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Research article

# Astragaloside IV mediates the effect and mechanism of KPNB1 on biological behavior and tumor growth in prostate cancer

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ABSTRACT

*Background:* and purpose Prostate cancer is an comparatively prevalent clinical malignant tumor in men, impacting the lives of millions of men globally. This study measured the expression of Karyopherin Subunit Beta 1 (KPNB1) in prostate cancer cells, and made an effort to investigate how astragaloside IV affects the biological behavior, tumor growth, and mechanism of action of prostate cancer through KPNB1.

*Methods:* Human prostate cancer and normal cells were obtained and KPNB1 expression levels in the two cells were determined using qPCR and WB. Prostate cancer cells were grouped according to the addition of astragaloside IV, KPNB1 inhibitor (importazole) alone and in combination. KPNB1, NF-κB, and cycle-related proteins were detected to be expressed at different levels in each group's cells by WB. MTT to assess the viability of the cells. To identify the cell cycle, use flow cytometry, and sphere formation experiment to observe sphere formation ability. Nude mice were purchased and subcutaneously inoculated with prostate cancer cells to establish a prostate cancer model, and grouped by tail vein injection of astragaloside IV and importazole. Tumor size was measured. KPNB1 and NF-κB expression in tumor tissues were detected by WB. The expression of proteins relevant to the cycle is observed by immunohistochemical methods. TUNEL was used to detect apoptosis of tissue cells.

*Results:* KPNB1 expression was upregulated in prostate cancer cells ($P < 0.05$). KPNB1, NF-κB, and cycle-related protein levels were decreased by astragaloside IV and importazole both separately and together. Decreased viability of the cells and a higher percentage of cell cycle arrest in the G0 phase, apoptosis was increased, and sphere formation was decreased ($P < 0.05$). In vitro implantation experiments found that the application of astragaloside IV and importazole resulted in tumor growth inhibition, decreased KPNBI, NF-κB, and cyclin expression in tumor tissues, and promoted apoptosis in tumor tissues ($P < 0.05$).

*Conclusion:* Prostate cancer cells' expression of KPNB1 is downregulated by astragaloside IV, which also prevents the cells from proliferating. It offers a conceptual framework for the use of astragaloside IV in the management of prostate cancer.

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## 1. Introduction

Currently the second most prevalent malignant tumor in males globally, prostate cancer ranks fifth in terms of the causes of cancer-related deaths in men [1,2]. Its morbidity and mortality show an increasing trend from year to year [3]. The prostate gland, the site of origin of prostate cancer, is located around the urethra and below the bladder and is an important male reproductive support organ whose main functions are urination and maintenance of sperm activity [4]. Currently, the main methods of treating prostate cancer include surgical resection [5], immunotherapy [6], chemotherapy [7], and androgen deprivation [8]. However, in its early phases of development, prostate cancer has not yet displayed any particular clinical symptoms [9]. Many patients present with metastatic symptoms such as bone pain before going to the hospital. The disease is often found to be predominantly localized in the middle and late stages and extensive metastatic type, resulting in unsatisfactory overall treatment and prognosis [10,11]. Therefore, it is crucial to study the biological behavior and potential therapeutic mechanisms of prostate cancer.

The application of phytomedicines in cancer treatment has garnered an abundance of attention over the past few years [12]. Astragalus, as a traditional Chinese medicine, has attracted much attention in tumor research for its multiple pharmacological effects [13]. Astragaloside IV, the most active compound in Astragalus, is associated with diverse pharmacological actions, including anti-inflammatory [14], antifibrotic [15], anticancer [16], and immune system regulation [17]. According to certain research, astragaloside IV has anticancer properties against a wide range of cancer types, such as liver cancer [18], stomach cancer [19], lung cancer [20], etc. Gong et al. [21] found that astragaloside IV could regulate pSmad3C/3L to elicit the Nrf2/HO-1 signaling cascade to inhibit hepatocellular carcinoma cell proliferation, migration, and invasion, attenuate hepatic fibrosis, and form an anticancer effect. Nevertheless, the impact of astragaloside IV in prostate cancer on its biological behavior and mechanism of action have not been fully elucidated.

Nucleus-cytoplasmic transport processes play a key role in normal cellular function as well as in tumor development [22]. Part of the nuclear transporter protein family, KPNB1 is essential for transporting various protein molecules across membranes to the nucleus [23]. In the cytoplasm, the nuclear transporter protein α identifies nuclear localization signals on target molecules. KPNB1 is a nuclear transporter protein receptor that attaches the nuclear transporter protein α-target molecule complex and anchors it to the nuclear membrane, initiating translocation through the nuclear pore to the nucleus. Guanosine triphosphate kinase interacts to KPNB1 in the nucleus, the nuclear transporter protein α-target molecule-KPNB1 complex de-aggregates, and free KPNB1 returns to the cytoplasm to participate in the next round of translocation [24]. KPNB1 not only mediates protein entry into the nucleus, but also participates in cell cycle regulation and mediates tumor development [25]. It has been found that KPNB1 promotes prostate cancer proliferation by promoting cMyc entry into the nucleus to up-regulate Cyclin D1 and Cyclin B1 cyclins [26]. KPNB1 expression in gliomas stimulates β-catenin entrance into the nucleus via the Wnt/β-catenin pathway, which in turn increases glioma cell proliferation and positively correlates with the malignant grade of gliomas [27].

