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# Activation of Caspase-1 by NLRP3 Inflammasome Drives Malignant Progression in Prostate Cancer

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## Abstract

Inflammation is increasingly recognized as a key contributor to the onset and progression of prostate cancer (PCa). In this study, we investigated the molecular mechanisms by which the NLR family pyrin domain-containing protein 3 (NLRP3) inflammasome influences PCa malignancy. NLRP3 expression in PCa tissues and cell lines was measured using quantitative real-time PCR (qRT-PCR) and fluorescence in situ hybridization (FISH). To explore its functional role, we performed gain- and loss-of-function experiments in LNCaP and PC3 cells, assessing proliferation, apoptosis, and migration via TUNEL, CCK-8, and Transwell assays. The interplay between caspase-1 and NLRP3 was further examined through rescue experiments, western blotting, and qRT-PCR. In vivo, the tumor-promoting effects of NLRP3 were evaluated using a subcutaneous xenograft model.Our results revealed that NLRP3 expression was significantly elevated in PCa tissues and cell lines. Functionally, activation of the NLRP3/caspase-1 inflammasome by ATP+ LPS enhanced proliferation and migratory capacity while reducing apoptosis in PC3 and LNCaP cells. Western blot analysis confirmed increased caspase-1 activity following inflammasome activation. Overexpression of NLRP3 accelerated malignant behaviors, whereas silencing NLRP3 had the opposite effect, mediated at least in part through caspase-1. Rescue experiments demonstrated that modulating caspase-1 expression could reverse the effects of NLRP3 overexpression or knockdown, establishing a regulatory link between the two proteins. Consistently, NLRP3 depletion in vivo significantly impaired tumor growth. Overall, these findings highlight the critical role of the NLRP3 inflammasome in driving PCa progression via caspase-1 activation. This study provides mechanistic insight into the NLRP3/caspase-1 axis and suggests it as a promising target for therapeutic intervention and clinical management of prostate cancer.

**Keywords:** Prostate cancer, Malignant progression, LNCaP, NLRP3

## Introduction

Prostate cancer (PCa) ranks among the most prevalent malignancies in men globally, with an estimated 191,930 new diagnoses and 33,330 deaths reported in the United States in 2020 [1, 2]. The incidence of PCa has steadily risen over the past decade and exhibits notable variation across different ethnic groups, with relatively lower rates in developing countries compared to certain Western nations [3, 4]. Despite advances in diagnosis and treatment, PCa remains a major health concern due to a complex interplay of genetic predisposition, lifestyle, diet, and environmental factors [5, 6]. Moreover, challenges such as overtreatment of indolent disease and limited options for metastatic PCa underscore the need for improved diagnostic and therapeutic strategies [7, 8]. Early detection and intervention are crucial for selecting optimal treatment approaches, including radical prostatectomy, hormone therapy, chemotherapy, and radiotherapy [8]. Accumulating evidence indicates that chronic inflammation significantly contributes to tumor cell proliferation, migration, angiogenesis, and chemotherapy resistance across various cancers [9], highlighting the importance of understanding cancer-

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associated inflammation to develop more effective and safer therapies.

Inflammation has been implicated in both tumor initiation and progression [9–11]. While acute inflammation serves as a protective response against pathogens, chronic inflammation can lead to tissue damage, DNA mutations, epigenetic alterations, and genomic instability, thereby facilitating cancer development [10, 12]. Inflammasomes are intracellular multiprotein complexes that trigger inflammatory responses upon sensing danger signals [13, 14]. Among these, the NLR family pyrin domain-containing protein 3 (NLRP3) inflammasome is the most extensively studied. NLRP3 activation follows a two-step process involving priming and subsequent activation. During priming, NF-κB signaling promotes the expression of NLRP3 and related proteins, which can then respond to endogenous or microbial stimuli [15]. The activation step is triggered by factors such as pore-forming toxins, viral RNA, ATP, or particulate matter [16]. Once activated, NLRP3 serves as a platform for caspase-1 activation, which mediates the maturation of pro-inflammatory cytokines including IL-1β and IL-18 [17]. Caspase-1-dependent cell death, known as pyroptosis, plays a key role in both inflammation and tissue repair, while IL-1β and IL-18 contribute to inflammatory signaling [17, 18].

Previous studies have highlighted the pivotal role of the NLRP3 inflammasome in cancer initiation and progression [19, 20]. For instance, Chung *et al*. reported that enhanced mitochondrial oxidative phosphorylation correlates strongly with NLRP3 inflammasome activation in nasopharyngeal carcinoma [20]. However, the molecular mechanisms and functional significance of NLRP3 in PCa remain incompletely understood. Identifying NLRP3-regulated target genes and elucidating their associated signaling pathways is therefore critical for advancing research on tumor-associated inflammation. In the present study, we observed that NLRP3 expression was elevated in PCa tissues and cell lines. Functional assays demonstrated that NLRP3 downregulation inhibited proliferation, migration, and apoptosis in PCa cells. Mechanistically, NLRP3 inflammasome promoted malignant progression by epigenetically upregulating caspase-1 expression. Collectively, these findings provide new insights into the role and regulatory mechanisms of the NLRP3 inflammasome, offering potential avenues for improving PCa diagnosis and treatment.

## Materials and Methods

*PCa samples and patients*

In this study, paired tumor and adjacent non-cancerous tissues were collected from 30 patients who underwent radical prostatectomy for prostate cancer. None of the participants had received radiotherapy or chemotherapy prior to surgery. Tumor specimens were classified and staged according to the International Union Against Cancer (UICC) TNM criteria. For each patient, tumor and corresponding non-tumor tissue samples were obtained following pathological confirmation. Immediately after excision, tissue fragments were snap-frozen in liquid nitrogen and stored at −80 °C until further experimental use. Written informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee of Nanjing Medical University.

*Reagents and cell culture*

Human prostate cancer cell lines (PC3, DU-145, 22RV1, LNCaP) and the immortalized normal prostate stromal cell line WPMY-1 were obtained from ATCC. PC3, DU-145, and 22RV1 cells were maintained in F-12K medium, while LNCaP and WPMY-1 were cultured in RPMI-1640 medium (Life Technologies, USA), all supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$.

adenosine triphosphate (ATP) and Lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human recombinant caspase-1 protein (BioVision, USA) was used to enhance caspase-1 activity, while the caspase-1 inhibitor Z-YVAD-FMK (PromoCell, Germany) was applied to suppress its activation. For inhibitor experiments, cells were pretreated with 10 µM Z-YVAD-FMK for 30 minutes before LPS stimulation. NLRP3 inflammasome activation was induced by treating cells with 1 µg/mL LPS for 8 hours, with or without 5 mM ATP added during the last 30 minutes.

