Effect of *Polygonatum Sibiricum* polysaccharides on nude mice model of prostate cancer PC-3 cells

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Abstract. The present study aimed to explore the influence of *Polygonatum sibiricum* polysaccharides (PSP) on the progression of prostate cancer PC-3 cell xenografts in nude mice, with a specific emphasis on analyzing the regulation of key proteins in the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) and nuclear factor-kappa B (NF-κB) signaling pathways. An androgen-independent PC-3 prostate cancer cell line was subcutaneously injected into immunocompromised BALB/c nude mice to establish a xenograft model. The mice were randomly allocated into five groups, each comprising six animals. Drug dosages were determined according to the body surface area ratio between humans and nude mice. The control group was given normal saline, whereas the docetaxel (DTX) group received docetaxel at a dosage of 5 mg/(kg·d). The PSP treatment groups were administered PSP at low [100 mg/(kg x d)], medium [200 mg/(kg x d)], and high [400 mg/(kg x d)] doses. Each treatment was delivered via gavage at a volume of 0.2 ml every other day for a 30-day period. Tumor volume and body weight were recorded every 3 days to evaluate the effect of PSP on xenograft growth, with tumor size and overall health status serving as the primary assessment criteria. A total of 4 h after drug administration, tumor volume was measured to calculate the tumor inhibition rate. Subsequently, apoptosis in tumor tissues was evaluated using the TUNEL assay. Immunohistochemistry was conducted to detect the expression levels of PI3K, Akt, NF-κB p65, their phosphorylated forms (p-PI3K, p-Akt and p-NF-κB p65), and caspase-3. At the initial stage of establishing the tumor-bearing nude mouse model of prostate cancer, all groups of nude mice displayed stable mental states, high levels of activity, regular feeding habits and heightened responsiveness to external stimuli. However, as the tumors progressed, a decline in activity, food intake and responsiveness to stimuli was observed across all groups. PSP inhibited the proliferation of PC-3 cells and induced apoptosis in tumor-bearing nude mice, presumably by downregulating PI3K, Akt and NF-κB p65, thereby suppressing the PI3K/Akt and NF-κB signaling pathways. Simultaneously, PSP upregulated the expression of caspase-3, which contributed to its antitumor effects in the PC-3 prostate cancer model.

Introduction

Prostate cancer, predominantly an epithelial malignancy originating in glandular tissue (95% as adenocarcinomas), represents one of the most prevalent cancers in men globally (1). Over the past decade, its incidence has surged at an annual rate of 5%, currently accounting for 14% cancer cases in men, ranking second in overall frequency (2). The rising occurrence in the country can be attributed to factors such as societal aging and significant dietary shifts. As the population ages, the risk of developing prostate cancer increases exponentially. Additionally, modern dietary patterns, characterized by high consumption of processed foods and low intake of fruits and vegetables, are considered to contribute to the development and progression of the disease. These lifestyle changes, combined with environmental factors, create a conducive environment for prostate cancer development (3). To advance more effective treatments, growing research focuses on integrating Chinese and Western medicine, which holds significance for enhancing quality of life of patients with prostate cancer (4). The dried rhizomes of *Polygonatum Sibiricum*, *Rhizoma Pinelliae*, or *Rhizoma Polygonatum* exhibit properties such as spleen strengthening, lung moistening, qi tonification (where enhancing qi means supplementing and regulating the body's vital energy), and yin nourishment (which involves nourishing the body's yin fluid) (5,6). Qi tonification and yin nourishment is a treatment concept in traditional Chinese medicine. This concept emphasizes the importance of restoring the body's internal balance to promote self-healing. By tonifying