Cellular homeostasis is hampered and tumor growth is encouraged by dysregulated nucleocytoplasmic shuttling. Many kinds of tumor types exhibit abnormal expression of KPNB1, which is believed to be linked to the genesis and spread of malignancies. KPNB1 shows aberrant expression in a variety of tumors, and is thought to be associated with the genesis and spread of malignancies [28]. Yet, it is unclear about its possible contribution to the etiology of prostate cancer. Therefore, in our study, we studied the expression and related effects of KPNB1 in prostate, and introduced KPNB1 inhibitor importazole. Importazole is a specifically targeted KPNB1 inhibitor whose main function is to inhibit KPNB1-mediated nucleo-cytoplasmic transport, thereby blocking the action of KPNB1 [26].

Prostate cancer cells were utilized as research subjects in this study, and it was discovered to be up-regulated in prostate cancer cells of KPNB1. The addition of astragaloside IV inhibited the levels of KPNB1, exploring the astragaloside IV mediated effects of KPNB1 in prostate cancer and its mechanism of action. Broaden the research horizons in the field of prostate cancer. To offer crucial hints for the creation of novel treatment approaches. With a view to improving the therapeutic effect and quality of life of prostate cancer patients.

## 2. Materials and methods

### 2.1. Reagents and instruments

Main reagents: Fetal bovine serum (164210, Procell, Wuhan China), penicillin-streptomycin mixture (PB180120, Procell, Wuhan China), RWPE-1 special medium (CM-0200, Procell, Wuhan China), astragaloside IV (AS-IV, $C_{41}H_{68}O_{14}$, purity≥98 %, Chengdu Pfield Biotechnology Co., LTD, Wuhan, China), importazole (HY-101091,MedChemExpress, New Jersey, US), reverse transcription reagent (TER017-1, Beijing Dingguo Changsheng Biotechnology Co., LTD, Beijing, China), RIPA Protein Lysate (WB-0071, Beijing Dingguo Changsheng Biotechnology Co., LTD, Beijing, China), BCA kit (BCA01, Beijing Dingguo Changsheng Biotechnology Co., LTD, Beijing, China), PVDF membrane (PVDF, Beijing Dingguo Changsheng Biotechnology Co., LTD, Beijing, China), ECL luminescent solution (CSB–K11714CL, Beijing Dingguo Changsheng Biotechnology Co., LTD, Beijing, China), KPNBI, NF-κB, Cyclin-B1, CDK1, Cyclin-D1, and β-actin antibody all purchased in abclonal, TUNEL kit (T2130, Solarbio, Beijing, China).

Main instruments and equipment: inverted microscope (MF52, Mshot, Guangzhou, China), constant temperature incubator (HF151, Healforce, Shanghai, China), centrifuge (H1650, Cence, Hunan, China), electrophoresis instrument (DYY-6D, Liuyi, Beijing, China), PCR instrument (CG-05, Healforce, Shanghai, China), gel imaging system (SH-523, Shenhuabio, Hangzhou, China), enzyme labeling instrument (FC,Thermofisher, Shanghai, China).

### 2.2. Cell culture and grouping

Human normal prostate cells RWPE-1 (K-SFM + 0.05 mg/mL BPE + 5 ng/mL EGF + 1 % P/S, CL-0200, Procell, Wuhan China), and prostate cancer cells PC3 (Ham's F–12K + 10 % FBS + 1 % P/S, CL-0185, Procell, Wuhan China), incubated in an incubator at 37 °C with 5 % $CO_2$. When the cells had grown to more than 80 % of the bottom of the bottle and the medium had turned yellow for a short time, they were washed twice with PBS at 37 °C. Trypsin digestion was performed until the cells were discrete into individual rounds and suspended in trypsin. After adding DMEM medium to stop the digestive process and centrifuge to discard the supernatant. Add 1 mL of DMEM medium containing serum. Count using a cell counter and add approximately $1 \times 10^6$ cells for passaging. All operations are aseptic to avoid cell contamination. Cells were grouped according to the addition of astragaloside IV and the KPNB1 inhibitor importazole: (1) model group: prostate cancer cells PC3, with the addition of the drug solvent DMSO; (2) model + astragaloside IV group: To the PC3 cells, astragaloside IV 20 μmol/L was added; (3) model + importazole group: Incorporation of the KPNB1 inhibitor importazole 8 μmol/L in PC3 cells; (4) model + astragaloside IV + importazole group: the drug astragaloside IV and the KPNB1 inhibitor importazole were added to PC3 cells. Cells were incubated and cultured for 48 h for subsequent experiments. Cells were incubated and cultured for 48 h as follow-up experiments. All the cells used in the study were identified by STR and tested for mycoplasma. The sites were correct and there was no mycoplasma infection. All the operations followed the principle of aseptic technique.

### 2.3. Real-time fluorescence quantitative PCR

Trizol method was utilized to extract RNA from the cells. Qualified RNA ready for reverse transcription was selected, reverse transcription reaction solution was prepared, gently mixed, and reverse transcription reaction was performed to synthesize cDNA. The KPNB1 mRNA relative level in cells was measured using the SYBR Green PCR Fluorescence Detection Kit. Table 1 displays the primer sequences, and the PCR reaction solution was prepared on ice using 18S rRNA as an internal control. Ct values were obtained for each group of reactions, the relative expression of KPNB1 mRNA in cells was computed using the $2^{-\Delta\Delta Ct}$ formula.

### 2.4. Western blotting test

Total proteins from each group of cells and testes were extracted with RIPA protein lysate, and protein concentration measurement with BCA kits. SDS-PAGE was utilized to separate the proteins, which were then transferred to PVDF membranes. Primary antibodies against KPNB1 (A23235, 1:10000), NF-κB (A11160, 1:500), Cyclin-B1 (A22435, 1:1000), CDK1 (A11420, 1:2000), Cyclin-D1 (A11022, 1:1000), and β-actin (AC026, 1:20000) in 1 % skimmed milk were added. After rinsing 3 times, ECL was added for chemiluminescence development and gel imaging. The destination bands were analyzed and processed for gray value using Image J image processing software.

