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

Huiyi Huang [a,1], Wenxia Chen [a,1], Qiulian Liu [b], Ting Wei [a], Weiliang Zhu [a], Hui Meng [a], Linlang Guo [a], Jian Zhang [a,*]

[a] Department of Oncology, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China
[b] Department of Oncology, First People's Hospital of Jiujiang City, Jiujiang 332000, China

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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.

* Corresponding author. Address: Department of Oncology, Zhujiang Hospital, Southern Medical University, 253 Gongye Road, Guangzhou 510282, China.
E-mail address: 13925091863@139.com (J. Zhang).
[1] These authors contributed equally to this work.

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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.