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qi and nourishing yin, traditional Chinese medicine aims to strengthen the body's defenses and improve its overall function. This approach is particularly relevant in the treatment of chronic diseases such as cancer, where the body's immune system is often compromised. Previous studies have shown that traditional Chinese medicines with such effects may have potential effects on improving the overall function of the body and may be involved in regulating physiological processes such as the immune system. Natural polysaccharides, which are abundant in traditional Chinese medicines, have become a major research focus in the development of functional foods with anti-tumor activity due to their low toxicity and limited side effects on the human body, indicating their promising application prospects (7-9). The potent bioactivity of PSP arises from its diverse constituents, including polysaccharides, alkaloids, flavonoids, steroidal saponins, lignans and amino acids. In recent years, mounting evidence has underscored the pivotal role of polysaccharides in *Polygonatum Sibiricum*. Among them, PSP, a natural polysaccharide primarily composed of mannose, galactose, glucose, fructose, rhamnose, arabinose, and galacturonic acid-along with trace amounts of xylose and glucuronic acid-exhibit diverse pharmacological activities. These include immunomodulatory, anti-tumor, antioxidant, and anti-aging effects (10,11). Studies indicate that PSP, a major component of *Polygonatum Sibiricum*, exerts multiple therapeutic effects, such as mitigating diabetic complications, alleviating nephropathy-induced renal impairment, preventing bone loss, and exhibiting anticancer properties (12,13). Specifically, in prostate cancer, PSP has been identified to inhibit the proliferation of cancer-associated fibroblasts and exhibit anti-prostate cancer effects (14,15). To further elucidate PSP's role and mechanism in prostate cancer treatment, *in vivo* experiments were conducted using a PC-3 cell nude mouse xenograft model, aiming to contribute to novel cancer therapies grounded in Chinese medicine.

Materials and methods

Laboratory animals and cells. A total of 30 BALB/c nude male mice (nu/nu), SPF (Specific Pathogen Free) grade, weighing (19.03±1.00) g at 6 weeks of age, were sourced from Beijing Specific Bio-Technology Co. Ltd. under license SCXK (Beijing) 2019-0010. Housing conditions included a temperature of 23±3˚C, relative humidity of 40-70%, and 12/12-h light/dark cycle. Food and water were provided *ad libitum*. All experimental procedures adhered to institutional animal experiment guidelines and were approved by the Animal Ethics Committee (approval no. HBNU20240622120; Zhangjiakou, China). The PC-3 cell line was supplied by BeiNaTrunLian Biotechnology Co. Ltd. (cat. no. BNCC350783; https://www.bncc.com/pro/p1/1/p_350783.html) and cultured in RPMI-1640 medium with 10% fetal bovine serum at 37˚C in a 5% CO_2 incubator under stable condition.

Construction and grouping of a nude mouse model of prostate cancer with hormonal tumor. Following the subcutaneous inoculation of 0.2 ml PC-3 cell suspension ($1x10^7$ cells/ml) resuspended in PBS into the lateral right forelimb of BALB/c nude mice, tumor diameters reached 3-5 mm after three weeks, confirming successful model establishment. Tumor growth and any pathological alterations at the inoculation site were monitored daily throughout the cell implantation period, alongside observations of physiological changes in the mice, including reduced appetite, decreased activity, and diminished responsiveness.

Following dosage conversion based on the body surface areas of humans and nude mice, the model nude mice were randomly assigned to five groups of six: The control group received saline, the DTX group was administered docetaxel at 5 mg/(kg x d), and the PSP (cat. no. S27804; Shanghai Yuanye Biotechnology Co., Ltd.) group was subdivided into low-dose [100 mg/(kg x d), medium-dose (200 mg/(kg x d)], and high-dose [400 mg/(kg x d)] groups. Each treatment was administered via gavage (0.2 ml) at 9:00 am every other day for 30 days. Throughout the intervention period, body weight and the long and short diameters of the transplanted tumors were recorded every three days, and tumor volume was calculated using the formula [Tumor Volume=(Long Diameter x Short Diameter2)/2]. Time-dependent changes in body weight and tumor volume were plotted. Observations included alterations in appetite, activity levels and responsiveness. A total of 4 h after the final gavage, cardiac blood was collected, spleens were excised, and tumors were removed for further experimentation. Tumor inhibition rate (%) was calculated as (1-mean tumor volume of the treatment group/mean tumor volume of the control group) x100%. The euthanasia procedures adhered to the AVMA Euthanasia Guidelines. After anesthesia with CO_2 at a displacement rate of 30-70% per min, cervical dislocation was carried out.