### 2.5. MTT experiment

Inoculate each group of cells into 96-well plates, replaced with medium containing 1 % bovine serum for synchronized digestion, with 6 replicate wells in each group, and incubated for 12, 24, and 48 h, respectively. Each well received an addition of MTT solution, which was then incubated for 4 h. After discarding the culture media with care, add 50 μL of DMSO. Shake well and then measure the OD value at the wavelength of 490 nm using an enzyme labeler.

### 2.6. Flow cytometry

Cells were digested with 0.125 % trypsin, centrifuged to discard the supernatant, washed twice using PBS, add 500 μL Annexin V Binding Buffer. Addition of the nucleus staining solution and fluorescein labeling. After mixing by slight vortexing, incubate at room temperature away from light for 15 min, then transfer to a flow-through tube for immediate cell cycle and apoptosis detection. Samples that cannot be detected immediately must be placed on ice, protected from light, and finished within 1 h. The samples should be incubated for 15 min at room temperature.

### 2.7. Sphere formation experiments

Tumor stem cells containing an adherent growing cancer cell line were isolated using trypsin-EDTA solution T3924 with 80–90 % cell confluent and in good condition. Cell suspension was centrifuged and the supernatant was discarded. Resuspend the cells by adding 4 mL of 3dGRO™ Spheroidal Medium S3077 and adjust the cell concentration. Take 4 mL of cells at a concentration of $1 \times 10^4$

**Table 1**
Primer sequences.

| Genetics | Forward | Reverse |
|---|---|---|
| KPNB1 | 5′-GAGCCCTTCCTCCTGTTCTCT-3′ | 5′-TTGAGGCTGTCTTGTTGTGGT-3′ |
| 18S rRNA | 5′-AGTCCCTGCCCTTTGTACACA-3′ | 5′-CGATCCGAGGGCCTCACTA-3′ |

cells/mL inoculate them in ultra-low adsorption multiwell plates and incubate at 37 °C, 5 % $CO_2$ for one week. Half of the culture volume of fresh spheroplast medium was added every 3 days, and passaging was performed when a dark central zone began to form in the medium. Transfer cells and medium to a 15 mL conical tube using a serum pipette. Tumor spheroids were collected and photographs were taken to record the size of the spheroids.

### 2.8. Tumor transplantation in nude mice

Twelve male BALB/c nude mice, aged four to six weeks, were bought and housed in an environment free of specific pathogens. Sterile feed and water were freely ingested before the experiment. After one week of acclimatization to the environment, animal experimental studies were started with subcutaneous inoculation of PC3 cell ($5*10^6$ cell/200μl) suspension to construct a prostatic cancer model. The nude mice were grouped according to whether they were injected with astragaloside IV and importazole in the tail vein or not: (1) model group: subcutaneous PC3 cell injections were administered to nude mice, and injected with the drug solvent DMSO in the tail vein; (2) model + astragaloside IV group: the prostatic cancer model was injected with astragaloside IV 20 μg/mL in the tail vein; (3) model + importazole group: the prostatic cancer model was injected with importazole 10 μL; (4) model + astragaloside IV + importazole group: the prostatic cancer model was injected with astragaloside IV and importazole. After the injection was completed, the mice continued to be reared. Tools such as vernier calipers were used to measure the length and width of the tumor directly, and the volume of the tumor was calculated according to the formula. In general, the tumor volume (V) can be calculated by formula V= (width 2* length)/2. The nude mice were put to death after the tumors were observed to reach the appropriate size. Tumor tissue were extracted to perform WB, immunohistochemistry, and tunel experiment.

### 2.9. Immunohistochemistry

Paraffin sections were dewaxed and hydrated for antigen repair with citrate. After endogenous peroxidase blocking, serum was blocked for 1 h, and primary antibodies (Cyclin-B1, CDK1, Cyclin-D1) were added. After the sections were incubated at 4 °C for the full night, the secondary antibody was added, and visualized with DAB for 3 min. Sections were dehydrated by xylene, gradient alcohol, and blocked with neutral gum. Keeping the exposure conditions consistent, observe with a microscope and take pictures.