*ELISA for cytokine detection*

PC3 and LNCaP cells were plated in 24-well plates and allowed to adhere overnight. Following LPS ± ATP treatment, cell culture supernatants and lysates were collected and centrifuged at 300 × g for 8 minutes at 4 °C to remove detached cells. The supernatants were further

concentrated using a 10 kDa cutoff centrifugal filter (Microcon, Merck-Millipore, Germany) at 12,000 × g for 30 minutes at 4 °C. Levels of IL-1β and IL-18 were quantified using human ELISA kits (R&D Systems, USA; MBL, Japan) according to the manufacturer's instructions.

*Lentiviral transfection*

For stable gene manipulation, lentiviral vectors encoding shRNA targeting NLRP3 (sh-NLRP3) or overexpression constructs (ov-NLRP3), along with corresponding negative controls (sh-NC and ov-NC), were packaged using the LV3-pGLV-h1-GFP-puro system (GenePharma, China). PC3 and LNCaP cells were transduced, and stable cell populations were selected with 4 μg/mL puromycin for two weeks. Selected cells were then used for functional assays including proliferation, migration, and apoptosis analyses.

*Immunohistochemistry (IHC)*

Paraffin-embedded tissue sections were deparaffinized, rehydrated, and washed with PBS. Antigen retrieval was performed by microwaving for 20 minutes, followed by cooling at room temperature for 1 hour. Endogenous peroxidase activity was blocked with 3% $H_2O_2$ for 15 minutes. Sections were incubated overnight at 4 °C with primary antibodies against NLRP3 and caspase-1 (Cell Signaling Technology, USA). After washing, sections were incubated with HRP-conjugated secondary antibody (1:100) for 1 hour, developed with DAB, and visualized under a light microscope.

*Immunofluorescence (IF) staining*

PC3 and LNCaP cells were fixed in 4% paraformaldehyde for 30 minutes, permeabilized with 0.5% Triton X-100 for 15 minutes, and blocked with 5% BSA for 1 hour at room temperature. Cells were incubated overnight at 4 °C with anti-NLRP3 antibody (1:200, Abcam, UK), followed by incubation with Alexa Fluor–conjugated secondary antibody (1:500, Invitrogen) for 1 hour. Nuclei were counterstained with Hoechst 33342 (Beyotime, China) for 30 minutes. Confocal images were captured using a Zeiss LSM5 Live microscope (Carl Zeiss, Germany).

*Cell proliferation assay (CCK-8)*

Cell proliferation was evaluated using the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Japan). PCa cells were seeded in 96-well plates at a density of 3,000 cells per well, with five replicate wells per group, and cultured in complete medium (200 μL per well) at 37 °C under 5% $CO_2$. At designated time points (0, 1, 2, 3, and 4 days), 10 μL of CCK-8 solution (5 mg/mL) was added to each well and incubated for an additional 4 hours. Absorbance at 450 nm was measured using a microplate reader, and growth curves were generated to assess cell proliferation.

*TUNEL assay for apoptosis*

Apoptotic cells were detected using the In Situ Apoptosis Detection Kit (Roche, Basel, Switzerland) on cells fixed with 4% paraformaldehyde, following the manufacturer's protocol. Nuclei of apoptotic cells were stained brown under a fluorescence microscope and counted manually in a blinded manner. The apoptotic index was calculated as the percentage of TUNEL-positive cells among the total number of cells. Counts were performed in five randomly selected high-power fields (×400) per slide, with approximately 100 cells analyzed per field.

*Transwell migration assay*

Cell migratory ability was assessed using Transwell chambers (24-well inserts) with uncoated membranes. Transfected PC3 and LNCaP cells were suspended in 200 μL of serum-free medium and added to the upper chamber. The lower chamber contained 400 μL of complete medium with 10% fetal bovine serum as a chemoattractant. Cells were allowed to migrate for 24 hours. Non-migrated cells on the upper surface were removed with a cotton swab, while cells that migrated to the lower surface were fixed in 4% formaldehyde and stained with crystal violet. Migrated cells were quantified under a light microscope.

*Quantitative real-time PCR (qRT-PCR)*

Total RNA was extracted from PCa cell lines and tissue samples using the RNeasy Mini Kit (ThermoFisher Scientific, USA) according to the manufacturer's instructions. Samples were lysed in 1 mL of TRIzol reagent, treated with DNase I to remove genomic DNA contamination, and purified. cDNA synthesis was performed using the PrimeScript Reverse Transcription Kit (Takara, Japan). Real-time PCR amplification was carried out using SYBR® Premix Ex Taq™ (Takara) on a StepOne Plus Real-Time PCR System (Applied Biosystems, USA). Each sample was assayed in triplicate and repeated twice. Data analysis was performed using Bio-Rad iQ5 software (version 2.0), with GAPDH as the

internal control. Relative expression levels of NLRP3 and caspase-1 were calculated using the 2^−ΔΔCt method. The primer sequences used for amplification were as follows:

NLRP3:
reverse, 5′-TTGTCTCCGAGAGTGTTGCC-3′;
forward, 5′-ATGTGGGGGAGAATGCCTTG-3′,
caspase-1:
forward, 5′-GCAATGAAGACGAAGGCGAC-3′,
reverse, 5′-GTGCCCGTGCGAGATTTTAG-3′;
GAPDH:
reverse, 5′-GAAGATGGTGATGGGATTTC-3′.
forward, 5′-GAAGGTGAAGGTCGGAGTC-3′,

*Protein detection by western blot*

Cells or tissue samples were lysed in appropriate lysis buffer and incubated on ice for 30 minutes. Lysates were centrifuged at 14,000 × g for 15 minutes at 4 °C to remove debris. Supernatants were collected, denatured by heating, and stored at −20 °C. Protein concentrations were quantified using the BCA Protein Assay Kit (Pierce, Rockford, USA). Primary antibodies targeting NLRP3, caspase-1, and pro-caspase-1 were obtained from Santa Cruz Biotechnology (USA), while HRP-conjugated goat anti-rabbit secondary antibody was sourced from Genscript. GAPDH served as the internal loading control. Equal amounts of protein were separated on SDS-PAGE gels and transferred onto PVDF membranes. Membranes were blocked in 5% skim milk for 1 hour at room temperature, incubated with primary antibodies overnight at 4 °C, washed, and then incubated with secondary antibodies for 1 hour. Band intensities were visualized and semi-quantified using Alpha SP imaging software.

