.1)	5 (15.6)		3 (42.9)	17 (36.2)	2 (11.8)	1 (7.7)	
Moderate (%) ++	2 (14.3)	22 (57.9)	10 (31.3)		1 (14.3)	21 (44.7)	10 (58.8)	2 (15.4)	
High (%) +++	0 (0)	8 (21.1)	17 (53.1)		1 (14.3)	9 (19.1)	5 (29.4)	10 (76.9)	
Total	14	38	32		7	47	17	13	

Higher level of GnT-V was detected more frequently in the advanced tumors.

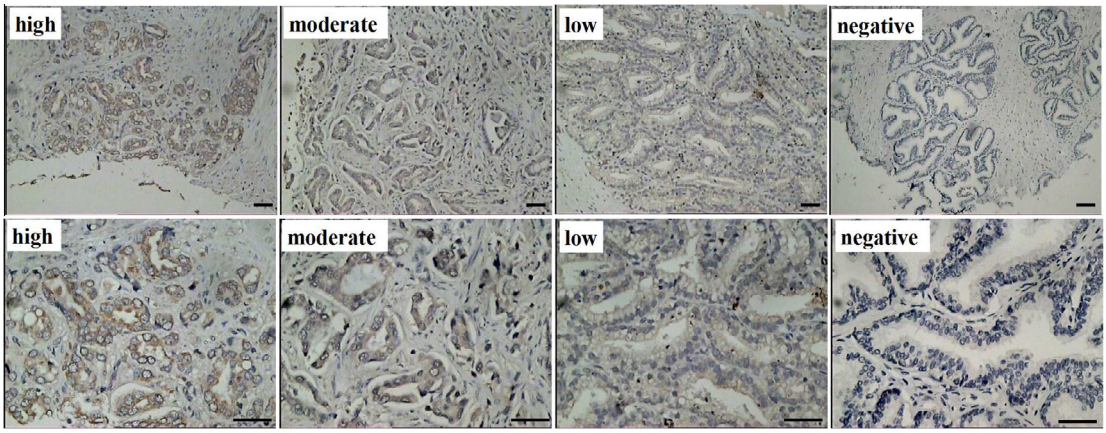

Fig. 1. The GnT-V protein expression in 84 PCa cases and 5 prostatic hyperplasia cases was detected using tissue microarrays technology. The pictures showed cytoplasmic positivity of GnT-V in PCa tissues. Scale bar, 50 μm.