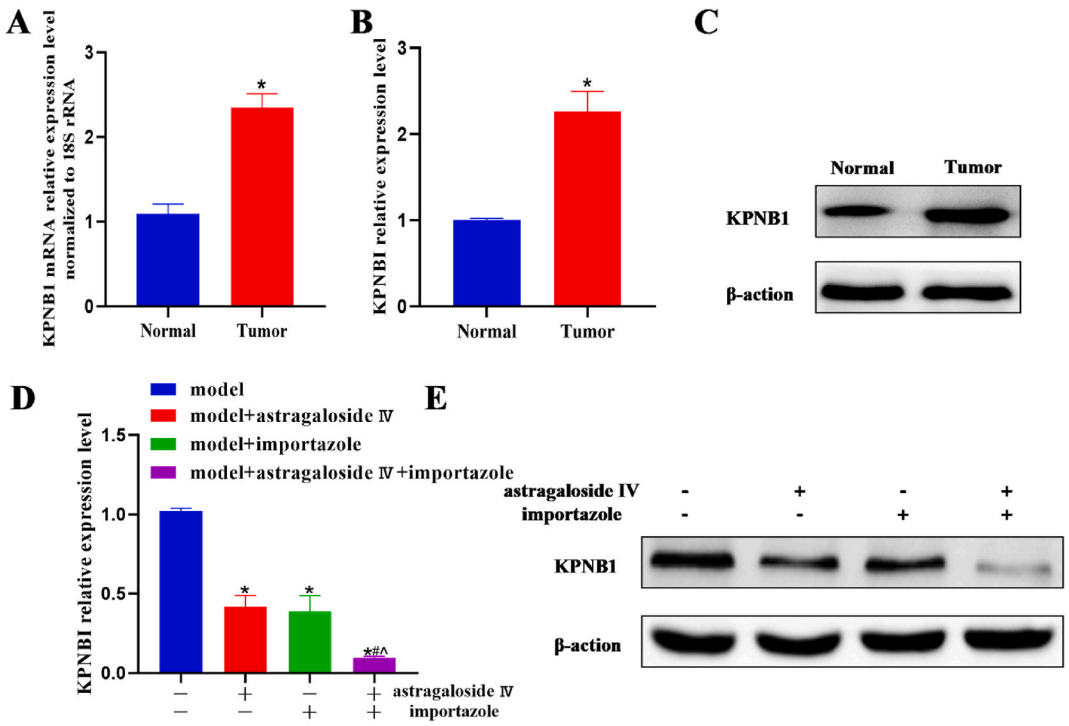


**Fig. 1.** Impact of astragaloside IV on KPNB1 in prostate cancer cells (n = 3). Note: A: KPNB1 mRNA expression in human prostate cancer and normal cells; B–C: Representative images and quantitative assessment of KPNB1 protein in human prostate cancer and normal cells (The original graphics as shown in Supplement 1), $^*P < 0.05$, compare with human normal prostate cells; D–E: Quantitative assessment and representative images of KPNB1 protein in prostate cancer cells (The original graphics as shown in Supplement 2), $^*P < 0.05$, $^{\#}P < 0.05$, $^{\wedge}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively.

### 2.10. TUNNEL experiment

Operate according to the TUNEL kit instructions, observe and take pictures under a confocal microscope. Select 40 × lens, observe 20 microscopic pictures, and calculate the number of blue and green cells in each of them to take the average value. The apoptosis index was used to determine the apoptosis between groups. Apoptotic index=(apoptotic cells/total cells) × 100 %.

### 2.11. Statistical analysis

SPSS 22 was used for data processing and analysis, and GraphPad Prism 9 was used for data plotting. The results were expressed as mean ± standard deviation. Comparisons between two groups were conducted using t-tests, while comparisons between several groups were made using one-way ANOVA. $P < 0.05$ indicates a statistically significant difference.

## 3. Results

### 3.1. Impact of astragaloside IV on prostate cancer cells' expression of KPNB1

In order to detect KPNB1 expression in human prostate cancer, qPCR and WB were used to identify KPNB1 expression in both human normal prostate cells and prostate cancer cells. The findings demonstrated that KPNB1 expression in prostate cancer cells was considerably higher in both mRNA (Fig. 1A) and protein (Fig. 1B–C) than in human normal prostate cells ($P < 0.05$).

WB examined the impact of astragaloside IV and importazole on KPNB1 levels in prostate cancer cells. The results found that astragaloside IV and importazole alone and in combination reduced the KPNB1 expression level ($P < 0.05$), and the reduction of KPNB1 expression level was most obvious with the combination of astragaloside IV and importazole (Fig. 1D–E). The combination of astragaloside IV and importazole reduced the KPNB1 expression level most significantly, indicating that in prostate cancer cells, astragaloside IV may lower KPNB1 expression.

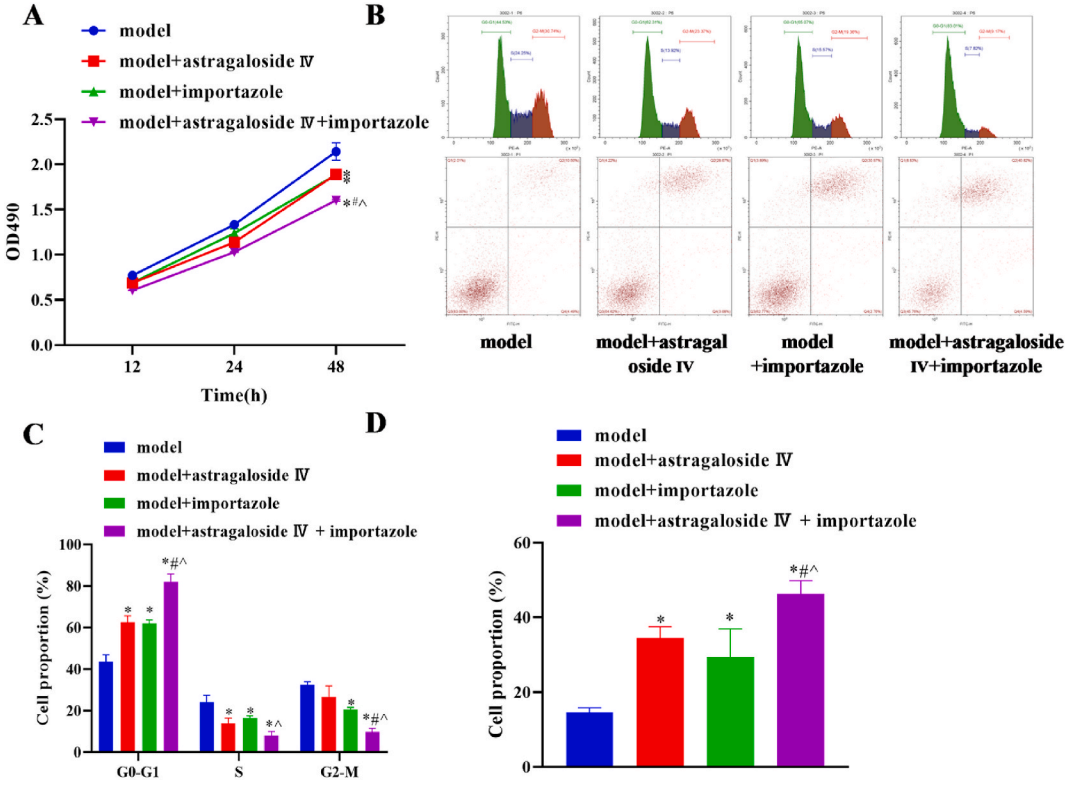


**Fig. 2.** Effects of astragaloside IV on cell viability, cycle and apoptosis via KPNB1 (n = 3). Note: A: Prostate cancer cell viability, $^{*}P < 0.05$,$^{\#}P < 0.05$, $^{\wedge}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively; B–D: Flow cytometry to detect the effect of cell cycle and apoptosis; $^{*}P < 0.05$,$^{\#}P < 0.05$, $^{\wedge}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively.