*In Vivo tumorigenicity in nude mice*

Animal studies were approved by the Animal Ethics and Use Committee of Nanjing Medical University. Eight-week-old male nude mice were randomly assigned to two groups (n = 5 per group). PC3 cells stably expressing sh-NLRP3 or sh-NC were injected subcutaneously into the axillary region. Tumor growth was measured weekly, and mice were euthanized six weeks after injection. Tumor volumes were calculated using the formula:

$$\text{tumor volume} = (\text{width}^2 \times \text{length})/2. \quad (1)$$

*Statistical analysis*

Data analyses were performed using SPSS 22.0 software. Categorical variables were analyzed with the $\chi^2$ test or Fisher's exact test, whereas multivariate relationships were evaluated via Cox regression analysis. Kaplan–Meier curves were used to examine survival outcomes. Comparisons between two groups were conducted using Student's t-test. Pearson correlation analysis was employed to assess the relationship between NLRP3 and caspase-1 expression levels in PCa tissues. All results are expressed as mean ± standard deviation (SD), with $P < 0.05$ considered statistically significant.

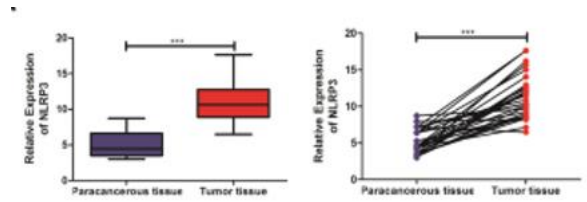
## Results and Discussion

*Elevated NLRP3 expression in PCa tissues and cell lines*

To investigate the association between NLRP3 expression and malignant progression in prostate cancer (PCa), data from PCa patient samples were analyzed. As shown in **Figure 1a**, NLRP3 expression was significantly higher in PCa tissues compared to normal controls ($P < 0.05$). Among the cohort, 12 patients were diagnosed with hormone therapy-refractory prostate cancer (HTR-PCa). A comparison between HTR-PCa and non-hormone therapy-refractory PCa (non-HTR-PCa) tissues revealed that NLRP3 expression was elevated in HTR-PCa samples relative to non-HTR-PCa **(Figure 1b).**

Consistently, qRT-PCR analysis demonstrated increased NLRP3 expression in PCa cell lines, with PC3 cells showing relatively higher levels and LNCaP cells exhibiting lower levels, which were subsequently used for functional assays **(Figure 1c).** According to predictions from the lncATLAS database (http://lncatlas.crg.eu/), NLRP3 is primarily localized in the cytoplasm of PCa cells, a finding confirmed experimentally via immunofluorescence staining **(Figure 1d).**

To further validate these findings in clinical samples, paired tumor and adjacent non-cancerous tissues from PCa patients were subjected to immunohistochemical analysis. The results indicated that NLRP3 was markedly upregulated in tumor tissues compared to matched paracancerous tissues **(Figure 1e).** Collectively, these data demonstrate that NLRP3 is highly expressed in both PCa tissues and cell lines, suggesting that it may function as an oncogenic factor in PCa.

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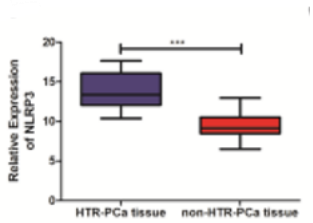


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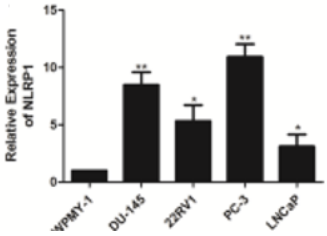


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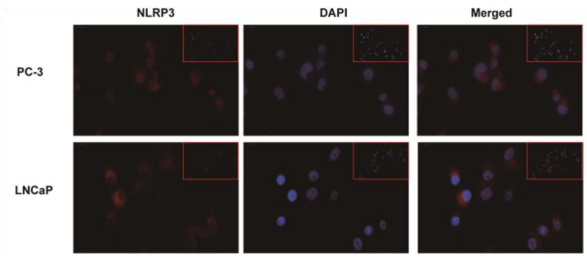


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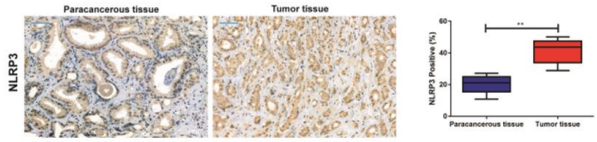



e)

**Figure 1.** NLRP3 is Highly Expressed in PCa Tissues and Cell Lines

a) The expression of NLRP3 was analyzed in paired tumor and adjacent non-tumor tissues from PCa patients using qRT-PCR, revealing a marked increase in tumor samples. b) In addition, qRT-PCR comparisons between hormone therapy-refractory PCa (HTR-PCa) tissues and non-HTR-PCa tissues showed that NLRP3 levels were higher in HTR-PCa samples. c) NLRP3 expression was also measured across PCa cell lines, with differential expression observed, guiding the selection of PC3 (high expression) and LNCaP (low expression) for subsequent functional studies. d) FISH analysis confirmed the primarily cytoplasmic localization of NLRP3 in PC3 and LNCaP cells (magnification: ×40). e) Immunohistochemical staining of patient samples further validated the elevated expression of NLRP3 in tumor tissues compared to matched adjacent tissues. Data are presented as mean ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.

*High NLRP3 expression is associated with advanced TNM stage and lymph node metastasis in PCa*

A total of 30 paired tumor and adjacent non-cancerous tissue samples were divided into high and low NLRP3 expression groups. Associations between NLRP3 levels and clinicopathological features—including age, serum PSA, Gleason score, tumor size, TNM stage, and lymph node metastasis—were evaluated. As summarized in **Table 1**, patients with elevated NLRP3 expression exhibited a higher TNM stage and more frequent lymph node involvement compared to those with low NLRP3 expression. These results suggest that NLRP3 may play a role in driving the malignant progression of prostate cancer.

