Expression and clinical value of NLRP1 and NLRC4 inflammasomes in prostate cancer

KE LIANG[1], ZUNJIN KE[1], JIANHONG HUANG[1] and XIJIONG ZHANG[2]

Departments of [1]Urology and [2]Pathology, The First People's Hospital of Pinghu, Pinghu, Zhejiang 314200, P.R. China

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Abstract. The present study explored the clinical value of the protein expression levels of nucleotide binding oligomerization-like receptor family pyrin domain containing 1 (NLRP1) and nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4 (NLRC4) inflammasomes in the diagnosis and treatment of prostate cancer. A total of 54 patients with prostatic hyperplasia and 58 patients with prostate cancer were recruited at The First People's Hospital of Pinghu between January and May 2022. Immunohistochemical staining was used to determine the protein expression levels of the NLRP1 and NLRC4 inflammasomes in addition to the proinflammatory cytokines IL-18 and IL-1β in the two groups of patients. The protein expression levels of NLRP1 and NLRC4 inflammasome were significantly increased in patients with prostate cancer compared with patients with prostate hyperplasia. The differences in expression of NLRP1 and NLRC4 inflammatory vesicles in prostate cancer of different stages were also compared based on data from The Cancer Genome Atlas. The protein expression level of NLRP1 demonstrated a significant positive correlation with IL-1β and IL-18 expression, and the protein expression level of the NLRC4 inflammasome was significantly positively correlated with IL-18 expression. The protein expression levels of both NLRP1 and NLRC4 demonstrated a significant positive correlation with the Gleason score of prostate cancer. The expression of NLRP1 in tumor (T)3/T4 was significantly higher compared with T1 and expression of the NLRC4 inflammasome in T2 and T3/T4 was significantly higher compared with T1. Expression of the NLRP1 and NLRC4 inflammasomes was significantly higher in patients with prostate cancer, compared with patients with prostatic hyperplasia. Therefore, expression of NLRP1 and NLRC4 may promote tumorigenesis by promoting the maturation and release of proinflammatory cytokines IL-1β and IL-18. Expression of the NLRP1 and NLRC4 inflammasomes demonstrated a significant positive correlation with the risk of prostate cancer. Expression of the NLRP1 and NLRC4 inflammasomes in middle- and advanced-stage tumors was higher compared with early-stage tumors. These results suggested that inflammasome expression may serve a significant role in the progression of tumors and could provide a fixed value for the risk assessment and prognosis prediction of prostate cancer.

Correspondence to: Dr XiJiong Zhang, Department of Pathology, The First People's Hospital of Pinghu, 500 Sangang Road, Pinghu, Zhejiang 314200, P.R. China
E-mail: zhangxijiong772@126.com

Abbreviations: NLRP, nucleotide binding oligomerization-like receptor family pyrin domain containing; NLRC4, nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4; PSA, prostate specific antigen; TPSA, total prostate specific antigen; FPSA, free prostate specific antigen; ASC, apoptosis-associated speck-like protein containing CARD; CARD, caspase recruitment domain; NLR, nucleotide-binding oligomerization domain-like receptor; H&E, hematoxylin and eosin; PYD, pyrin domain; LRR, leucine-rich repeat; TME, tumor microenvironment; IL, interleukin; TCGA, The Cancer Genome Atlas; PV, prostate volume; BMI, body mass index; TNF-α, tumor necrosis factor alpha; AR, androgen receptor; NF-kB, nuclear factor-kappa B; caspase-1, cysteinyl aspartate specific proteinase-1; T, tumor; FIIND, function to α-find domain; G, grade

Key words: prostate cancer, benign prostatic hyperplasia, inflammasomes, tumor, clinical value