### 3.2. Effect of astragaloside IV on cell viability, cycle and apoptosis via KPNB1

Impact of importazole and astragaloside IV addition on the proliferation and viability of prostate cancer cells as determined by the MTT assay. The results revealed that the cell viability of model + astragaloside IV group, model + importazole group, and model + astragaloside IV + importazole group were lower than that of the model group ($P < 0.05$), and the cell viability of the model + astragaloside IV + importazole group had the greatest decrease (Fig. 2A). It indicated that astragaloside IV inhibited KPNB1 expression, which in turn inhibited the growth and viability of prostate cancer cells.

The effects of astragaloside IV and importazole on the prostate cancer cell cycle and apoptosis were examined using flow cytometry. The results found that astragaloside IV and importazole alone and in combination caused cycle blockage at G0 phase and increased apoptotic cells (Fig. 2B–D, $P < 0.05$), and the magnitude of the change was most significant in the combination of astragaloside IV and importazole. It indicated that astragaloside IV impeded cycle progression by decreasing KPNB1, exacerbated cell cycle block, causing apoptosis in prostate cancer cells.

### 3.3. Effect of astragaloside IV on NF-κB and cyclins in cells via KPNB1

The expression of NF-κB and cyclin was detected in prostate cancer cells. It was found that the addition of astragaloside IV and importazole alone and in combination decreased the expression levels of NF-κB, Cyclin-B1, CDK1, and Cyclin-D1 ($P < 0.05$), and the combination of the addition showed the greatest reduction (Fig. 3A–E).

### 3.4. Effect of astragaloside IV on cell sphere formation via KPNB1

The effects of adding astragaloside IV and importazole on the sphere formation of prostate cancer cells were detected. The experimental results showed that the addition of astragaloside IV and importazole alone and in combination reduced the number of cell sphere formations, and the reduction was the largest in the combination; the difference in the number of sphere formations between the model + astragaloside IV group and the model + importazole group was not obvious (Fig. 4A–B). It indicated that astragaloside IV inhibited the sphere formation ability of prostate cancer cells by decreasing KPNB1.

### 3.5. Effect of astragaloside IV on tumor growth via KPNB1

In order to detect the effect of astragaloside IV on tumor growth, experiments on subcutaneous injection of prostate cancer cells in nude mice were performed. It was found that astragaloside IV and importazole alone and in combination resulted in smaller tumor size and weight reduction ($P < 0.05$), and the smallest size and lightest weight of the tumors were found in the combination of astragaloside IV and importazole (Fig. 5A–D), which indicated that the inhibitory effect of astragaloside IV on the tumor growth of prostate cancer might be related to the mediation of KPNB1.

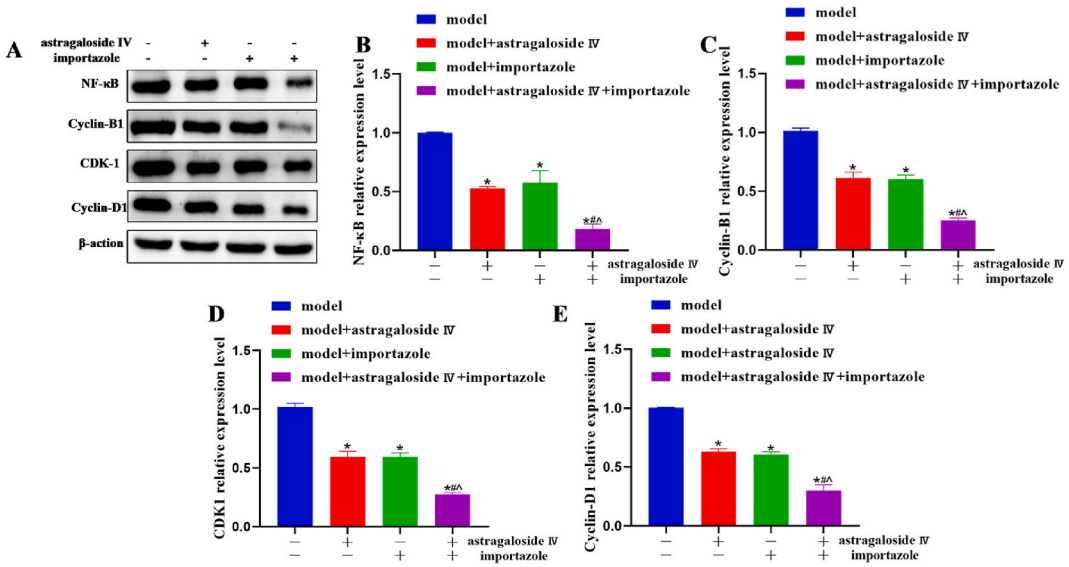


**Fig. 3.** Effects of astragaloside IV on NF-κB and cyclins in cells via KPNB1 (n = 3). Note: A: Representative images of NF-κB, Cyclin-B1, CDK1, Cyclin-D1 and endogenous β-action proteins in cells (The original graphics as shown in Supplement 3); B–F: Quantitative assessment of NF-κB, Cyclin-B1, CDK1, Cyclin-D1 and endogenous β-action proteins in cells, $^{*}P < 0.05$, $^{\#}P < 0.05$, $\hat{}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively.