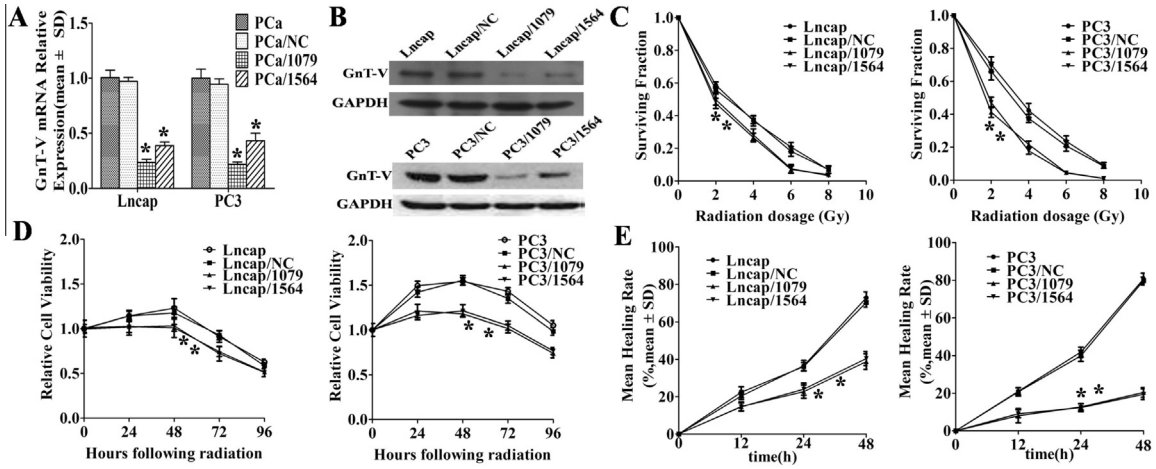

Fig. 2. The GnT-V stably suppressed cell lines PCa/1079 and PCa/1564 were successfully constructed and the proliferation and migration of cells were assessed. (A) The expression of GnT-V mRNA in PCa/1079 and PCa/1564 cells was greatly decreased compared with the control groups by RT-PCR analysis. (B) The expression of GnT-V at the protein level in PCa/1079 and PCa/1564 cells was also decreased compared with the control groups by Western blot. (C) The cell colonies were stained with crystal violet and counted after 14 days. Survival fractions of PCa/1079 and PCa/1564 were notably lower than that in the control groups after radiation. (D) PCa/1079 and PCa/1564 showed a significant reduction of cell viability following 6 Gy radiation compared with the control groups by CCK-8 assay. (E) GnT-V-inhibition groups showed a significant reduction of cell mobility at all three time point compared with the control groups by Scratch assay. Healing rates of the control groups were higher than that of PCa/1079 and PCa/1564 at different time point. Data are presented using mean ± SD, $^{*}P < 0.05$.

3.5. Knockdown of GnT-V decreased radiation-induced G2/M phase arrest

G2/M phase cells in Lncap/NC and Lncap/1079 were about 3.86% and 3.36% before radiation by the dection of flow cytometer and the difference had no statistical significance ($n = 9$, $P > 0.05$). A radiation-dose-dependent G2/M arrest was significant in Lncap/NC after radiation, while this phenomenon was not obvious in Lncap/1079. The results in PC3 cells were similar to Lncap cells ($n = 9$, $P < 0.05$) (Fig. 3F). These finding suggested that down-regulation of GnT-V could result in decreased radiation-induced G2/M phase arrest.

3.6. Suppression of GnT-V enhanced PCa radiation sensitivity in vivo

In PCa xenograft nude mice, the PCa/1079 (Lncap/1079 and PC3/1079) tumors grew significantly slowly compared with the control groups ($n = 6$, $P < 0.05$) (Fig. 4A and B). GnT-V mRNA and protein of tumors from PCa/1079 were decreased compared with the control groups by qRT-PCR, Western blot and immunohistochemisty ($n = 3$, $P < 0.05$) (Fig. 4C–E). The PCa xenograft nude mice from PCa/1079 treated with radiation showed longer survival time than that in the control groups ($n = 10$, $P < 0.05$) (Fig. 4F). Moreover, the increased Bax and decreased Bcl-2 protein expression in PCa/1079 after radiation could be confirmed by Western blot and immunohistochemisty similarly in in vivo studies ($n = 3$, $P < 0.05$) (Fig. 4G and H).

4. Discussion

In our research, the expression of GnT-V was detected positively correlated with Gleason score and T stage in PCa tissues, while negative in prostatic hyperplasia cases. These results demonstrated that GnT-V might be involved in the malignant potential of PCa.

Cell lines of PCa/1079 and PCa/1564 with down-regulation of GnT-V were developed by transferring shRNA into Lncap and PC3 cells separately. In vitro, PCa/1079 cells showed decreased cell proliferation, migration and increased apoptosis compared with the control groups after radiation. Furthermore, the PCa/1079 tumors grew more slowly than that in the control groups after radiation