**Table 1.** Correlation of NLRP3 Expression with Clinicopathological Features in Prostate Cancer Patients

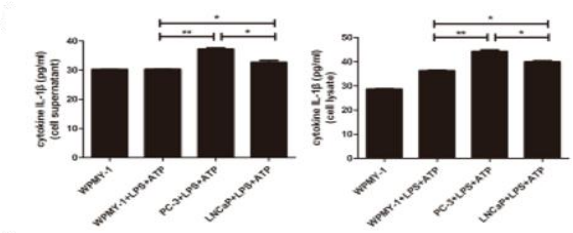
| Variables | Patient number (*N*) | NLRP3 expression | | *P*-value |
|---|---|---|---|---|
| | | Low (*n* = 12) | High (*n* = 18) | |
| **Age (years)** | | | | 0.765 |
| **≤60** | 14 | 6 | 8 | |
| **>60** | 16 | 6 | 10 | |
| **Tumor size (cm)** | | | | 0.171 |
| **≤2** | 12 | 3 | 9 | |
| **>2** | 18 | 9 | 9 | |
| **Gleason score** | | | | 0.879 |
| **≤6** | 12 | 5 | 7 | |
| **>6** | 18 | 7 | 11 | |
| **TNM stage** | | | | **0.044*** |
| **I and II** | 11 | 7 | 4 | |
| **III and IV** | 19 | 5 | 14 | |
| **Lymph node metastasis** | | | | **0.018*** |
| **No** | 10 | 7 | 3 | |
| **Yes** | 20 | 5 | 15 | |
| **Serum PSA (ng/ml)** | | | | 0.171 |

| | | | |
|---|---|---|---|
| **≤10** | 12 | 3 | 9 |
| **>10** | 18 | 9 | 9 |

Expression levels were categorized as low or high based on the median value of the samples, and associations were analyzed using the Pearson $\chi^2$ test, with *P < 0.05 considered statistically significant.

*LPS + ATP induces NLRP3/caspase-1 inflammasome activation in prostate cancer cells*

To determine whether NLRP3 inflammasome formation could be triggered by LPS and ATP, we examined their effects in PC3 and LNCaP cells. As shown in **Figure 2a** and 2B, ELISA measurements indicated that treatment with LPS + ATP led to a significant increase in IL-1β and IL-18 levels in both the culture supernatant and cell lysates, relative to the WPMY-1 control. Similarly, comparisons between treated and untreated PC3 and LNCaP cells demonstrated that extracellular IL-1β and IL-18 concentrations were substantially elevated following LPS + ATP exposure **(Figures 2c and 2d).** These observations collectively suggest that LPS + ATP efficiently promotes the assembly and activation of the NLRP3/caspase-1 inflammasome in prostate cancer cell lines.

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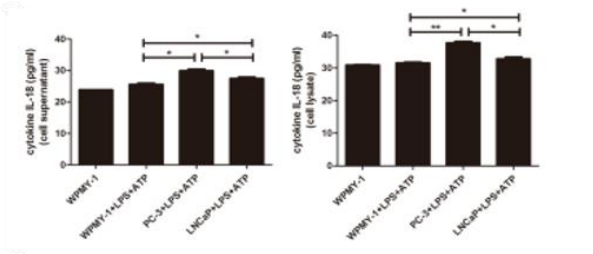


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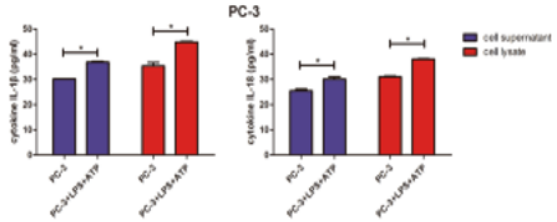


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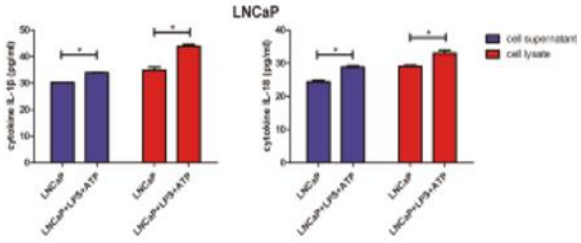


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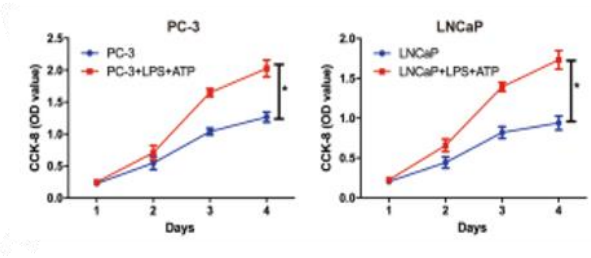


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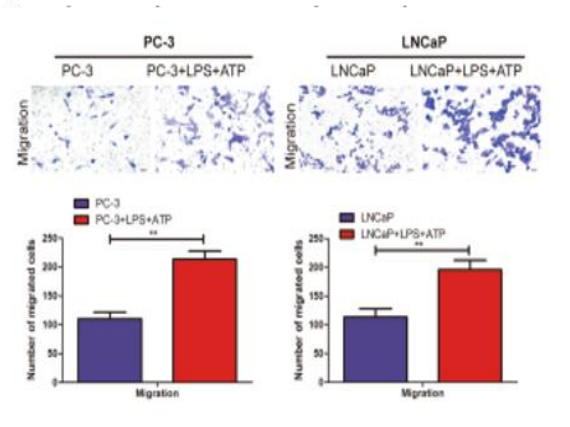


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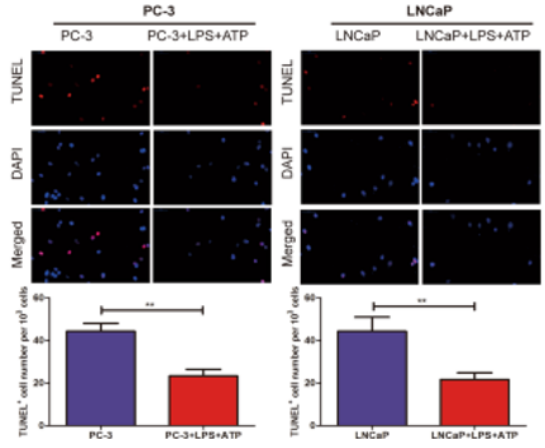


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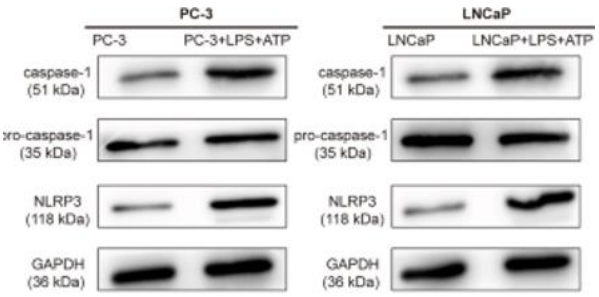



h)

