Research Article

Therapy of Prostate Cancer by Nanoyam Polysaccharide

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We evaluated the effect and mechanism of yam polysaccharide on the proliferation of the prostatic cancer cell line and tumor-bearing mice. The effect of nanoyam polysaccharide on prostatic cancer cell line PC-3 was measured using the scratch adhesion test and flow cytometry. The growth effect induced by nanoyam polysaccharide was detected with the CCK-8 test. The levels of caspase-3 protein were determined with Western blot. In our data, nanoyam polysaccharide presented inhibitory effect on the proliferation of PC-3. The scratch adhesion test showed that the rate of wound healing in the intervention group was significantly lower than that in the control group (p < 0.05). Flow cytometry assay showed that, after treatment with nanoyam polysaccharide, the apoptosis rate in the intervention group was significantly lower than that in the control group (45.8% ± 2.6%, 25.8% ± 3.1%; p < 0.05). Western blot assay showed upregulated levels of caspase-3 in the intervention group, compared to the control group (p < 0.05). Our results suggested that nanoyam polysaccharide strongly suppressed the growth of prostatic cancer by inducing the overexpression of caspase-3 and may be a potent anticancer strategy.

1. Introduction

Prostate cancer, defined as one of the most common malignant tumors in male, ranks the second in global morbidity and the sixth in mortality [1]. Previously, prostate cancer was usually treated with surgery and local radiotherapy. However, due to the invisibility of early prostate cancer, most patients are diagnosed in the middle and late stage and miss the best opportunity for treatment. Testicular resection combined with endocrine therapy was mainly used in patients with advanced cancer. Patients may develop androgen-independent prostate cancer during endocrine therapy [2, 3].

With the continuous in-depth study of Chinese traditional pharmacology, which is often used in the comprehensive treatment of cancer and has achieved good results, Chinese medicine has become an important part of tumor treatment in China. Studies have shown that traditional Chinese medicine played multiple roles in preventing tumorigenesis and antitumor therapy, such as increasing efficacy and reducing toxicity, suppressing tumor recurrence and metastasis [4, 5]. Some studies have found that nanoyam polysaccharides could be used as antitumor active substances [6–8], but little research has been performed on prostate cancer.

Previous studies indicated that the hot water extraction could be used to separate pure Chinese yam polysaccharide...
There exists bioactive ingredients such as β-1,3-glucose, α-1-galactose, and α-1,6-galactose in the purified yam polysaccharide, which could be encapsulated into nanostructure. Nanoparticles are prepared by natural polymers with the size of approximately 100 nm [10]. In this study, the antitumor activity of nanoencapsulated yam polysaccharide was investigated in prostate cancer in vitro and in vivo.

2. Methods and Materials

2.1. Cell Line and Animals. Human prostatic cancer cell line was purchased from Shanghai Cell Institute, Chinese Academy of Sciences. Cells were cultured in medium RPMI1640 (Gibco) containing 10% bovine fetal serum (Thermo Fisher Scientific). Forty healthy male 5-6-week-old BALB/c mice (body weight, 18-22 g) were purchased from the Centre of Experimental Animals at Fudan University. All mice were kept on a 12 h light/dark cycle and given free access to food and water. The mice were divided into four groups randomly: control group (n = 10), low-dose group (n = 10), medium group (n = 10), and high-dose group (n = 10). The animal experiment and all associated procedures were approved by the Animal Ethical and Welfare Committee at Fudan University.

2.2. Reagents. Nanoyam polysaccharides were purchased from Shanxi Ciyuan Biotech Company. Caspase-3 primary antibody (ab13847, 1:1000, Abcam) and GAPDH (#4970, 1:1000, Abcam) were purchased. HRP-conjugated rabbit anti-mouse IgG were from Jackson ImmunoResearch Laboratories Inc. (West Grove, Pennsylvania, USA). FITC-Annexin V/PI kit was purchased from Sigma (APOAF-20TST, USA). Flow cytometry assay was performed in FACS-san440 (BD, USA). CCK-8 kit was purchased from Beyotime.