Detection of apoptosis in PC-3 cell-transplanted tumor tissues by TUNEL method. Paraffin-embedded tissue sections, with a thickness of 5 μm, were employed. The sections were immersed in xylene at room temperature for 5 min and this process was repeated once to ensure the thorough elimination of paraffin. Subsequently, they were soaked in 100% ethanol at room temperature for 5 min and the soaking was replicated. Next, the sections were immersed in gradient ethanol (90, 80, 70%) successively for 3 min each at room temperature. After gentle rinsing with PBS, the excess liquid around the samples on the slides was carefully removed using filter paper. At this juncture, a paraffin or hydrophobic pen could be utilized to demarcate the sample distribution contour, which was advantageous for the subsequent permeability treatment and equilibration labeling procedures. During the experiment, it was essential to prevent sample desiccation. The treated samples were placed in a humid box to maintain their moisture content. The 2 mg/ml Proteinase K solution was diluted with PBS in a ratio of 1:100 to obtain a final concentration of 20 μg/ml. A total of 100 μl of the diluted Proteinase K solution was added to each sample to ensure complete coverage and then incubated at room temperature for 20 min. The samples were gently rinsed with PBS 2-3 times to eliminate the excess liquid, and the liquid around the samples on the slides was carefully absorbed. Thereafter, in accordance with the protocol of the TUNEL method apoptosis detection kit (cat. no. BL644C; Beijing Lanjieke Science and Technology Co., Ltd.), TUNEL labeling and re-staining were carried out. Subsequently, the apoptosis of sample cells was observed, and images were captured under a fluorescence microscope

(Model Ts2-FL; Beijing Heng Sanjiang Instrument Sales Co., Ltd.). Image analysis was conducted using ImageJ 1.8.0 software (National Institutes of Health).

Expression of proteins related to PI3K/Akt and NF-κB signaling pathways detected by IHC. All procedures were performed in strict accordance with the provided protocols. Tumor tissues from nude mice were excised, followed by fixation (in 4% paraformaldehyde for 24 h at room temperature), dehydration (using an 80, 90, 95 and 100% ethanol series, with each concentration applied for 2 h), clearing, paraffin embedding and sectioning. The sections underwent deparaffinization, gradient alcohol hydration, rinsing, and placement in PBS buffer. Antigen retrieval was conducted as per antibody instructions. A peroxidase blocker was then applied, incubated at room temperature for 10 min, and rinsed with PBS. The primary antibodies (1:200; cat. nos. AF6241, AF3241, AF6261, AF0016, AF5006, AF2006 and AF6311; Affinity Biosciences) were applied and incubated overnight at 4˚C. Afterward, reaction enhancement solution was added and incubated for 20 min at room temperature, followed by the secondary antibody, Goat Anti-Rabbit IgG H&L (HRP) (1:1,000; cat. no. ab6721; Abcam) under similar conditions. Color development was achieved with DAB (3,3'-Diaminobenzidine) (1:20; cat. no. AR1027; Boster Biological Technology) for 5 min, after which the sections were examined under a microscope. Hematoxylin counterstaining was performed for 3 min. Following staining, sections were dehydrated, cleared, sealed, and observed under the microscope (Model Ts2-FL; Beijing Heng Sanjiang Instrument Sales Co., Ltd.), with images captured for analysis. ImageJ 1.8.0 software (National Institutes of Health) was used for subsequent analysis.