Introduction

Prostate cancer is the second most common type of malignancy in men and also has the fifth highest mortality rate in men with cancer (1). In the past 20 years, due to an aging society and changes in Chinese diet and lifestyle, the incidence of prostate cancer has gradually increased in China, and the standardized incidence and mortality of prostate cancer in China are 6.15/100,000 and 2.48/100,000 individuals (2). Prostate cancer is the most common male genitourinary malignancy in China, higher than bladder cancer (3). However, the pathogenesis of prostate cancer is still poorly understood and may be related to genetic background, age, ethnicity, lifestyle, diet, chronic infection and inflammatory response (4). Previous studies have reported that chronic inflammation is strongly associated with the development of prostate tumors (4,5). Inflammation also plays a key role in tumorigenesis and development, interfering with the ability of the immune system to target tumor cells and affecting the response of the tumor to therapy (6). A previous study reported that untreated chronic lower urinary and reproductive tract infections can lead to prostatic inflammatory

hyperplasia, which can lead to the development of prostate tumors (4). A previous study on chronic inflammation and prostate tumors by Gurel *et al* (5) reported that chronic prostate infection was associated with prostate cancer and high-grade malignancies, even in men with levels of prostate-specific antigen (PSA) in the expected range. Therefore, prostate cancer can be considered to be closely related to the chronic inflammatory response.

The innate immune system is the body's first line of defense against invading pathogens or danger signals. The innate immune system can induce an inflammatory response, activating phagocytic cells to rapidly recognize and eliminate invading microorganisms and can also induce and activate the acquired immune response (7). The innate immune system, using pathogen-associated and damage-associated molecular pattern recognition, activate inflammatory signaling pathways and cause inflammation (8). Nucleotide-binding oligomerization domain-like receptors (NLRs) in the cytoplasm are activated in response to intracellular danger signals and form inflammasomes (8). Inflammatory bodies are comprised of NLRs, the apoptosis-associated speck-like protein (ASC) containing a caspase recruitment domain (CARD) and the effector protein cysteinyl aspartate specific proteinase-1 (caspase-1) (9). Effects of the inflammasome are closely related to a number of human diseases associated with dysfunctional immunoregulation, including autoimmune diseases (8), asthma (10), psoriasis (11), lupus nephritis (12), intestinal inflammation (13) and Alzheimer's disease (14), and have also been reported to serve crucial roles in tumor progression, prognosis and treatment response (15,16). In previous years, the pathogenic mechanism of inflammasome action has been reported to occur through activation of caspase-1, which promotes the expression of inflammatory factors and thus serves a role in the inflammatory response (9). The classic inflammatory response pathway is mainly activated through caspase-1-mediated induction of active subunits p10 and p20, which promote maturation of inflammatory cytokines, such as IL-1β and IL-18, and initiate inflammation-associated programmed cell death, namely pyroptosis. Activated caspase-1 provides host cells with a dual defense mechanism by releasing mature cytokines and removing infected or damaged cells (17). Inflammasomes initiate appropriate immune responses after infection or aseptic injury to resist pathogenic injury and avoid damage to the host. NLRP1, NLRP3, NLRC4 and other inflammasomes participate in the maintenance and resolution of chronic inflammatory response (18).

In a previous study, numerous inflammasomes were reported to be found in prostate tissue, including nucleotide binding oligomerization-like receptor family pyrin domain containing (NLRP)1, NLRP3 and nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4 (NLRC4) inflammasomes (19). Although the mechanisms of action of these inflammasomes are not currently fully understood, the process of inflammation may be closely related to tumor development (1). Presently, the pathogenesis of prostate cancer is unclear. Therefore, studying the process of inflammation in the context of prostate cancer and exploring its role in the occurrence and development of tumors can provide insight into the mechanism of prostate cancer development and a future direction for new diagnostic and treatment options.

Materials and methods

Ethical approval. In the present study, data were collected from 112 patients who underwent prostate puncture biopsy at the Urology Department of The First People's Hospital of Pinghu (Pinghu, China) from January to May 2022. All patients signed the informed consent forms. Ethical approval was obtained from The First People's Ethics Committee of Pinghu (Pinghu, China; approval no. 002).

Patient inclusion and exclusion criteria. The patient inclusion criteria used in the present study were as follows: i) All patients were >18 years of age; ii) prostate specific antigen (PSA) 4-10 ng/ml and/or free/total PSA (F/TPSA) <0.16; iii) PSA >10 ng/ml; iv) rectal ultrasound or prostate MRI reported suspicious lesions; and v) the digital rectal examination reached the prostate nodules. The patient exclusion criteria used in the present study were as follows: i) Patients in the acute stage of urinary tract or suffering from systemic infection; ii) patients with serious heart complications or major heart disease; iii) poor control of hypertension and diabetes; iv) patients with severe perianal rectal lesions; v) patients with severe hemorrhagic disease; vi) preoperative long-term oral administration of a 5A-reductase specific inhibitor; and vii) prior history of prostate surgery.