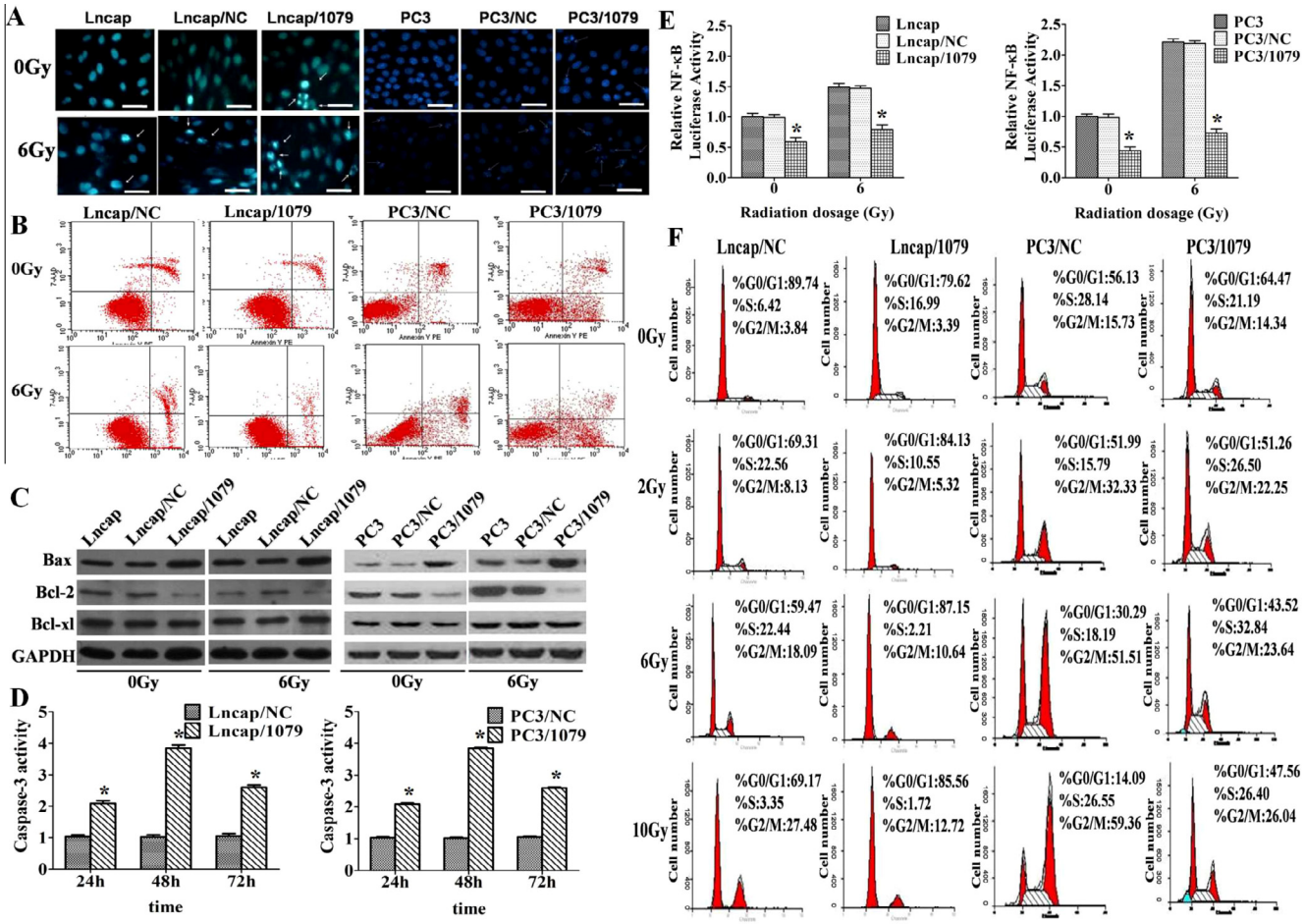

Fig. 3. Suppression of GnT-V expression increased radiation sensitivity of PCa cell lines and the underlying mechanisms were discussed. (A) Apoptotic morphology was observed in PCa, PCa/NC and PCa/1079 before and after radiation. Arrows indicated the appearance of nuclear condensation and fragmentation in cells. Scale bar, 50 μm. (B) Flow cytometer was utilized to analyze the number of apoptotic cells. The apoptosis rates in PCa/1079 groups were higher than that in the control groups. (C) The expression of Bcl-2 family proteins in PCa, PCa/NC and PCa/1079 cells was measured before and after radiation. The increased Bax and decreased Bcl-2 expression in PCa/1079 after radiation could be observed by Western blot. (D) Caspase-3 activity in PCa cell lines was measured using the caspase-3 colorimetric assay kit. The absorbance value of caspase-3 activity in the PCa control groups was defined as 1. Caspase-3 activity of PCa/1079 was higher than that in the control groups after radiation. (E) GnT-V down-regulation inhibited activation of NF-κB transcription induced by radiotherapy. (F) A radiation-dose-dependent G2/M arrest was observed in PCa/NC cells after radiation, while this phenomenon could not be observed in PCa/1079 cells. Data are presented using mean ± SD, $^{*}P < 0.05$.