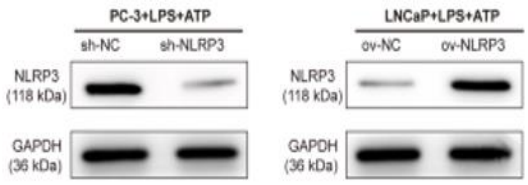
**Figure 2.** LPS and ATP Trigger NLRP3 Inflammasome Activation and Promote Malignant Traits in PCa Cells a–b) ELISA analysis showed that treatment with LPS combined with ATP markedly elevated levels of cleaved IL-1β and IL-18 in both the culture supernatants and cell lysates of PC3 and LNCaP cells, whereas no significant change was observed in WPMY-1 cells. c–d) Single treatments with LPS or ATP alone did not significantly alter cytokine levels, indicating that the synergistic effect of LPS and ATP is required for NLRP3 inflammasome activation. e) Cell proliferation assays (CCK-8) revealed that LPS + ATP exposure enhanced proliferation in both PCa cell lines. F Transwell migration assays demonstrated increased migratory ability following the combined stimulation (magnification: ×40). g) TUNEL staining indicated that apoptotic rates were reduced after LPS + ATP treatment (magnification: ×40). h) Western blotting confirmed that protein levels of NLRP3 and caspase-1 were upregulated in response to LPS + ATP. Data are presented as mean ± SD, scale bar: 20 μm, *$p < 0.05$, **$p < 0.01$.

*Activation of the NLRP3 Inflammasome Accelerates Malignant Progression in PC3 and LNCaP Cells*

CCK-8 analysis **(Figure 2e)** demonstrated that stimulation with LPS combined with ATP significantly increased the proliferative capacity of PC3 and LNCaP cells, whereas treatment with LPS or ATP alone did not produce a similar effect. Transwell migration assays further showed that exposure to LPS + ATP markedly enhanced the migratory potential of prostate cancer cell lines compared with single-agent treatments **(Figure 2f).** To evaluate apoptotic responses, TUNEL staining was performed, revealing that LPS + ATP stimulation significantly reduced apoptosis in both PC3 and LNCaP cells **(Figure 2g).** Collectively, these findings indicate that activation of the NLRP3 inflammasome promotes proliferation and migration while suppressing apoptosis in PCa cell lines. Consistent with these functional changes, western blot analysis showed that NLRP3 and caspase-1 protein levels were significantly elevated following LPS + ATP treatment compared with LPS or ATP alone **(Figure 2h)**, ($P < 0.05$).

*NLRP3 overexpression or knockdown differentially regulates malignant behaviors in PCa cells*

To further investigate the role of NLRP3 in prostate cancer progression, CCK-8, Transwell, and TUNEL assays were conducted in PC3 and LNCaP cells following genetic manipulation of NLRP3 expression. Successful construction of NLRP3 overexpression and knockdown models was confirmed by western blotting **(Figure 3a)** and qRT-PCR analysis **(Figure 3b).** ELISA results showed that, under LPS + ATP stimulation, levels of IL-1β and IL-18 in both cell lysates and culture supernatants were significantly increased in NLRP3-overexpressing cells and markedly reduced in NLRP3-knockdown cells compared with ov-NC and sh-NC controls, respectively **(Figures 3c and 3d).** CCK-8 assays revealed that silencing NLRP3 significantly suppressed cell proliferation, whereas NLRP3 overexpression enhanced proliferative activity in both PC3 and LNCaP cells under LPS + ATP treatment **(Figure 3e).** Similarly, Transwell assays showed reduced or enhanced migratory capacity in sh-NLRP3 or ov-NLRP3 cells, respectively, relative to their corresponding controls **(Figure 3f).** TUNEL staining further indicated that apoptosis was increased following NLRP3 knockdown but decreased in NLRP3-overexpressing cells compared with sh-NC and ov-NC groups **(Figure 3g)**. In line with these results, western blot analysis demonstrated that NLRP3 and caspase-1 expression levels were significantly decreased or increased in sh-NLRP3 or ov-NLRP3 cells, respectively, under LPS + ATP stimulation **(Figure 3h).** Overall, these data confirm that activation of the NLRP3/caspase-1 inflammasome axis promotes malignant progression in prostate cancer cells.

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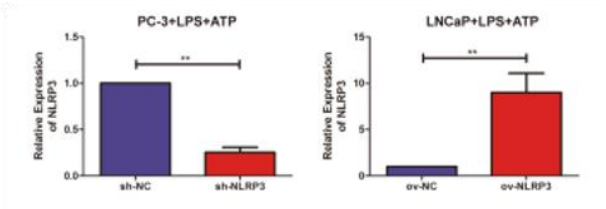


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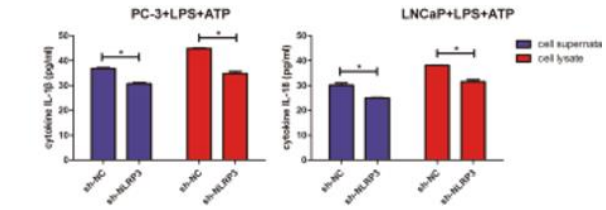


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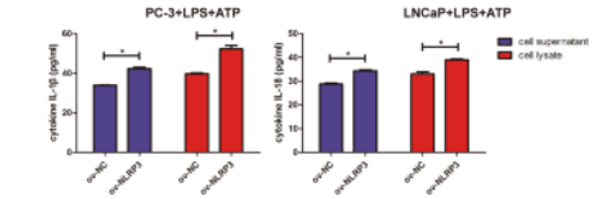


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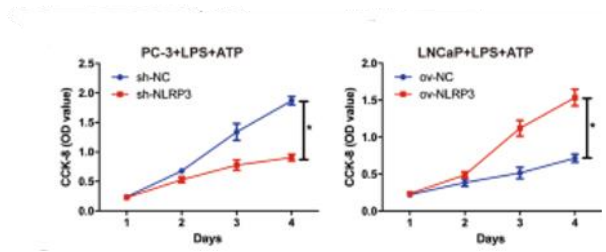


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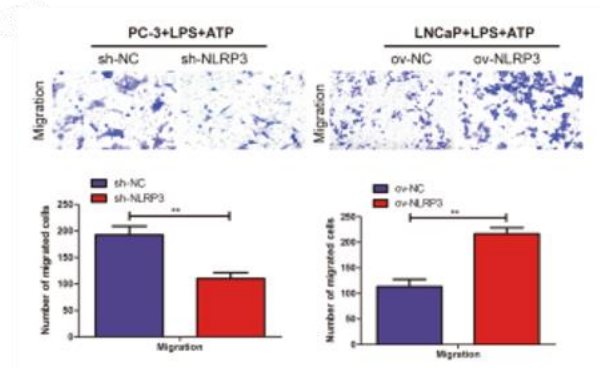