Sample collection. Between January and May 2022, a total of 112 patients who met the aforementioned inclusion criteria underwent transrectal prostatic needle biopsies. An ultrasound system was used for intraoperative positioning and an automatic biopsy gun was used to perform 12 needle punctures (six needles on each side of the prostate). Tissue samples obtained from the punctures were preserved in a 10% formaldehyde solution (at room temperature for 12-24 h) for pathological examination. According to the results, patients were divided into two groups: The prostate hyperplasia and prostate cancer groups.

Immunohistochemistry. A paraffin-embedded continuous slice (4-8˚C; section thickness, 3 mm) and hematoxylin and eosin (H&E) staining was performed to analyze tissue structure and extent of the lesions in patients with prostatic hyperplasia and prostate cancer. Immunohistochemical staining was performed as previously described (20). Briefly, conventional dewaxing was performed, then samples were placed in citrate buffer solution (pH 6.0), heated in a pressure cooker (>95˚C) for antigen repair then washed thrice with PBS (pH 7.4; Xiamen Tongling Biomedical Technology Co., Ltd.) for 3 min after natural cooling occurred. For rehydration, the slices were soaked in: i) Anhydrous alcohol for 3 min twice; ii) 95% alcohol 3 min twice; and iii) 85% alcohol 3 min. The slices were then washed thoroughly with double steamed water. Endogenous peroxidase activity was inactivated by incubating samples with H_2O_2 (3%; 18-28˚C) for 10 min, then washed thrice with PBS for 3 min. IL-1β (cat. no. AP8531C; 1:100; Abcepta Biotech Ltd. Co.), NLRC4 (cat. no. bs20016R; 1:200; BIOSS), IL-18 (cat. no. AP20583c; 1:100; Abcepta

Table I. Comparison of basic data from patients with prostate cancer or prostate hyperplasia.

Patient characteristic	Prostate hyperplasia (n=54)	Prostate cancer (n=58)	T-value	P-value
Age, years	72.98±6.57	72.52±6.89	1.151	0.252
BMI	22.84±2.93	23.19±2.90	-0.652	0.516
Prostate volume, mm	38.26±18.23	39.35±18.56	-0.235	0.815
International Prostate Symptom Score	14.81±5.15	15.09±5.16	-0.289	0.773

Data are presented as the mean ± standard deviation.

Biotech Ltd. Co.,) and NLRP1 (cat. no. 862764; 1:100; Zenbio; Chengdu Zhengneng Biotechnology Co., Ltd.) primary antibody working solutions were incubated with samples at 37˚C for 1 h, then washed thrice with PBS for 3 min. HRP goat anti-mouse/anti-rabbit secondary antibody (cat. no. DD13; 1:200; Xiamen Tongling Biomedicine Technology Co., Ltd.) was added to samples and incubated at 37˚C for 30 min, then washed thrice in PBS for 3 min. Samples were stained with 3,3'-diaminobenzidine (Tongling Biomedicine Technology) and incubated at room temperature. Slides were subsequently stained with H&E (18-28˚C for 5-10 min), washed with water then dehydrated (85% alcohol twice for 3 min each time, 98% alcohol twice for 3 min each time, and anhydrous alcohol twice for 3 min each time), before samples were sealed using clear, neutral gum. Samples were imaged using a light microscope (Olympus Corporation).

Immunohistochemical grade determination. Two senior pathologists reviewed and scored the radiographs (20). Positive protein expression was indicated by a brownish-yellow color in the cell membrane or cytoplasm. The proportion of positive cells and the intensity of the stain were subsequently scored. The proportion of positive cells was scored as follows: Five fields of view (magnification, x200) were observed on each sample section and the percentage of positive cells was calculated. Positive proportion of cells <5%=0 points, 5-25%=1 point, 26-50%=2 points, 51-75%=3 points and 76-100%=4 points. The stain intensity of positive cells was scored as follows: 0 for colorless, 1 for light yellow, 2 for brown and 3 for dark brown. The two scores were multiplied to give the final score. The expression levels of NLRP1 and NLRC4 in patients with prostate hyperplasia and prostate cancer were compared, and their expression characteristics were analyzed. Protein expression levels of proinflammatory cytokines IL-1β, IL-18 and the NLRP1 and NLRC4 inflammasomes were determined and correlation analysis performed. Microscope images were produced using cellSens (Olympus Corporation).