Statistical analysis. Data analysis was performed using SPSS 25.0 statistical software (IBM Corp.). For normally distributed variables, results were presented as the mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA (Analysis of Variance) was applied for comparisons when variances were homogeneous, followed by LSD for pairwise comparisons among groups. In cases of heterogeneity of variance, Dunnett's test was employed. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Changes in growth status and tumor weight volume of transplanted tumors in nude mice. At the early stage of tumor model development, nude mice across all groups exhibited stable physiological conditions, characterized by consistent activity levels, regular food intake and a maintained responsiveness to external stimuli. Their overall health remained within acceptable parameters. Post-administration of PSP, the general condition of the mice improved, with more pronounced effects observed at higher doses. Over time, both body weight and tumor volume showed a consistent upward trend across all groups ($P<0.05$). However, no statistically significant differences in body weight or tumor volume were detected between groups at the same time points (Fig. 1A and B). By day 21 post-inoculation, the grafted tumors had reached diameters of 3-5 mm, confirming the successful establishment of the nude mouse model. Following drug intervention according to group assignments, the weight of nude mice initially showed a slight increase before stabilizing, with no statistically significant differences in weight between groups at equivalent time points (Fig. 1A). Tumor volumes in all groups progressively increased over time after drug administration ($P<0.05$), with significant differences in tumor growth observed across groups subjected to varying doses of PSP at corresponding time points (Fig. 1B). This indicates that PSP has a certain inhibitory effect on tumor growth, and the degree of inhibition is related to the dose; he inhibitory effect on tumor volume growth increased proportionally to the dose of PSP. After 30 days of intervention, tumor samples collected 4 h post-final administration were used to calculate the inhibition rate, and yielding tumor inhibition rates of 44.00, 16.26, 24.58, and 34.82% in the DTX and PSP groups, respectively, compared with the control group (Fig. 1C). These data further verified that PSP can effectively inhibit the growth of PC-3 cell xenografts in nude mice, although its inhibitory effect was slightly weaker than that of docetaxel. After the treatment was completed, the nude mice were sacrificed, and the tumors were harvested (Fig. 1D). PSP exhibited marked inhibition of tumor cell proliferation in a time- and dose-dependent manner.

Apoptosis of PC-3 cells in various groups of hormonal nude mice. The TUNEL assay results indicated that, compared with the control group, no significant change in the apoptosis rate of PC-3 cells was observed in the low-dose group (Fig. 2). However, a substantial increase in apoptosis was evident in the DTX, medium-, and high-dose groups, with the DTX group showing a significantly higher apoptosis rate than the PSP group ($P<0.01$). Both TUNEL data demonstrated a dose-dependent relationship, where the medium-dose group revealed higher apoptosis than the low-dose group, and the high-dose group exceeded the medium-dose group ($P<0.05$). This clearly demonstrated that PSP could promote the apoptosis of PC-3 cells in a dose-dependent manner. As the dose of PSP increases, more PC-3 cells undergo apoptosis, which is an important mechanism for PSP to inhibit tumor growth.

IHC results of PI3K/Akt and NF-κB signaling pathway-related proteins in tumor tissues of nude mice in each group. Immunohistochemical analysis of the transplanted tumor tissues revealed no statistically significant variations in PI3K, Akt and P65 protein expression levels (Fig. 3A-C). However, compared with the control group, p-PI3K levels in the DTX and PSP groups were reduced by 14.84, 0.48, 10.80 and 22.18%, respectively (Fig. 3D, $P<0.01$). Similarly, p-Akt levels revealed reductions of 10.14, 0.68, 7.20 and 12.40% (Fig. 3E, $P<0.001$), while p-P65 levels decreased by 14.87, 5.13, 5.27 and 10.30% (Fig. 3F, $P<0.001$). Among the three, p-PI3K demonstrated the most pronounced reduction, with all proteins exhibiting dose-dependent effects. By contrast, caspase-3 levels in the DTX and PSP groups increased by 27.27, 7.7, 15.2 and 24.4% compared with the control group (Fig. 3G, $P<0.001$). These results suggested that PSP may inhibit the PI3K/Akt and NF-κB signaling pathways by reducing the phosphorylation levels of PI3K, Akt and P65, without affecting their total protein levels. At the same time, the increase in caspase-3 expression indicates that PSP promotes apoptosis in PC-3 cells through this signaling pathway regulation mechanism. While