in vivo. Additionally, the PCa xenograft nude mice of PCa/1079 showed longer survival time than that in the control groups after radiation. These results indicated that targeted suppression of GnT-V increased the PCa cells radiation sensitivity both in vitro and in vivo.

Apoptosis is an important mechanism causing PCa cells to die when cells were subjected to radiotherapy and faulty apoptosis is a known mechanism of resistance to radiotherapy [20]. Bcl-2 family contain pro-apoptotic proteins including Bax and anti-apoptotic proteins such as Bcl-2, Bcl-xl [21]. It was reported that the elevated Bax/Bcl-2 ratio could lead to the increase of caspase-3 activity [22]. As the member of central apoptosis regulators, caspase-3 makes a direct effect on cell apoptosis, which may result in the modulation in radiation sensitivity [23]. Down-regulation of GnT-V could induce apoptosis and enhance radiation sensitivity in nasopharyngeal carcinoma but rendered human neuroblastoma cells more resistant to retinoic acid-induced apoptosis [17,24]. The mechanism for the bidirectional activity of GnT-V in cell apoptosis is still unclear. In our research, the apoptosis rate was increased after down-regulation of GnT-V, and there was further increased in PCa/1079 after radiation. GnT-V down-regulation enhanced Bax and reduced Bcl-2 expression in PCa/1079. Moreover, the further increasing of Bax and decreasing of Bcl-2 after radiation gave rise to elevated Bax/Bcl-2 ratio. In addition, caspase-3 activity in PCa/1079 cells was up-regulated after radiation compared with that in PCa cells. These results indicated that inhibition of GnT-V may increase Bax and decrease Bcl-2 expression, therefore, causing increased caspase-3 activity and enhanced radiation sensitivity.

NF-κB is a super family of transcription factors which plays a prominent role in development, progression and treatment-resistant of cancer [25]. It was reported that the proliferation and invasion of tumors were inhibited by suppression of NF-κB. Thus, suppression of these signaling pathways may promote the effects in the treatment of cancer [26]. It was also known that inhibition of the PI3K/AKT-NF-κB pathway with curcumin enhanced radiation-induced apoptosis in human Burkitt's lymphoma [27]. Besides, NF-κB activity also modulating the Bcl-2 family proteins resulting in affecting cancer cell apoptosis [28,29].Therefore, by comparing with the NF-κB transcriptional activity before and after radiation, our results suggested that down-regulation of GnT-V inhibited the NF-κB pathway, which regulated Bax and Bcl-2 genes, leading to the increase of apoptosis and made the cells become more sensitive to radiotherapy. The radiosensitization effect of down-regulation of GnT-V is due, in part, to the inhibition of the NF-κB pathway.