Differences in inflammatory cell expression between different prostate cancer groups. Based on data from The Cancer Genome Atlas (TCGA) dataset (https://portal.gdc.com), differences in the expression of NLRP1 and NLRC4 inflammasomes in patients with different stages of prostate cancer were compared to further explore the clinical value of the diagnosis and treatment of prostate cancer (The data of 495 patients were collected, including 177 patients in the T1 stage, 205 patients in the T2 stage and 113 patients in the T3/T4 stage).

Statistical analysis. SPSS (version 25.0; IBM Corp.) statistical software was used for data analysis and the mean ± standard deviation was calculated. Data with a non-normal distribution were presented as the median ± interquartile range. An unpaired Student's t-test was used to compare data from normally distributed groups and a Wilcoxon rank sum test was used when data were not normally distributed. According to the normal distribution of measurement data in both groups, Pearson correlation was used to conduct a correlation analysis. The difference in expression of NLRP1 or NLRC4 in different stages of prostate cancer was also analyzed. The Kruskal-Wallis test was used to compare the significant differences between the three groups, and the Dunn post hoc test was used for pairwise comparisons between the groups. $P<0.05$ was considered to indicate a statistically significant difference. The R (version 4.0.3) GTEx package (https://www.gtexportal.org/home/datasets) was used for statistical analysis.

Results

Comparison of basic patient characteristics. Characteristics of patients with prostatic hyperplasia (n=54) and prostate cancer (n=58) were assessed. The mean age of patients with prostatic hyperplasia was 72.98±6.57 years and those with prostate cancer was 72.52±6.89 years. There was no significant difference in the age, BMI, prostate volume (PV) and International Prostate System Score (21) between the two groups of patients ($P>0.05$), which demonstrated that the clinical data were comparable (Table I).

H&E staining. Arrangement of the glands in the patients with prostatic hyperplasia were regular and the outlines of individual glands were well defined (Fig. 1). There was no infiltration of lymphocytes, plasma cells or other inflammatory cells in samples of patients with prostatic hyperplasia. In the group of patients with prostate cancer, minor acinar structures were observed, some of the glands were damaged and not precisely defined, solid flakes, nests or single-cell structures were observed and some nuclei were distinct. There was no infiltration of lymphocytes or plasma cells observed in samples of patients with prostate cancer.

Expression of inflammasomes. NLRP1 and NLRC4 inflammasome expression was observed in prostate hyperplasia and prostate cancer tissue, primarily in the cytoplasm and membrane of cells, and were diffusely distributed (Fig. 2). Compared with patients with prostate hyperplasia, the

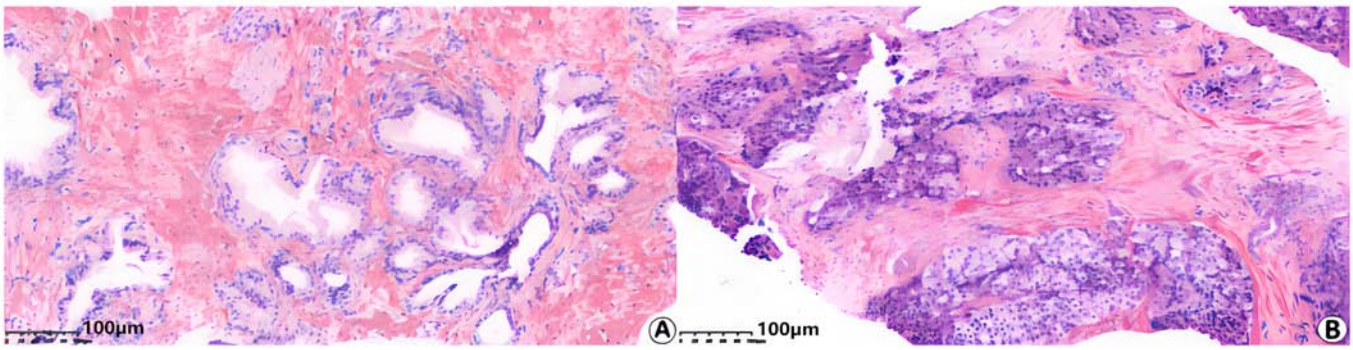

Figure 1. Hematoxylin and eosin staining of samples from patients with (A) prostate hyperplasia or (B) prostate cancer (magnification, x200).