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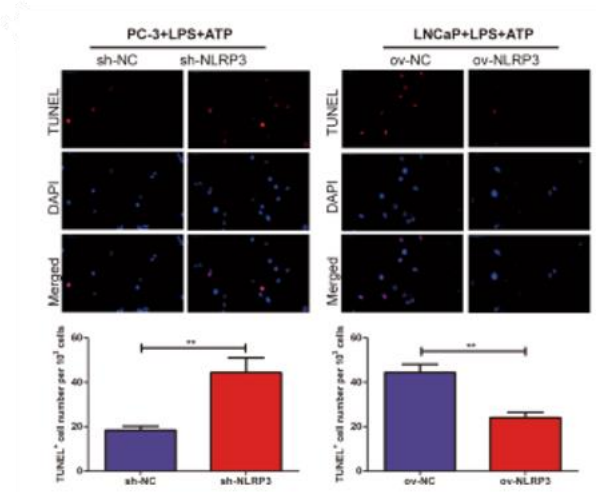


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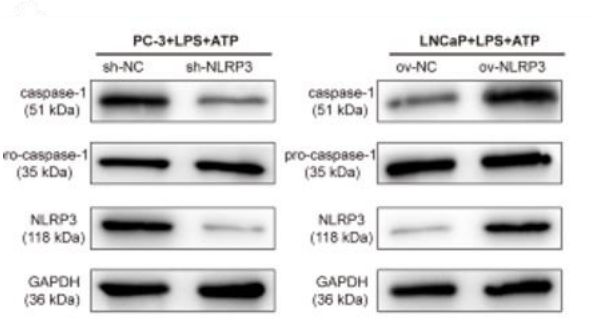


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**Figure 3.** Modulation of NLRP3 Influences Malignant Behavior in PCa Cells

a-b) Western blot and qRT-PCR analyses confirmed efficient overexpression and knockdown of NLRP3 in PC3 and LNCaP cell lines. c-d) ELISA results showed that IL-1β and IL-18 levels in both the supernatant and cell lysates were significantly reduced following NLRP3 knockdown (sh-NLRP3), whereas overexpression of NLRP3 (ov-NLRP3) led to a substantial increase in these cytokines relative to controls. e) CCK-8 assays demonstrated that NLRP3 overexpression enhanced cell proliferation, while knockdown impaired proliferation. f) Transwell migration assays revealed that cell motility was promoted in ov-NLRP3 cells and suppressed in sh-NLRP3 cells (magnification: ×40). g) TUNEL assays indicated that apoptosis was reduced upon NLRP3 overexpression and increased following NLRP3 knockdown (magnification: ×40). h) Western blotting confirmed corresponding changes in NLRP3 and caspase-1 protein levels after modulation of NLRP3

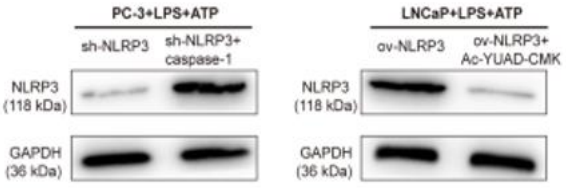
expression. Data are presented as mean ± SD, *p < 0.05, **p < 0.01.

*NLRP3 promotes oncogenic activity via caspase-1 activation in PCa cells*

To elucidate the molecular mechanism by which NLRP3 drives malignant progression, we investigated its regulation of caspase-1. PC3 and LNCaP cells were treated with the caspase-1 inhibitor Z-YVAD-FMK to suppress endogenous caspase-1 activity, resulting in a significant decrease in NLRP3 expression, as verified by western blot and qRT-PCR. Conversely, treatment with recombinant human caspase-1 protein increased NLRP3 levels in PCa cells **(Figures 4a and 4b).**

ELISA assays demonstrated that co-treatment of sh-NLRP3 cells with recombinant caspase-1 under LPS + ATP stimulation restored IL-1β and IL-18 secretion, which was otherwise reduced by NLRP3 knockdown alone **(Figure 4c).** Similarly, combining ov-NLRP3 with Z-YVAD-FMK markedly attenuated cytokine levels relative to ov-NLRP3 alone under LPS + ATP stimulation **(Figure 4d).** Functional assays further showed that recombinant caspase-1 and Z-YVAD-FMK could counteract the effects of NLRP3 modulation on proliferation, migration, and apoptosis in PCa cells, as assessed by CCK-8, Transwell, and TUNEL assays **(Figures 4e–4g).** Western blotting confirmed that NLRP3 and caspase-1 protein levels were restored or suppressed in cells treated with sh-NLRP3 + recombinant caspase-1 or ov-NLRP3 + Z-YVAD-FMK, respectively, compared with cells with NLRP3 modulation alone **(Figure 4h).**

Collectively, these findings indicate that the NLRP3 inflammasome promotes oncogenic phenotypes in prostate cancer cells through activation of caspase-1.

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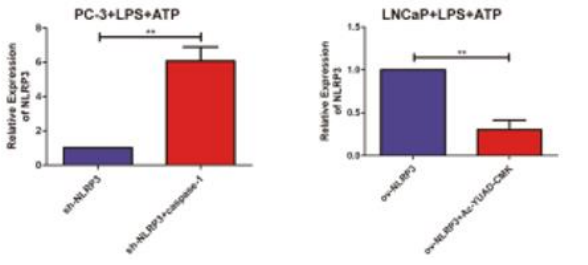


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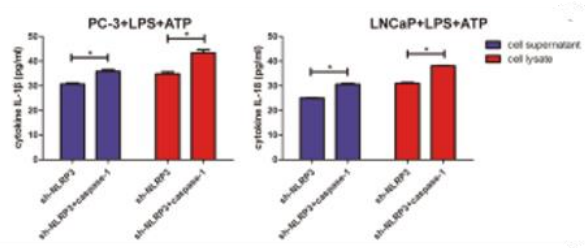


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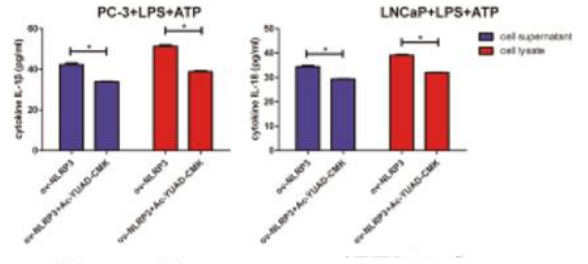


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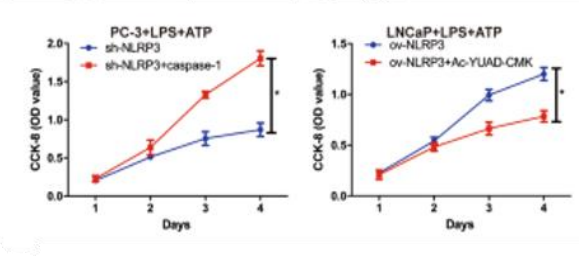