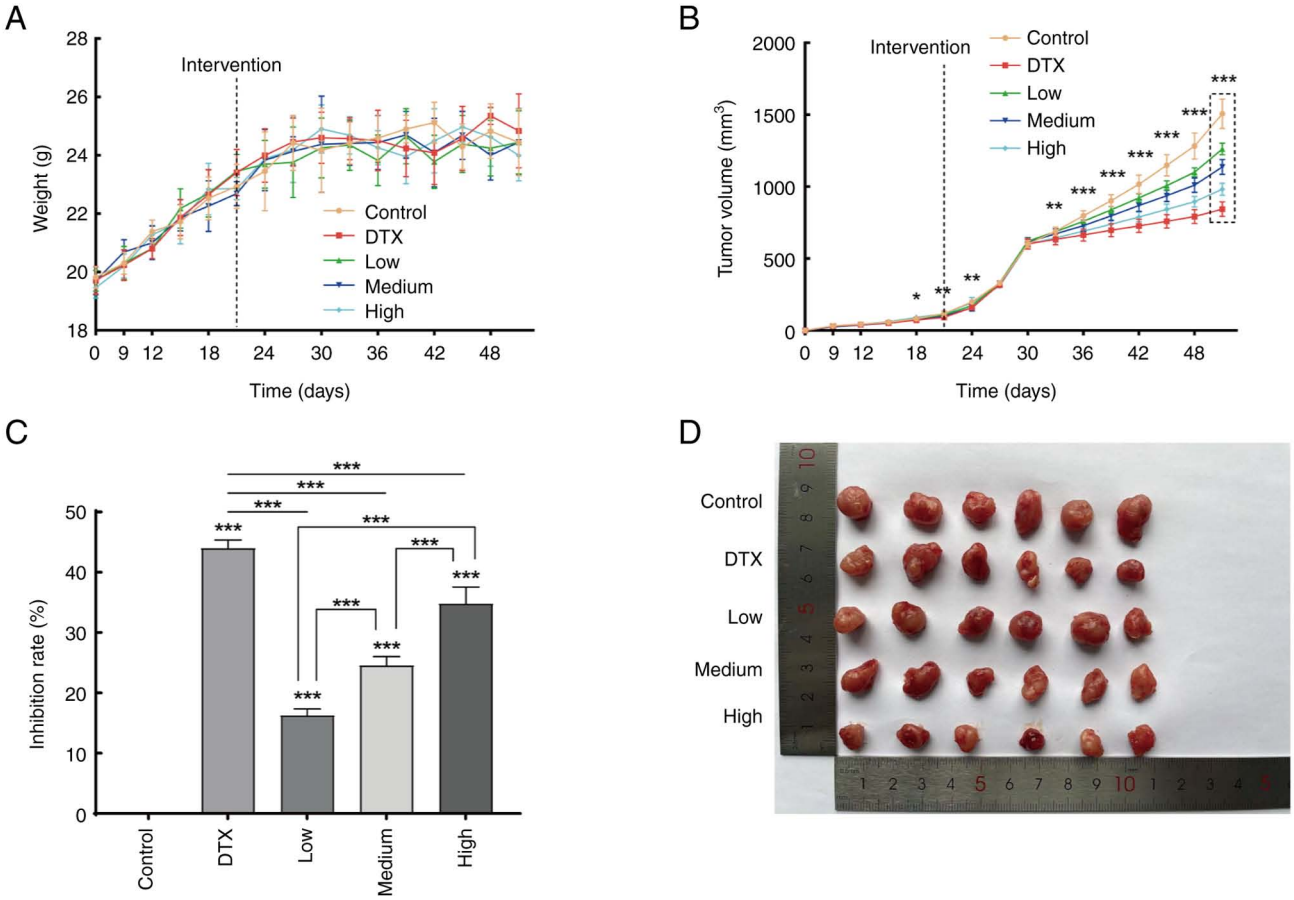

Figure 1. Construction and grouping of a nude mouse model of prostate cancer with hormonal tumor. (A and B) Changes in body weight and tumor volume of nude mice in each group from the initiation of cell inoculation to the completion of drug intervention, as well as (C) the tumor suppression rate measured 4 h after the final drug administration. (D) Images of dissected tumors from nude mice. *P<0.05, **P<0.01 and ***P<0.001. DTX, docetaxel.