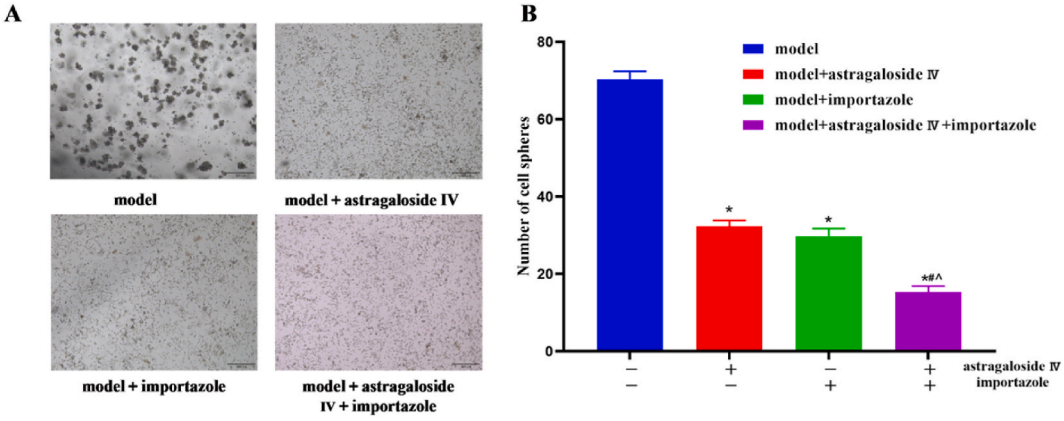


**Fig. 4.** Effect of astragaloside IV on cell sphere formation via KPNB1 (n = 3). Note: A–B: Effect of astragaloside on sphere formation in each group of cells, $^{*}P < 0.05$,$^{\#}P < 0.05$, $\hat{}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively.

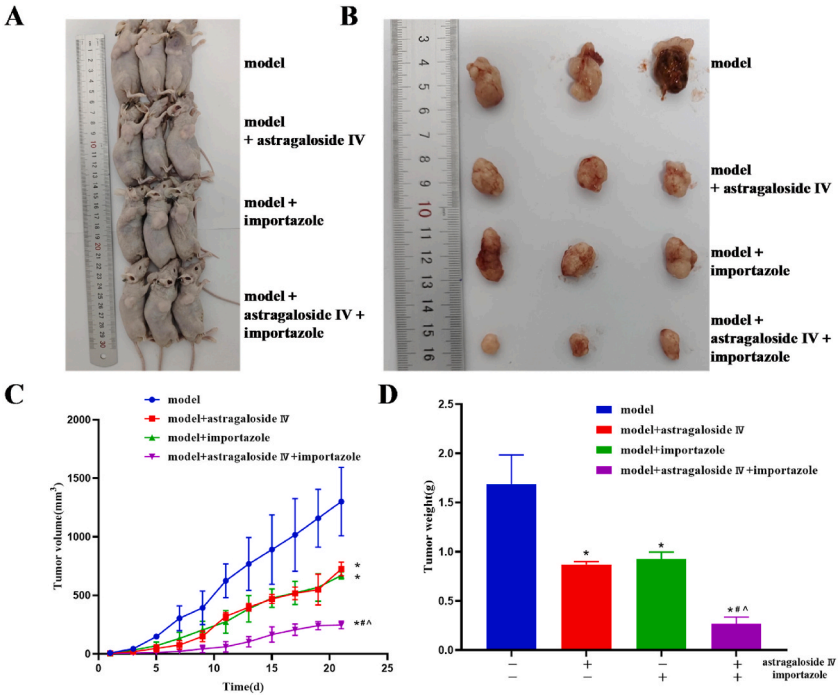


**Fig. 5.** Effect of astragaloside IV on tumor growth via KPNB1 (n = 3). Note: A–B: Prostate cancer tumor tissue in each group; C–D: Prostate cancer tumor volume and weight in each group, $^{*}P < 0.05$,$^{\#}P < 0.05$, $\hat{}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively.

### *3.6. Effect of astragaloside IV on tumor tissue cycle and apoptosis via KPNB1*

WB detected the expression levels of KPNB1 and NF-κB in tumor tissues. It was found that astragaloside IV and importazole alone and in combination inhibited KPNBI and NF-κB expression ($P < 0.05$), and the magnitude of inhibition was most significant in combination (Fig. 6A–C).

Immunohistochemistry was performed to observe the expression of cyclins in prostate cancer tissues. It was found that compared with the model group, astragaloside IV and importazole alone and in combination down-regulated the expression levels of Cyclin-B1, CDK1, and Cyclin-D1 ($P < 0.05$), and the down-regulation of the combination was the largest (Fig. 6D–G, which further confirmed that astragaloside IV, by reducing the expression of KPNB1 in the tumor tissues that inhibited the expression of cyclin.