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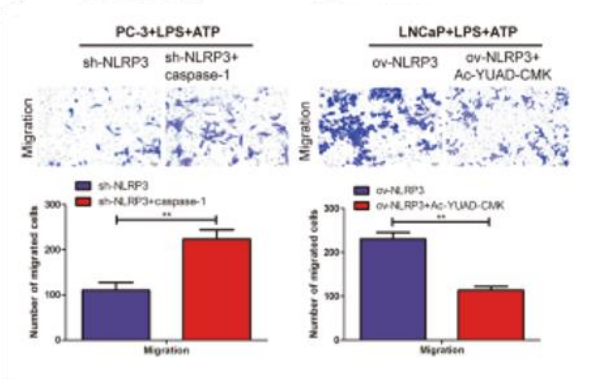


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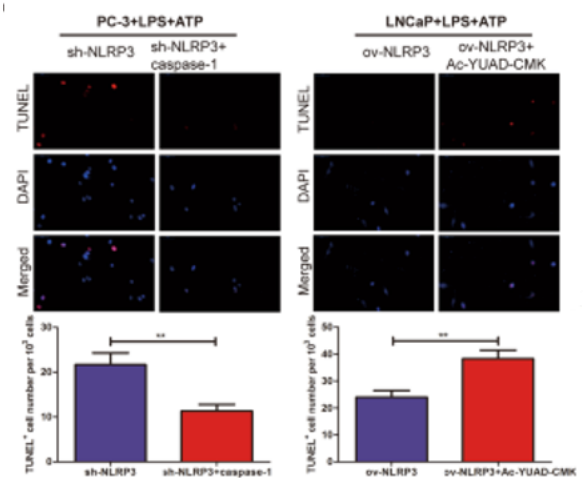


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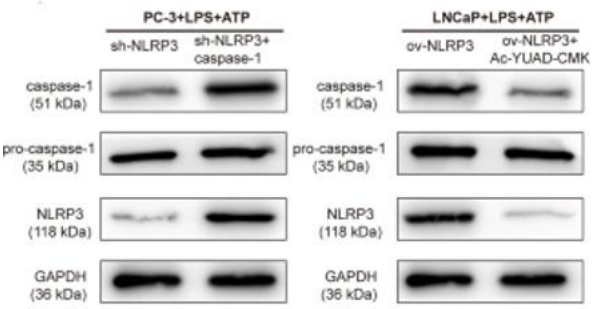


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**Figure 4.** NLRP3 Inflammasome Drives Oncogenic Activity via Caspase-1 in PCa Cells

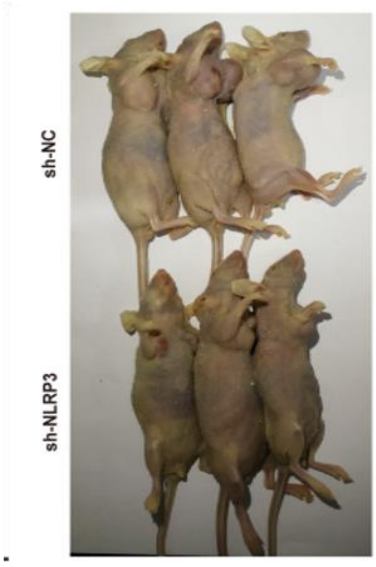
a-b) Western blot and qRT-PCR analyses confirmed efficient modulation of NLRP3 in PC3 and LNCaP cells following combined treatments: sh-NLRP3 with recombinant caspase-1 and ov-NLRP3 with the caspase-1 inhibitor Z-YVAD-FMK. c–d ELISA assays revealed that IL-1β and IL-18 levels in both the supernatants and lysates were restored in sh-NLRP3 cells when supplemented with recombinant caspase-1, while the addition of Z-YVAD-FMK to ov-NLRP3 cells significantly suppressed these cytokines compared with ov-NLRP3 alone. e) CCK-8 assays showed that proliferation was rescued in sh-NLRP3 cells by recombinant caspase-1 and attenuated in ov-NLRP3 cells by Z-YVAD-FMK. f) Transwell migration assays indicated that cell motility followed a similar pattern: enhanced by recombinant caspase-1 in sh-NLRP3 cells and reduced by Z-YVAD-FMK in ov-NLRP3 cells (magnification: ×40). g) TUNEL staining revealed that apoptosis was reversed in sh-NLRP3 cells with recombinant caspase-1 and increased in ov-NLRP3 cells treated with Z-YVAD-FMK (magnification: ×40). h) Western blotting confirmed that NLRP3 and caspase-1 protein levels were correspondingly restored or suppressed under these treatments. Data are presented as mean ± SD, *p < 0.05, **p < 0.01.

These findings demonstrate that the oncogenic effects of NLRP3 in prostate cancer cells are largely mediated through activation of caspase-1.

*Knockdown of NLRP3 suppresses PCa tumor growth in vivo*

To examine the role of NLRP3 in tumorigenesis, PC3 cells stably transfected with sh-NLRP3 or sh-NC were subcutaneously injected into the left flank of nude mice. Tumor growth was markedly delayed in mice receiving sh-NLRP3-transfected cells compared to controls **(Figures 5a and 5b).** Correspondingly, the body weights of mice bearing sh-NLRP3 tumors were slightly reduced relative to the sh-NC group **(Figure 5c).** Analysis of excised tumors revealed decreased NLRP3 and caspase-1 protein levels following NLRP3 knockdown **(Figures 5d and 5e).** Immunohistochemical staining confirmed a significant reduction of NLRP3 and caspase-1 expression in sh-NLRP3 xenografts compared with sh-NC xenografts **(Figure 5f).**

Overall, these in vivo data support the conclusion that NLRP3 contributes to prostate cancer tumor growth, likely through regulation of the caspase-1 pathway.