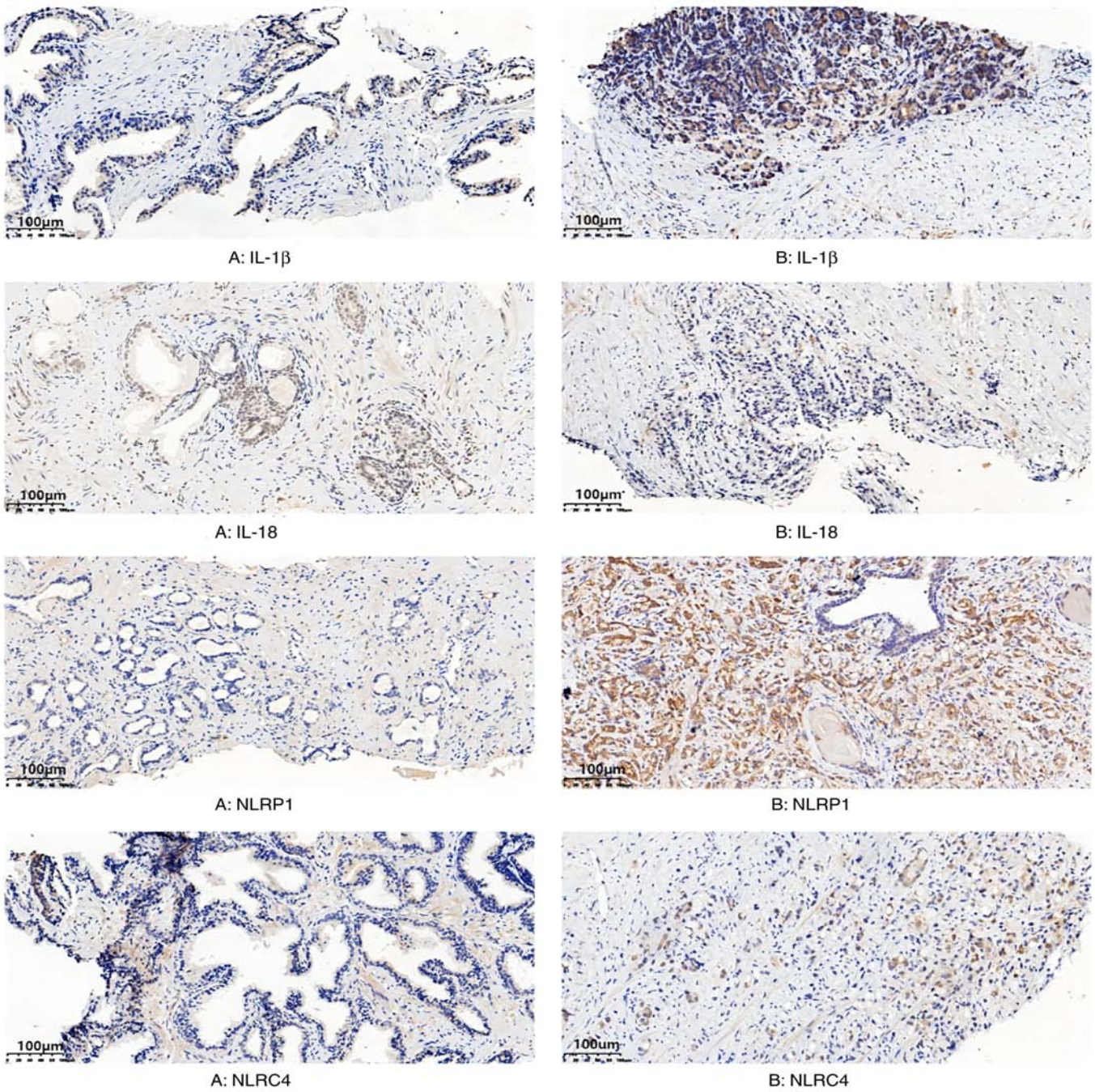

Figure 2. Immunohistochemical staining for protein expression levels of NLRP1, NLRC4, IL-1β and IL-18 in patients with (A) prostate hyperplasia or (B) prostate cancer. NLRP1, nucleotide binding oligomerization-like receptor family pyrin domain containing 1; NLRC4, nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4; IL, interleukin.