According to the findings of the Tunel experiment, the model + astragaloside IV group, the model + importazole group, and the

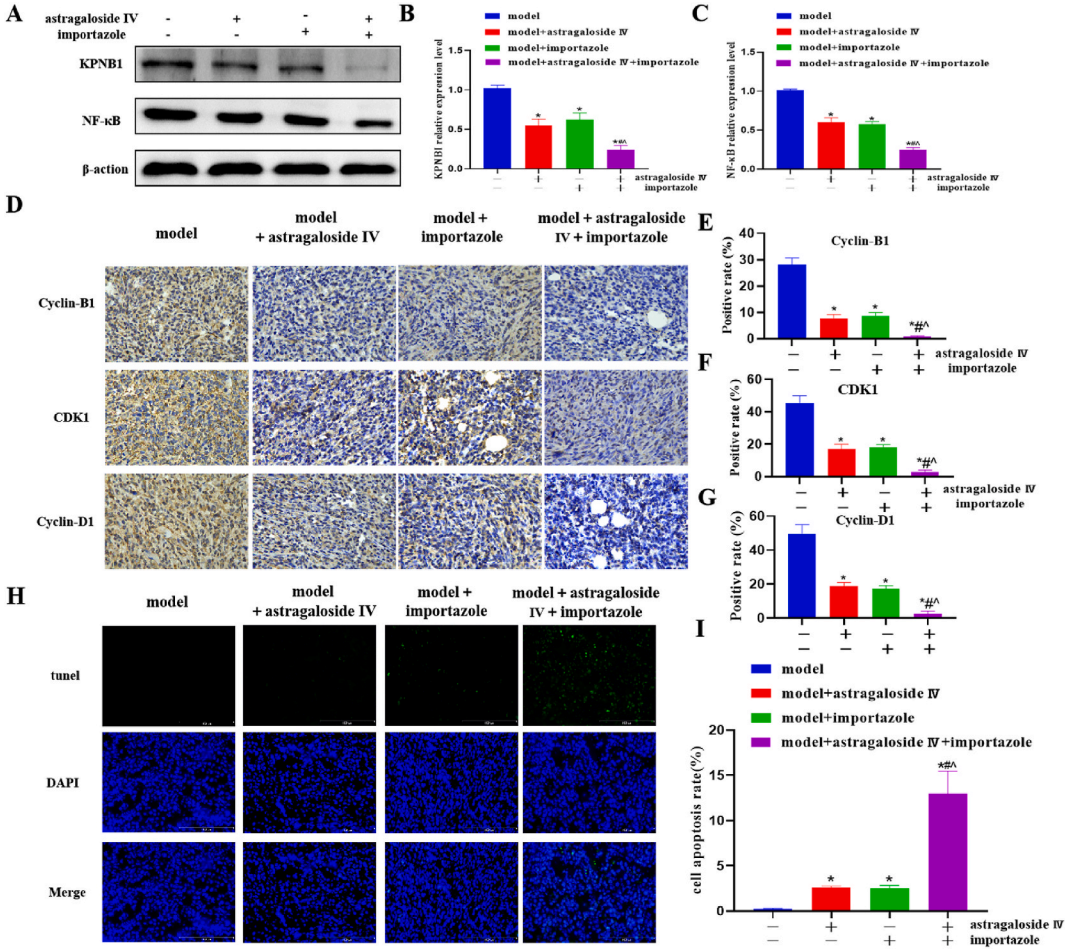


**Fig. 6.** Apoptosis and the tumor tissue cycle are affected by astragaloside IV via KPNB1 (n = 3). Note: A–C: Representative images and quantitative assessment of KBNB1 and NF-κB in tumor tissues of each group by Western blot (The original graphics as shown in Supplement 4); D–G: Representative images and quantitative assessment of cyclin Cyclin-B1, CDK1, Cyclin-D1 in prostate cancer tissues of each group by immunohistochemistry experiments; H–I: Apoptosis of tumor tissues of each group by tunel experiments, $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\wedge}P < 0.05$ denote comparison with the model group, model + astragaloside IV group, and model + importazole group, respectively.

model + astragaloside IV + importazole group all had a significantly greater apoptosis rate than the model group ($P < 0.05$), and the apoptosis rate of the model + astragaloside IV + importazole group was the most elevated (Fig. 6H–I). indicating that astragaloside IV could promote apoptosis in prostate cancer tumor tissues by down-regulating KPNB1.

## 4. Discussion

Prostate cancer belongs to epithelial malignant tumors and occurs in the genitourinary system, which seriously affects the quality of life and life and health of middle-aged and elderly male patients [29]. The worldwide incidence of prostate cancer has surpassed 1.41 million cases, and the number of patients who succumbed to prostate cancer has reached 370,000 [30]. The development of prostate cancer involves a variety of complex factors, including genetics, age, environment, hormones and lifestyle [31]. Prostate cancer is androgen-dependent, and it develops and grows in part due to the androgen receptor [32]. Androgens bind to the androgen receptor, control downstream target gene expression, and further advance the progression of prostate cancer [33]. Debridement has some efficacy, but the disease is prone to evolve into Castration-Resistant Prostate Cancer, which ultimately result in the patient's death [34]. Chemotherapy has adverse side effects such as significantly reducing patients' autoimmunity and causing liver and kidney damage [35], thus affecting the therapeutic effect of patients.

Certain substances in traditional Chinese medicine can be utilized in the prevention and cure of cancer [36], and have the advantages of fewer side effects, improving immunity, promoting apoptosis, and prolonging survival time [37], but their mechanism of action in prostate cancer is still unclear. This study aimed to examine the possible mode of action of astragaloside IV and its impact on the growth and biological characteristics of prostate cancer tumors. The experiment detected that cancer cells have elevated KPNB1 expression, and down-regulated KPNB1 after the addition of astragaloside IV to the cancer cells. A mouse prostate cancer model was

constructed, and after astragaloside IV was injected into the tail vein, KPNB1 was down-regulated in the tumor tissues, and the tumor size became smaller. It is suggested that astragaloside IV has an inhibitory effect on prostate cancer progression and that it is associated with KPNB1.