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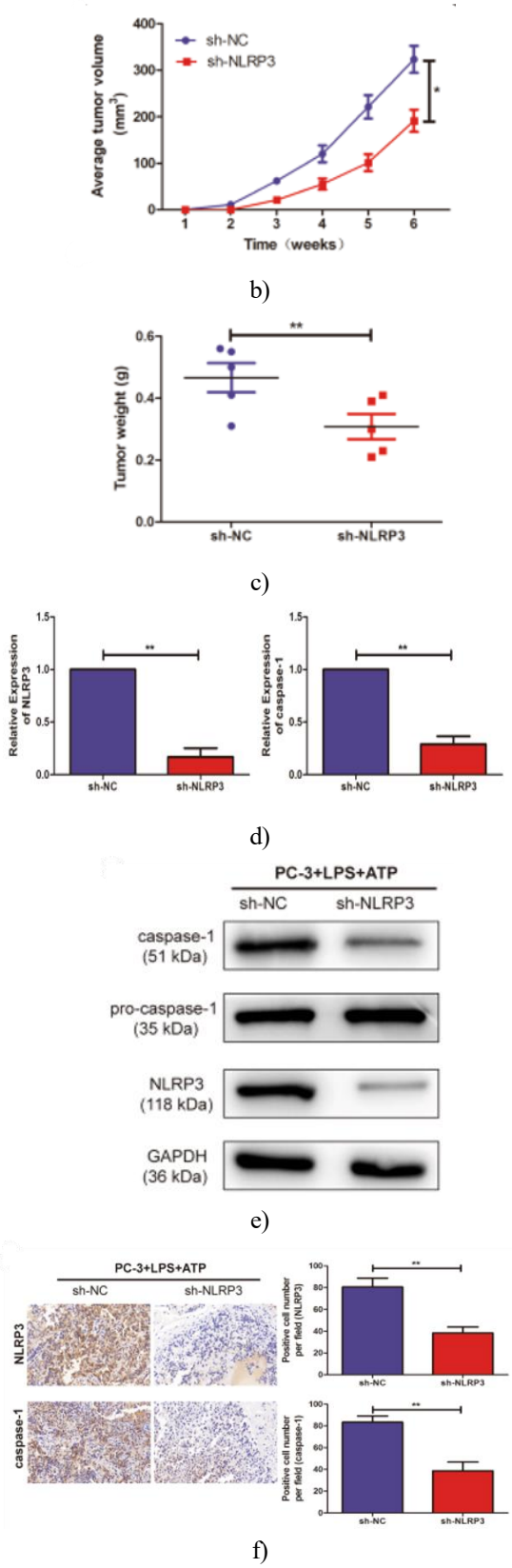


**Figure 5.** NLRP3 Knockdown Suppresses Tumorigenicity in Nude Mice

a) Representative images of tumors derived from sh-NC and sh-NLRP3 PC3 cells in nude mice are shown. b) Tumor growth curves over time demonstrate that tumors from sh-NLRP3-transfected cells grew more slowly compared with sh-NC controls. c) Tumor weight measurements at the endpoint indicate a significant reduction in mass in the sh-NLRP3 group. d) qRT-PCR analysis of excised tumor tissues revealed decreased expression of NLRP3 and caspase-1 in sh-NLRP3 xenografts. e) Western blotting confirmed the reduction of NLRP3 and caspase-1 protein levels in tumors from sh-NLRP3 mice relative to controls. f) Immunohistochemical staining further validated the diminished expression of NLRP3 and caspase-1 in sh-NLRP3 xenografts (magnification: ×40). Data are presented as mean ± SD, *$p < 0.05$, **$p < 0.01$

Prostate cancer (PCa) is one of the most frequently diagnosed malignancies worldwide, ranking second in incidence and fifth in mortality [1, 3]. Although the prevalence of PCa in China is lower than in Western countries, its rate of increase is considerably faster [3]. As China faces an aging population, PCa is expected to become a major health threat to elderly men [3, 8]. Consequently, both the clinical management and basic research of PCa have become pressing priorities [4, 6]. Previous studies have highlighted the role of inflammasomes, which are activated by microbial or viral infections and sense both exogenous and endogenous danger signals, in initiating inflammatory responses through cytokines, chemokines, and extracellular matrix remodeling [12, 14, 15]. Recent evidence suggests that inflammasomes also contribute to multiple stages of tumor development, impede immune-mediated tumor suppression, and reduce treatment efficacy [9, 10, 13]. Among inflammasomes, NLRP3 is widely expressed in epithelial cells, macrophages, keratinocytes, and dendritic cells [16, 17]. The NLRP3 inflammasome exhibits broad tissue expression, cell specificity, and strong functional potential, making it a promising target for molecular diagnostics and tumor-targeted therapies [18, 20]. Based on next-generation sequencing of PCa tissues in the Chinese population, we sought to further explore tumor-associated inflammasomes, characterize their molecular mechanisms, and identify NLRP3 as a potential biomarker for early detection. Such insights could improve understanding of PCa cell behavior and aid in the development of novel therapeutic strategies [21]. Activation of the NLRP3 inflammasome typically

involves a priming step, such as stimulation with a TLR agonist like LPS, followed by a secondary signal such as ATP [22]. In PC3 and LNCaP cells, LPS + ATP stimulation increased proliferation and migration while reducing apoptosis.

NLRP3 has long been implicated in numerous physiological and pathological processes, including cancer progression [19, 20, 23]. In this study, qRT-PCR analysis revealed elevated NLRP3 expression in PCa tissues and cell lines. Functionally, NLRP3 expression correlated with malignant progression, suggesting its role as a potential oncogene in PCa. CCK-8, TUNEL, and Transwell assays demonstrated that NLRP3 activation enhanced proliferation and migration while inhibiting apoptosis in PC3 and LNCaP cells. In vivo, silencing NLRP3 significantly suppressed tumor growth in nude mice, though the precise molecular mechanisms remained unclear.

One reported mechanism of NLRP3 inflammasome function involves recruitment of proteins or RNAs to target genes, exerting regulatory effects indirectly [23, 24]. To investigate its role in PCa pathogenesis, we performed subcellular fractionation, which revealed that NLRP3 predominantly localized to the nucleus in PC3 and LNCaP cells, suggesting transcriptional regulation. qRT-PCR and western blot analyses showed that caspase-1 levels decreased upon NLRP3 knockdown and increased with NLRP3 overexpression. Given that NLRP3 inflammasome activation triggers caspase-1–mediated cytokine release, we examined whether caspase-1 deficiency affected cellular responses [25]. Our results indicate that NLRP3 epigenetically upregulates caspase-1 in PCa cells.

## Conclusion

We further observed that NLRP3 expression in PCa tissues and cell lines positively correlated with caspase-1 levels. Reverse experiments in vitro and in vivo demonstrated that the tumor-promoting effects of NLRP3 are mediated through caspase-1 activation. In conclusion, this study elucidates a molecular mechanism by which the NLRP3 inflammasome drives PCa malignancy via caspase-1. These findings provide new insights for the research and clinical management of PCa, positioning NLRP3 inflammasome as a potential prognostic biomarker and therapeutic target.

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**Conflict of Interest:** None

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**Ethics Statement:** None

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