2.3. Tumor Xenograft Mouse Model. All animal experiment protocols were approved by the Institutional Review Board at the Immune Disease Institute. Breast cancer xenograft model was established in nude mice by the injection of PC-3 cells. Next, the mice received intravenous injection of PBS (as negative control), low-dose nanoyam polysaccharide, medium-dose nanoyam polysaccharide, and high-dose nanoyam polysaccharide every day for 4 weeks. The tumor growth and body weight of the mice were monitored until day 30 when the mice were sacrificed and the tumor tissues were excised for analysis.

2.4. Scratch Adhesion Test. PC-3 cell suspension was prepared at a density of $2 \times 10^5$ cells/mL. 2 mL cell suspension was inoculated in a 6-well plate and cultured for 12 h. After the cells were adhered to the wall, a straight line was drawn along the diameter of the hole with the nozzle of a 200 μL micropipette and the floating cells were washed away by PBS. The cells were divided into two groups. Normal medium was added to the control group, and nanoyam polysaccharide (80 mg/L) was added to the intervention group for 48 h after culture. The scratch was recorded at 0 h, 12 h, 24 h, and 48 h after microscopy. The relative ratio of the cell scratch spacing between the control group and the intervention group was calculated using the IPP software. The experiment was repeated three times, and the average value was calculated. The scratch healing rate = $\frac{0 \text{ h width} - x \text{h width}}{0 \text{ h width}}$.

2.5. CCK-8 Assay. A cell counting kit-8 (CCK-8) analysis kit (Thermo Fisher Scientific) was used to determine the PC-3 viability. The CCK-8 kit was put into each well 0, 12, 24, and 48 h, respectively, based on the manufacturer’s protocol, and we recorded the absorbance at 450 nm.

2.6. Flow Cytometry Assay. Cells were obtained from the cell line, and mice were stained with FITC-labeled Annexin V and PI. After incubation at room temperature for 15 min, samples were washed once with PBS buffer. At least 10,000 cells were assayed by two-color FCM using flow cytometry. Data were analyzed by the Cell Quest software (Becton Dickinson, Mountain View, CA).

2.7. Western Blot. Cells were then harvested and homogenized in ice-cold sodium dodecylsulfate (SDS) lysis buffer. After collection of total cell lysates, equal amount of

![Figure 1: (a, b) The tumor weight and tumor volume of mice in different groups. Data were presented as mean ± SD. *p < 0.05, compared with the control group.](image-url)
protein was separated by 10% SDS-PAGE and blotted onto a PVDF membrane. The PVDF membranes were blocked with Tris-buffered saline (TBS) containing 5% skimmed milk powder for 1 h and then incubated at 4°C overnight with caspase-3 mAb. After that, the membranes were washed with 16Tris-buffered saline/Tween-20 (TBS/T) buffer for three times (5 min each time) and incubated with HRP-conjugated polyclonal secondary antibody for 1 h at room temperature. The membranes were developed with the enhanced plus chemiluminescence assay (Pierce, USA) according to the manufacturer’s instructions. Images were analyzed by the Image Pro Plus 6.0 software. The caspase-3 expressions were normalized to the GAPDH loading control.

2.8. Statistical Analysis. The data were presented as mean ± SD. All statistical analysis was performed using SPSS11.0. Unpaired Student’s t-test was used for the comparisons between two different groups. p values less than 0.05 were considered significant.

Figure 2: Flow cytometry assay and CCK-8 assay for prostatic cancer cells. (a, b) The representative data of flow cytometry assay for apoptosis of cell line PC-3. (c) The average percent of apoptosis of PC-3 cells was calculated. (d) CCK-8 results for tumor cells in mice. All experiments were triplicated. Data were presented as mean ± SD. *p < 0.05, compared with the control group.