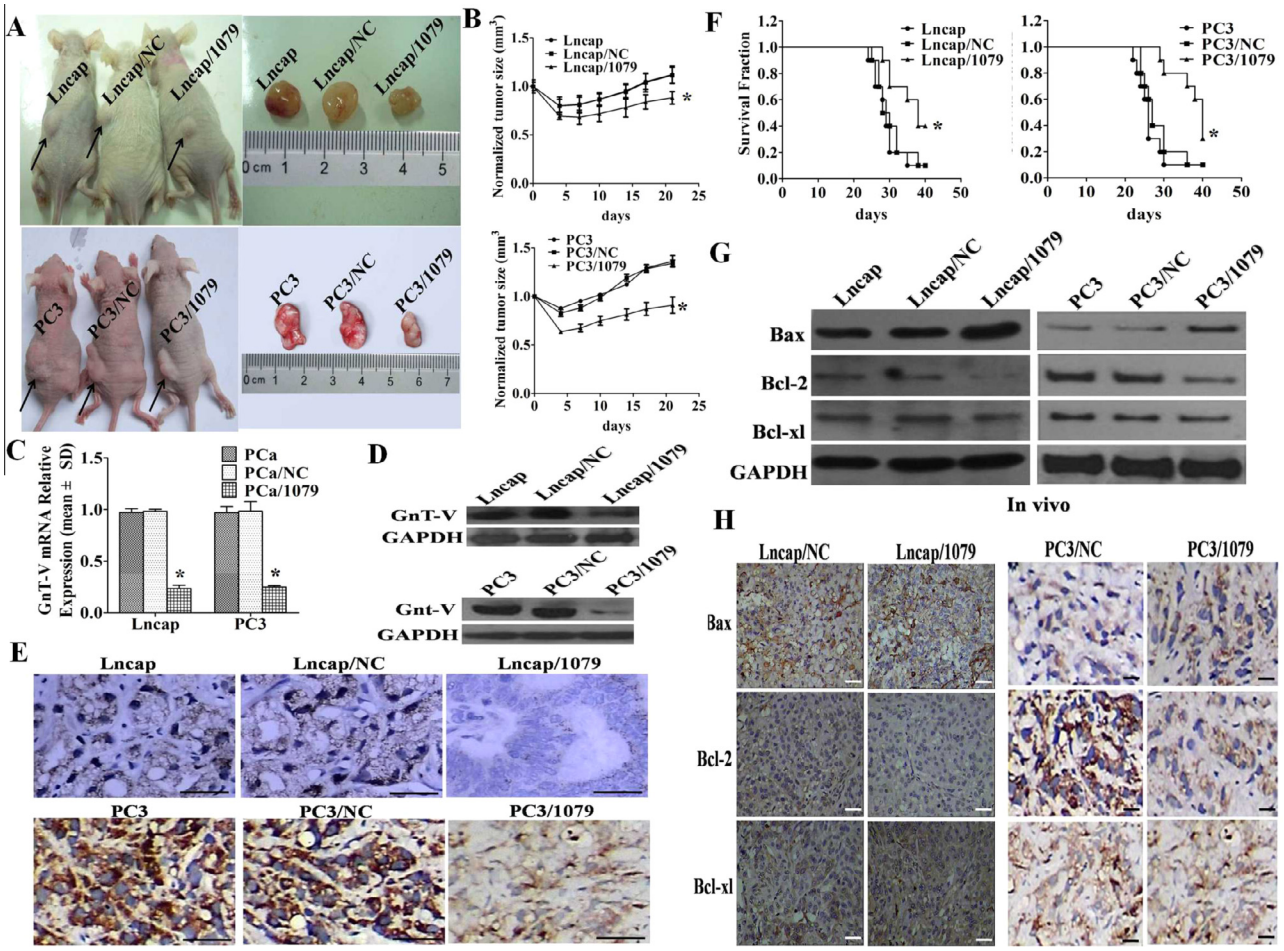

Fig. 4. In vivo, down-regulation of GnT-V decreased tumorigenicity after radiation. (A) The PCa xenograft nude mice were killed and photographed. Arrows indicated the tumors. The PCa/1079 (Lncap/1079 and PC3/1079) tumors grew significantly slowly compared with the control groups. (B) Growth curves of the PCa, PCa/NC and PCa/1079 tumors after radiation were constructed. Expression of GnT-V in tumors of six groups was measured using (C) qRT-PCR, (D) Western blot and (E) immunohistochemistry. Scale bar, 50 μm. (F) Survival rate was recorded and a survival curve was constructed. The PCa xenograft nude mice from PCa/1079 showed longer survival times than that in the control groups. In vivo studies, the Bcl-2 family proteins expression in the PCa, PCa/NC and PCa/1079 cells was detected by (G) Western blot and (H) immunohistochemistry. Scale bar, 50 μm. The results were consistent with that in vitro after radiation. Data are presented using mean ± SD, $^{*}P < 0.05$.