Table II. Expression of inflammasome in patients with prostate cancer or prostate hyperplasia.

Inflammasome	Prostate hyperplasia (n=54)	Prostate cancer (n=58)	T-value	P-value
NLRP1, points	3.28±1.43	6.60±3.25	6.919	>0.001
NLRC4, points	0.09±0.56	1.26±2.30	3.629	>0.001
IL-18, points	1.43±1.92	1.72±2.34	0.735	0.464
IL-1β, points	3.22±1.11	6.59±1.97	11.005	>0.001

IL, interleukin; NLRP1, nucleotide binding oligomerization-like receptor family pyrin domain containing 1; NLRC4, nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4.

Table III. Correlation analysis of the expression NLRP1 and NLRC4 with IL-18 and IL-1β expression.

	IL-18		IL-1β	
Inflammasome	r	P-value	r	P-value
NLRP1	0.208	0.020	0.568	<0.001
NLRC4	0.145	0.108	0.379	<0.001

IL, interleukin; NLRP1, nucleotide binding oligomerization-like receptor family pyrin domain containing 1; NLRC4, nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4.

expression of NLRP1 and NLRC4 inflammasomes in patients with prostate cancer was significantly increased (P<0.001; Table II). The protein expression level of IL-18 in patients with prostatic hyperplasia was not significantly different compared with patients with prostate cancer (P>0.05; Table II). However, the protein expression level of IL-1β in patients with prostate cancer was significantly increased compared with patients with prostatic hyperplasia (P<0.001; Table II).

Correlation analysis of the expression of NLRP1 and NLRC4 with IL-18 and IL-1β. The protein expression levels of NLRP1, NLRC4 and the proinflammatory cytokines IL-18 and IL-1β in tissue samples were analyzed and a correlation analysis was performed. Protein expression levels of IL-18 and IL-1β demonstrated a significant positive correlation with the protein expression levels of NLRP1, with a Pearson correlation coefficient of 0.208 and 0.568, respectively (P<0.05; Table III). The protein expression level of NLRC4 demonstrated a significant positive correlation with IL-1β protein expression level, with a Pearson correlation coefficient of 0.379 (P<0.01).

Correlation analysis of NLRP1 and NLRC4 expression with the prostate cancer index. In the group of patients with prostate cancer, the protein expression levels of NLRP1 and NLRC4 demonstrated a significant positive correlation with the Gleason score, with a Pearson correlation coefficient of 0.578 and 0.279, respectively (P<0.05; Table IV). The protein expression levels of IL-18 and IL-1β were not significantly correlated with the Gleason score (P>0.05) and the protein expression levels of NLRP1, NLRC4, IL-18 and IL-1β were not significantly correlated with TPSA, FPSA and F/TPSA (P>0.05).

Comparison of NLRP1 and NLRC4 expression in different stages of prostate cancer. The expression levels of NLRP1 and NLRC4 in different stages of prostate cancer were obtained from the TCGA database (22). The expression level of NLRP1 in G3 was significantly higher compared with G1 (P<0.05; Fig. 3). The expression level of NLRC4 was significantly increased at higher stages of prostate cancer, with the expression levels in cases with G2>G1 (P<0.05) and G3>T1 (P<0.001). However, there is currently a lack of reliable evidence to support this finding which needs to be confirmed by statistical analysis on a larger sample size of patients with prostate cancer.

Discussion

Prostate cancer is a common malignancy of the genitourinary system in older men (>60 years old), with the highest incidence and the third highest mortality rate of all types of cancers among men worldwide (23). For nearly 20 years, with the improvement of living standards, including the formation of a sedentary lifestyle and high-fat diet, as well as the aging of the population, the incidence of prostate cancer has increased yearly, which has brought an increasing social burden. Prostate cancer has therefore become a major public health problem in the male population (24).