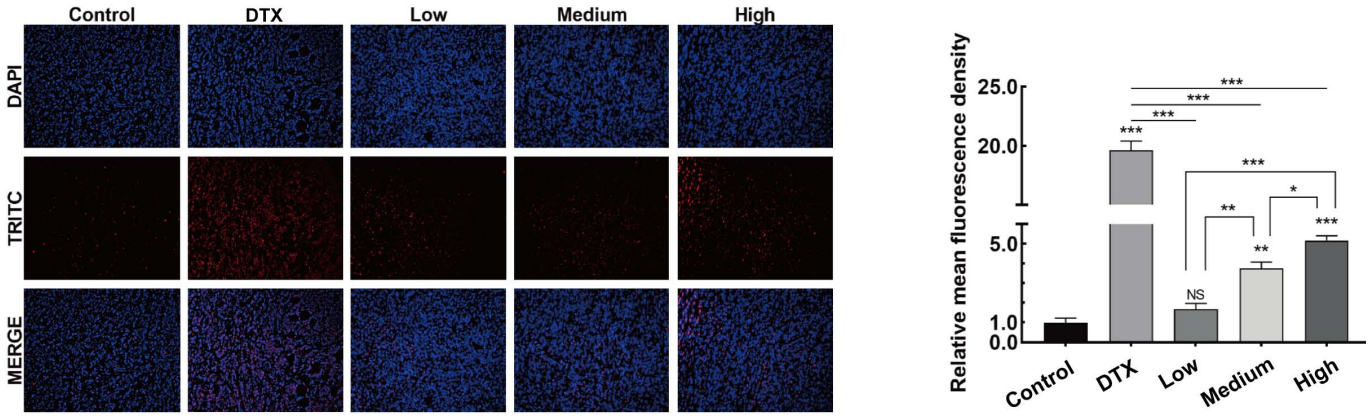

Figure 2. Detection of apoptosis in PC-3 cell-transplanted tumor tissues by TUNEL method. Fluorescence of apoptotic cells transplanted tumors across groups and the relative mean fluorescence intensity (using the control group as a baseline). *P<0.05, **P<0.01 and ***P<0.001. NS, not significant (P>0.05); DTX, docetaxel.

PSP reduced the phosphorylation of PI3K, Akt and P65, it significantly elevated Caspase-3 expression, without affecting the total protein levels of PI3K, Akt and P65.

Discussion

Prostate cancer patients commonly experience symptoms such as frequent urination, urgency, difficulty in urination and hematuria. As the disease progresses to advanced stages, metastasis often develops, posing severe risks to men's physical, mental and overall well-being. According to the International Agency for Research on Cancer (IARC) of the WHO, metastatic prostate cancer drastically reduces the five-year survival rate from 95 to 34%, significantly worsening patient prognosis (16). Conventional endocrine therapy and chemotherapy remain limited due to tumor heterogeneity