Radiation-induced G2/M arrest in tumor cells may allow cells to repair genetic lesions, which is critical in preventing cells from death [30]. Numerous studies have confirmed that the abrogation of the G2/M block lead to the radiation sensitization in p53-mutated cells [31]. Tumor cells treated with pentoxifylline that inhibited the G2/M arrest, were sensitized to radiation [32]. In our research, a radiation-dose-dependent G2/M arrest after radiation was not found in PCa/1079, however, this phenomenon was observed in PCa/NC. These results showed that down-regulation of GnT-V may decrease radiation-induced G2/M arrest, resulting in enhanced radiation sensitivity.

In conclusion, this study suggests that the GnT-V expression is inversely correlated with radiation sensitivity in PCa cells and the underlying mechanisms may be associated with the increase of Bax/Bcl-2 ratio, NF-κB transcription blockage and the reduction of cell cycle G2-M arrest.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

[1] R. Siegel, J. Ma, Z. Zou, et al., Cancer statistics, 2014[J], CA Cancer J. Clin. 64 (2014) 9–29.
[2] S.A. Mangar, R.A. Huddart, C.C. Parker, et al., Technological advances in radiotherapy for the treatment of localised prostate cancer, Eur. J. Cancer 41 (2005) 908–921.
[3] M.K. Brawer, Radiation therapy failure in prostate cancer patients: risk factors and methods of detection, Rev. Urol. 4 (Suppl. 2) (2002) S2–S11.
[4] A.V. D'Amico, K. Cote, M. Loffredo, et al., Determinants of prostate cancer-specific survival after radiation therapy for patients with clinically localized prostate cancer, J. Clin. Oncol. 20 (2002) 4567–4573.
[5] A. Pollack, G.K. Zagars, L.G. Smith, et al., Preliminary results of a randomized radiotherapy dose-escalation study comparing 70 Gy with 78 Gy for prostate cancer, J. Clin. Oncol. 18 (2000) 3904–3911.
[6] J.A. Talcott, J. Manola, J.A. Clark, et al., Time course and predictors of symptoms after primary prostate cancer therapy, J. Clin. Oncol. 21 (2003) 3979–3986.
[7] K.M. Woods Ignatoski, N.K. Grewal, S.M. Markwart, et al., Loss of Raf kinase inhibitory protein induces radioresistance in prostate cancer, Int. J. Radiat. Oncol. Biol. Phys. 72 (2008) 153–160.
[8] M. Zhang, M. Siedow, G. Saia, et al., Inhibition of p21-activated kinase 6 (PAK6) increases radiosensitivity of prostate cancer cells, Prostate 70 (2010) 807–816.
[9] Z. Kong, D. Xie, T. Boike, et al., Downregulation of human DAB2IP gene expression in prostate cancer cells results in resistance to ionizing radiation, Cancer Res. 70 (2010) 2829–2839.
[10] T. Handerson, J.M. Pawelek, Beta1, 6-branched oligosaccharides and coarse vesicles: a common, pervasive phenotype in melanoma and other human cancers, Cancer Res. 63 (2003) 5363–5369.
[11] H.B. Guo, H. Johnson, M. Randolph, et al., Specific posttranslational modification regulates early events in mammary carcinoma formation, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 21116–21121.