The pathogenesis of prostate cancer remains unclear and may be related to genetic background, age, ethnicity, lifestyle, diet, chronic infection and inflammatory responses. Previous studies have reported that chronic inflammation is associated with the occurrence of prostate tumors and serves a key role in certain stages of tumor development (4,5). The inflammatory response can be generated by the activation of inflammasome expression, thus inflammasomes may serve a role in the occurrence and development of prostate cancer (4). Inflammasomes are involved in the construction of the tumor microenvironment (TME) as they can induce the release of proinflammatory cytokines IL-18, IL-1β, TNF-α and human Toll-like receptor 4 and promote the proliferation of peripheral cells, thus constructing the TME, which is conducive to the occurrence of cancer (25). Inflammasomes can also promote the growth and survival of cancer cells. The IL-6/androgen receptor (AR) pathway serves an essential role in the occurrence and development of prostate cancer (26). Activation of

Table IV. Correlation analysis of NLRP1 and NLRC4 expression with the prostate cancer index.

	Gleason score		TPSA		FPSA		F/TPSA	
Inflammasome	r	P-value	r	P-value	r	P-value	r	P-value
NLRP1	0.578	0.000	0.183	0.169	-0.004	0.977	-0.078	0.560
NLRC4	0.279	0.034	0.028	0.832	-0.060	0.654	-0.106	0.429
IL-18	0.132	0.323	0.171	0.200	-0.060	0.655	-0.134	0.316
IL-1β	0.117	0.382	-0.360	0.786	0.157	0.245	-0.201	0.130

IL, interleukin; NLRP1, nucleotide binding oligomerization-like receptor family pyrin domain containing 1; NLRC4, nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4; TPSA, total prostate specific antigen; FPSA, free prostate specific antigen.

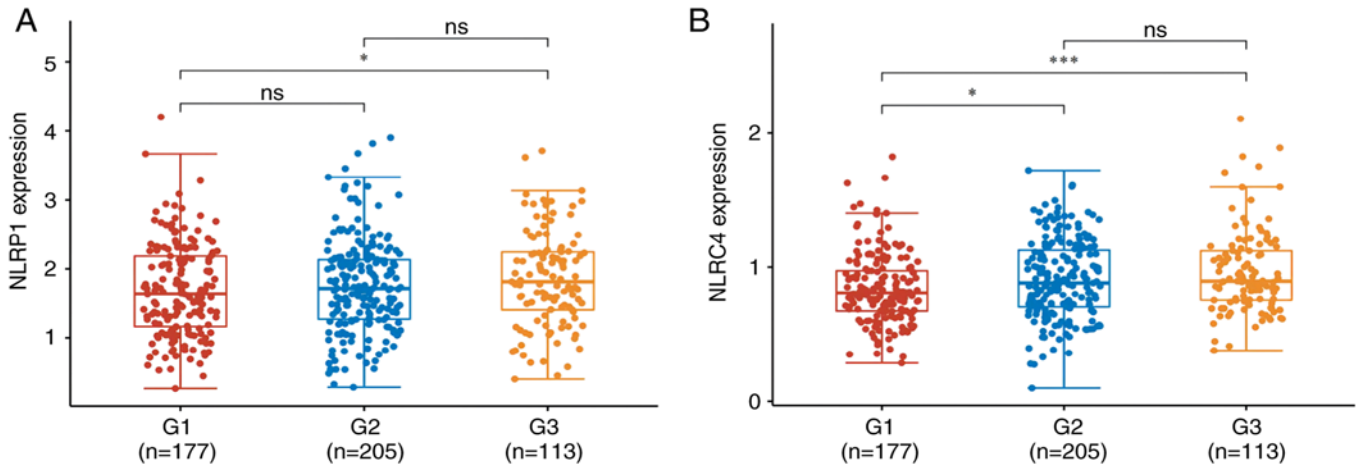

Figure 3. Protein expression levels of (A) NLRP1 and (B) NLRC4 inflammasomes in different stages of prostate cancer. *P<0.05 and ***P<0.001. NLRP1, nucleotide binding oligomerization-like receptor family pyrin domain containing 1; NLRC4, nucleotide-binding oligomerization domain leucine-rich repeat and caspase recruitment domain-containing 4; G, grade; ns, not significant.

the inflammasomes promotes the maturation and release of proinflammatory mediators IL-6 and IL-18 (27). Under the regulation of NF-kB, AR can be activated to induce PSA expression and promote the growth and proliferation of tumor cells (6). Inflammasomes can also promote angiogenesis, as NLRC4 mediates production of proinflammatory factor IL-1β, which acts on adipocytes and can mediate vascular endothelial growth factor A expression, which promotes the formation of blood vessels associated with trophoblast tumor cells (27).