Figure 3: Scratch adhesion test at 12 h, 24 h, and 48 h was conducted. All experiments were triplicated. Data were presented as mean ± SD. *p < 0.05, compared with the control group.
3. Results

3.1. Effect of Nanoyam Polysaccharide on Tumor Growth. The effect of nanoyam polysaccharide intervention on tumor growth in tumor-bearing mice was observed by detecting the volume and weight of the tumor body. Compared with the control group, different doses of nanoyam polysaccharide intervention could significantly inhibit the tumor weight of PC-3 tumor-bearing mice \((p < 0.05)\), as shown in Figure 1(a). In the HD group and MD group, the tumor weight inhibition rate in the LD group was 20.60\%±0.3\%, 38.07\%±0.4\%, and 27.08\%±0.1\%, respectively. Compared with the negative control group, the nanoyam polysaccharide could significantly inhibit the volume of prostate cancer in tumors-bearing mice \((p < 0.05)\), as shown in Figure 1(b).

3.2. Effect of Nanoyam Polysaccharide on Cell Viability. To determine the effect of nanoyam polysaccharide on apoptosis of prostatic cancer cells, Annexin V/PI staining was performed. As shown in Figures 2(a) and 2(b), apoptosis of prostatic cancer cells in the intervention group was significantly higher than that in the control group \((45.8\%±2.6\%, 25.8\%±3.1\%; p < 0.05)\). CCK-8 assay indicated that in the HD group and MD group, tumor cell activity in the LD group decreased to 81.80\%±1.1\%, 55.15\%±1.6\%, and 62.85\%±0.9\% of the control group, respectively, and the difference was significant \((p < 0.05)\), as shown in Figure 2(d).

3.3. Effect of Nanoyam Polysaccharide on Cell Migration. Scratch adhesion test showed that the rate of wound healing in the intervention group at 12 h, 24 h, and 48 h were significantly lower than that in the control group \((p < 0.05)\), indicating that nanoyam polysaccharide had an inhibitory effect on prostatic cancer cells.

3.4. Effect of Nanoyam Polysaccharide on Protein Levels. The expression of caspase-3 protein of prostate cancer in mice was detected by Western blotting. Compared with the control group, the expression of caspase-3 in prostate cancer tissues was downregulated in the HD group, MD group, and LD group, with statistical difference \((Figure 4, p < 0.05)\).

4. Discussion

This study investigated the anticancer effect of polysaccharides of nanoyam. PC-3 cell line is one of the representative cell lines of human prostate cancer. In vitro study showed that the nanoyam polysaccharide presented inhibitory on the human prostate cancer cell line PC-3. Furthermore, the scratch test showed that nanoyam polysaccharide can significantly inhibit the migration of pc-3 cells. By using flow cytometry analysis, we found that the intervention of nanoyam polysaccharide could induce apoptosis of pc-3 cells. Subsequently, in vivo study of PC-3 tumor-bearing mice, we explored the inhibitory effect of nanoyam polysaccharide on human prostate cancer cells and its potential mechanism. Four weeks after the intervention of nanoyam polysaccharide, the weight and volume of the tumor were significantly reduced. The results of CCK-8 assay showed that the nanoyam polysaccharides had an inhibitory effect on the proliferation of prostate tumors, which was consistent with the results of in vitro flow cytometry analysis.

Tumor development and prognosis are closely related to apoptosis. Abnormal apoptosis is considered as one of the causes of tumor formation, and inducing apoptosis of tumor cells has become a new trend of tumor treatment over the years [11, 12]. Classic apoptotic pathways include exogenous death receptor pathways and endogenous mitochondrial cytochrome release pathways. Existing studies have shown that these two pathways are interrelated, rather than independent, and both of these pathways will eventually activate the apoptotic end-effector caspase 3 [13, 14]. In this study, the protein level of caspase-3 in tumor cells of PC-3 tumor-bearing mice was detected by Western blot. We found that the intervention of nanoyam polysaccharide could inhibit tumor development and metastasis by inducing the upregulated expression of caspase-3 protein. The cell apoptosis signal pathway of cancer cells is very complex, and the nanoyam polysaccharide contains many active components.
Therefore, the signal mechanism of nanoyam polysaccharide inducing cell apoptosis needs further exploration.

To sum up, nanoyam polysaccharides are potential therapeutic agents for prostate tumors by inducing high levels of caspase-3 protein expression and inhibiting the proliferation of prostate tumors.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions
Cheng Peng, Bo Han, Zhaohui Zhai, and Yanping Shen contributed equally to this work.

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References