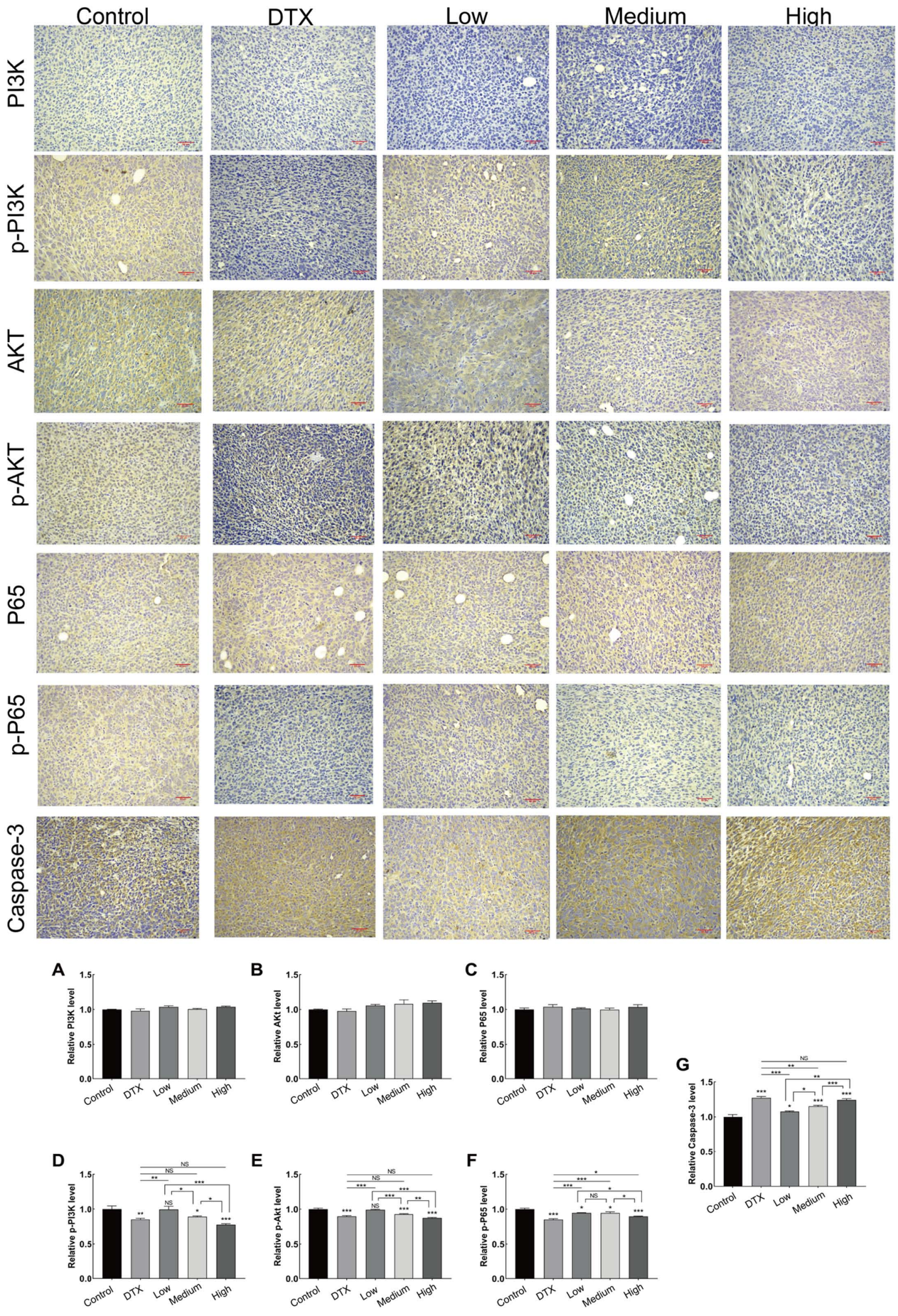

Figure 3. Immunohistochemical analysis of (A) PI3K, (B) Akt, (C) p65, (D) p-PI3K, (E) p-Akt, (F) p-P65 and (G) Caspase-3 proteins (light; magnification, x100; DAB; Scale bars, 50 μm). *P<0.05, **P<0.01 and ***P<0.001. NS, not significant (P>0.05); DTX, docetaxel; p-, phosphorylated.

and the development of drug resistance (17). However, advancements in prostate cancer research and genetic testing have uncovered new therapeutic targets, driving progress in precision treatments for metastatic prostate cancer (18). It has been indicated that certain Chinese medicines exhibit anticancer properties by inhibiting tumor cell growth (19). The bioactive components of these medicines include polysaccharides, flavonoids, terpenoids, phenolic compounds and alkaloids (20-25). Research demonstrates that natural polysaccharides, such as those from *Lycium barbarum*, safflower, *Ganoderma lucidum* and astragalus, exert antitumor effects by enhancing cytokine activity, boosting immune system responsiveness, and regulating multiple signaling pathways (26-29). Furthermore, modern pharmacological studies confirm that PSP possesses a wide range of biological functions, including anticancer, anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant and anti-aging properties (12,30).