An increasing amount of research has demonstrated the anticancer benefits of herbal remedies on malignant tumors [38]. For example, astragalus methyl glycoside has the effect of inhibiting AOM/DSS-induced colitis-associated tumorigenesis [39]. Numerous malignancies have been treated with astragaloside IV on an extensive basis. Some studies have reported that astragaloside IV promotes apoptosis, reduces cell growth activity, and prevent the formation of tumors in hepatocellular carcinoma cells by controlling the miR-150-5p/β-catenin axis [40]. Wang et al. [41]determined the expression of major cycle-related genes after astragaloside IV treatment of colorectal cancer and found that astragaloside IV down-regulated Cyclin D1 and CDK4, and inactivated NF-kB. Similar to the existing studies, according to our research, astragaloside IV mediates KPNB1 to produce anti-tumor actions, decreasing the viability of prostate cancer cells, inducing apoptosis, and downregulation NF-κB and the cyclin Cyclin-B1, CDK1, and Cyclin-D1 to provide an effective drug for the cure of prostate cancer.

Through the nuclear pore complex, KPNB1 transports proteins and RNA into and out of the nucleus and mediates the participation of transcription factors such as NF-κB and cellular signaling in a variety of cellular pathways [42], and regulates a variety of pathological processes [43]. KPNB1 dysregulation transports key oncogenic molecules across the nuclear membrane to the nucleus. Important carcinogenic chemicals are transported to the nucleus through the nuclear membrane by dysregulation of KPNB1. [44], which may be involved in post-transcriptional regulatory mechanisms [45], and has a significant impact on the progression and spread of cancer [46]. According to certain research reducing KPNB1 in lung adenocarcinoma cells induced apoptosis and inhibited the proliferation of cancer cells [47]. Some studies have suggested that the suppression of cell growth and promotion of apoptosis by KPNB1 is associated with a reduction in NF-κB p65 [48,49], and some studies have suggested an association with mitotic arrest [50]. Astragaloside IV was discovered to suppress KPNB1 in our experimental investigation on prostate cancer cells, and the reduction of KPNB1 induced apoptosis, and promoted cell-cycle blockade, and decreased the expression of NF-κB and cyclin. Subcutaneous injections of prostate cancer cells were made in male nude mice, and the tail vein was used to administer astragaloside IV and KPNB1 inhibitor importazole. Observation of tumor growth showed that astragaloside IV and importazole obviously inhibited tumor growth and promoted tumor cell apoptosis. Examination of KPNB1, NF-κB and cyclin levels in tumor tissues revealed that the expression of these proteins was significantly down-regulated. Astragaloside IV mediated KPNB1 and prevented the progression of prostate cancer, according to both in vitro and in-animal experiments.

In addition, we also observed that astragaloside alone has a significant inhibitory effect on cancer cells, and the growth of tumors is also slowed down. This may be due to the anti-inflammatory and antioxidant activity of astragaloside, which can remove free radicals and reduce the damage of cells caused by oxidative stress, thus slowing the growth and proliferation of prostate cancer cells, inhibiting inflammatory response and reducing the release of inflammatory mediators. Thereby reducing the growth and spread of prostate cancer cells. This is also one of the reasons why the combined inhibition effect of astragaloside on KPNB1 is better than that of Astragaloside alone and KPNB1 inhibition intervention. Astragaloside mediates the role of KPNB1, and the reason may be that Astragaloside directly or indirectly regulates the expression of KPNB1 gene by influencing the activity of transcription factors or cell signaling pathways. Transcription factors are key proteins that regulate gene transcription, they can bind to the promoter region of a gene to promote or inhibit gene transcription, and KPNB1 may be a downstream target for multiple cell signaling pathways. Astragaloside may influence KPNB1 gene expression by regulating specific transcription factors or cell signaling pathways. KPNB1 plays an important role in the transport between the nucleus and cytoplasm, and is involved in the regulation of a variety of cell signaling pathways. Regulating the expression of KPNB1 can affect a series of tumor-related signaling pathways, thereby inhibiting the proliferation and survival of prostate cancer cells.

In conclusion, the research we conducted revealed that KPNB1 expression was linked to the advancement of prostate cancer, astragaloside IV reduced prostate cancer cell viability and cyclin levels promoted apoptosis and inhibited tumor growth by down-regulating KPNB1. Our findings prove that astragaloside IV reduces KPNB1, which may be a prospective biomarker for for prostate cancer prevention, providing a new target for the application of astragaloside IV in prostate cancer. However, there are some limitations to our study. Although we found that astragaloside inhibited the proliferation and survival of prostate cancer cells by down-regulating the expression of KPNB1, its specific mechanism of action still needs to be further studied. In addition, our study did not involve the combined application of astragaloside and other therapeutic means, and the effect and mechanism of the combined treatment are also worthy of further investigation. In future studies, clinical trials may be conducted to evaluate the safety and efficacy of astragaloside in the treatment of prostate cancer, and to explore the use of astragaloside in combination with other treatments to improve treatment effectiveness. In addition, the potential value of KPNB1 as a biomarker for prostate cancer needs to be further explored to guide clinical application and the development of individualized treatment strategies.

## Data availability statement

The datas used and/or analyzed during the current study will be made available on request. The datas are available from the corresponding author.

## Ethical statement

Animal experiments in this study were approved by the Institutional Animal Care and Use Committee, Zhejiang Center of Laboratory Animals (approval number: ZJCLA-IACUC-20010509).

## CRediT authorship contribution statement

**Quan Ma:** Writing – original draft, Conceptualization. **Xiaojun Lu:** Writing – original draft, Conceptualization. **Wei Tian:** Software, Conceptualization. **Yongliang Chen:** Investigation, Formal analysis. **Xiaozhou He:** Writing – review & editing, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33904.

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