[12] Y.S. Kim, O.L. Son, J.Y. Lee, et al., Lectin precipitation using phytohemagglutinin-L (4) coupled to avidin-agarose for serological biomarker discovery in colorectal cancer, Proteomics 8 (2008) 3229–3235.
[13] A.K. Chakraborty, J.M. Pawelek, GnT-V, macrophage and cancer metastasis: a common link, Clin. Exp. Metastasis 20 (2003) 365–373.
[14] P. Guo, Q.Y. Wang, H.B. Guo, et al., N-acetylglucosaminyltransferase V modifies the signaling pathway of epidermal growth factor receptor, Cell. Mol. Life Sci. 61 (2004) 1795–1804.
[15] J. Gu, T. Isaji, Y. Sato, et al., Importance of N-glycosylation on alpha5beta1 integrin for its biological functions, Biol. Pharm. Bull. 32 (2009) 780–785.
[16] S.S. Pinho, R. Seruca, F. Gartner, et al., Modulation of E-cadherin function and dysfunction by N-glycosylation, Cell. Mol. Life Sci. 68 (2011) 1011–1020.
[17] T. Wei, Y. Li, E. Zhuo, et al., Down-regulation of GnT-V inhibits nasopharyngeal carcinoma cell CNE-2 malignancy in vitro and in vivo, Cancer Lett. (2011) 151–161.
[18] T. Wei, Q. Liu, F. He, et al., The role of N-acetylglucosaminyltransferases V in the malignancy of human hepatocellular carcinoma, Exp. Mol. Pathol. 93 (2012) 8–17.
[19] F.L. He, Q. Ma, J. Zhang, shRNAs aiming at glycosyltransferase inhibit invasive and proliferative ability of LoVo cell line in vitro, Prog. Biochem. Biophys. 36 (2009) 1071–1078.
[20] M.J. Szostak, N. Kyprianou, Radiation-induced apoptosis: predictive and therapeutic significance in radiotherapy of prostate cancer (review), Oncol. Rep. 7 (2000) 699–706.
[21] N.N. Danial, BCL-2 family proteins: critical checkpoints of apoptotic cell death, Clin. Cancer Res. 13 (2007) 7254–7263.
[22] X.M. Yin, Z.N. Oltvai, S.J. Korsmeyer, BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax, Nature 369 (1994) 321–323.
[23] S. Mazumder, D. Plesca, A. Almasan, Caspase-3 activation is a critical determinant of genotoxic stress-induced apoptosis, Methods Mol. Biol. 414 (2008) 13–21.
[24] K. Inamori, J. Gu, M. Ohira, et al., High expression of N-acetylglucosaminyltransferase V in favorable neuroblastomas: involvement of its effect on apoptosis, FEBS Lett. 580 (2006) 627–632.
[25] M. Karin, Nuclear factor-kappaB in cancer development and progression, Nature 441 (2006) 431–436.
[26] B.B. Aggarwal, R.V. Vijayalekshmi, B. Sung, Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe, Clin. Cancer Res. 15 (2009) 425–430.
[27] Q. Qiao, Y. Jiang, G. Li, Inhibition of the PI3K/AKT-NF-κB pathway with curcumin enhanced radiation-induced apoptosis in human Burkitt's lymphoma, J. Pharmacol. Sci. 121 (2013) 247–256.
[28] E. Pikarsky, R.M. Porat, I. Stein, et al., NF-kappaB functions as a tumour promoter in inflammation-associated cancer, Nature 431 (2004) 461–466.
[29] H. Kim, H. Chung, H.J. Kim, et al., Id-1 regulates Bcl-2 and Bax expression through p53 and NF-kappaB in MCF-7 breast cancer cells, Breast Cancer Res. Treat. 112 (2008) 287–296.
[30] J.L. Schwartz, J. Cowan, D.J. Grdina, et al., Attenuation of G2-phase cell cycle checkpoint control is associated with increased frequencies of unrejoined chromosome breaks in human tumor cells, Radiat. Res. 146 (1996) 139–143.
[31] A.M. Strunz, P. Peschke, W. Waldeck, et al., Preferential radiosensitization in p53-mutated human tumour cell lines by pentoxifylline-mediated disruption of the G2/M checkpoint control, Int. J. Radiat. Biol. 78 (2002) 721–732.
[32] T. Theron, A. Binder, F. Verheye-Dua, et al., The role of G2-block abrogation, DNA double-strand break repair and apoptosis in the radiosensitization of melanoma and squamous cell carcinoma cell lines by pentoxifylline, Int. J. Radiat. Biol. 76 (2000) 1197–1208.