Previous *in vitro* experiments demonstrated that PSP inhibited PC-3 cell proliferation, induced apoptosis, and significantly alleviated the S-phase cell cycle arrest. Further analysis identified reduced expression of p-Akt, p-PI3K, p-p65 and cyclin, alongside increased Caspase-3 activity, suggesting potential anti-prostate cancer effects through modulation of the PI3K/Akt and NF-κB signaling pathways (31). This provided substantial support for the present study. In the current research, an *in vivo* prostate cancer model was established by grafting PC-3 cells into nude mice, followed by drug intervention to evaluate the effects of PSP on tumor cell proliferation, apoptosis and the expression of pathway-related proteins. The results obtained were largely consistent with prior *in vitro* findings. The present study established a prostate cancer model by subcutaneously inoculating PC-3 cells into BALB/c nude mice. Tumor volumes in all groups expanded progressively over time, reaching diameters of 3-5 mm after three weeks, confirming the successful construction of the model. Following treatment with varying doses of PSP, tumor volumes significantly reduced, and apoptotic rates in tumor cells significantly increased, demonstrating a dose-dependent relationship. These results suggest that PSP effectively inhibit PC-3 cell proliferation and promote tumor cell apoptosis, with docetaxel exerting a more potent effect. Cancerous organisms often exhibit elevated levels of cytokines compared with their healthy counterparts. These findings suggest that *PSP*, a key component of the traditional Chinese medicine Huang Jing, hold potential as a therapeutic agent for prostate cancer.

Tumorigenesis involves complex mechanisms across various pathways, with the PI3K/Akt signaling pathway playing a central role in prostate cancer progression. Activation of downstream molecules via this phosphorylation cascade significantly influences all prostate cancer phenotypes (32). Moreover, the PI3K/Akt pathway is frequently activated in metastatic prostate cancers resistant to desmoplastic therapies (33). Aberrant activation leads to unchecked tumor cell growth and proliferation, making this pathway a key therapeutic target (34,35). PSP modulates the PI3K/Akt/mTOR and Toll like receptor 4/NF-κB pathways, while also influencing apoptosis-related protein expression, thereby exerting antitumor effects by inhibiting tumor cell proliferation and metastasis, promoting apoptosis and autophagy, suppressing angiogenesis, and regulating immune responses (36-38). In the present study, treatment with various doses of PSP significantly reduced the expression of p-PI3K, p-Akt and p-P65 in nude mice, while levels of PI3K, Akt and P65 remained unchanged, suggesting that PSP's effect on PC-3 prostate cancer cells may involve the suppression of PI3K/Akt and NF-κB pathway activation. Additionally, elevated Caspase-3 expression indicated that PSP promoted apoptosis in PC-3 cells within the nude mouse model.

Intervention studies with PSP demonstrated a marked decrease in the expression of p-Akt, p-PI3K and p-P65 proteins, alongside a significant increase in Caspase-3 expression in PC-3 cells from homozygous nude mice. These outcomes indicate that PSP may suppress the activation of PI3K, Akt and P65, thereby inhibiting both the PI3K/Akt and NF-κB signaling pathways, while concurrently promoting Caspase-3 expression. This combination effectively curtails the proliferation, migration and invasiveness of PC-3 prostate cancer cells, ultimately driving apoptosis. These findings suggest that PSP may hold considerable therapeutic potential for prostate cancer treatment. However, the antitumor mechanisms involved are multifaceted, and the present study addresses only select aspects. Furthermore, the limited sample size underscores the need for more comprehensive investigations into its precise mechanisms. The preliminary data suggest that the underlying mechanism may involve the dual modulation of the PI3K/Akt and NF-κB signaling pathways. A key limitation of the present study lies in its focus solely on the PC-3 cell line, leaving uncertainty about its applicability to other prostate cancer cell lines, such as LNCaP or DU145, or BPH cell lines. Future studies will aim to assess the effects of PSP across a broader range of cell lines and expand investigations in animal models to explore its therapeutic potentials.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

GZ and YT confirm the authenticity of all the raw data. GZ conceived the study and directed the project and analyses. CL and YT wrote the manuscript. YZ and CL constructed and grouped prostate cancer with hormone tumor nude mouse model. SD, CL and YT performed the TUNEL and immunohistochemistry assays. YT, JW and SY performed

bioinformatical analysis including data processing and statistics. GZ and YZ revised and edited manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. HBNV20240622120) by the Animal Welfare Ethics Committee of Hebei North University (Zhangjiakou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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