As the first identified inflammasome, NLRP1 belongs to the NLR protein family and is comprised of pyrin domain (PYD), leucine-rich repeat (LRR), function to α-find domain (FIIND) and C-terminal CARD, in addition to other domains and can independently activate caspase-1 (16). Mutation of the PYD domain of NLRP1 can lead to cancer and familial bryophyte-like keratosis, whilst a gain-of-function mutation in the FIIND domain can lead to systemic inflammation, arthritis and dyskeratosis (28). In the absence of external stimuli, NLRP1 suppresses autoregulation (29). Once stimulated, the N-terminal PYD domain of NLRP1 binds to ASC, initiating a cascade reaction and participating in numerous inflammatory diseases by regulating the innate and adaptive immune responses (30). In a previous study of prostatitis and prostatic hyperplasia in rats, Kashyap *et al* (31) reported that assembly and activation of NLRP1 in the prostate promoted the production of proinflammatory cytokines IL-1β and IL-18 after the autoproteolysis of caspase-1 and maturation. Glinskii *et al* (32) reported that the expression of the NLRP1 inflammasome is involved in the occurrence of prostate tumors, and NLRP1 expression is enhanced in highly metastatic prostate cancer cells in prostate cancer. In the present study, the NLRP1 inflammasome demonstrated a significant increase in expression in prostate cancer compared with prostatic hyperplasia. This result suggested that NLRP1 may be involved in the development of prostate cancer. The expression of NLRP1 demonstrated a significant positive correlation with the expression of IL-18 and IL-1β, which was consistent with the results of previous studies (6). Thus, further indicating that activation of the NLRP1 inflammasome may promote the maturation and release of inflammatory cytokines IL-1β and IL-18, serving an essential role in the occurrence and development of prostate cancer.

The NLRC4 inflammasome, also known as ICE-protease-activating factor, belongs to the NLR protein family (31). NLRC4 is comprised of CARD, neuronal apoptosis inhibitory protein (NAIP), MHC class II transcription activator,

incompatibility locus protein from *Podospora anserina* and telomerase-associated protein and LRR domains (31). NLRC4 can directly bind to pro-caspase-1 through CARD-CARD interactions, which triggers caspase-1 processing and activation (33). In addition, the adaptor molecule ASC, which contains apoptosis-associated blotch-like proteins encoded by the *PYCARD* gene, can also facilitate such interactions (31). ASC contains both PYD and CARD domains, and CARD can activate caspase-1, promote the protein expression of pro-IL-1β and pro-IL-18 and eliminate infected cells through apoptosis (34). In animal experiments, NLRC4 has been reported to serve a defensive role in maintaining intestinal stability, and NAIP5-NLRC4 influence can be activated to promote apoptosis of infected cells when infected with *Escherichia coli* and *Salmonella typhimurium* (35). Expression of IL-18 was increased in patients with active idiopathic thrombocytopenia compared with normal and reactive-idiopathic thrombocytopenia, which may be related to activation of the NLRC4 inflammasome (17). However, previous studies of NLRC4 have focused on the maintenance of intestinal balance and immune disorders, therefore information on the expression of NLRC4 in prostate disease is currently lacking. In the present study, the NLRC4 inflammasome demonstrated a significant increase in expression in patients with prostate cancer compared with prostatic hyperplasia, which indicated that NLRC4 inflammasomes may be involved in the occurrence and development of prostate cancer. Expression of NLRC4 demonstrated a significant positive correlation with IL-1β expression. These results demonstrated that the expression of IL-1β was closely related to NLRC4 expression. Therefore, NLRC4 inflammasome expression may be involved in the development and progression of prostate cancer by promoting the production of the pro-inflammatory cytokine IL-18 following auto-proteolysis of caspase-1.

Previous studies have reported that IL-18 expression promotes tumorigenesis (26). The present study demonstrated that IL-18 was not expressed in patients with prostate cancer. However, Dupaul-Chicoine *et al* (36) reported that NLRP3 inflammatory vesicle-mediated IL-18 production could inhibit the proliferation of hepatic colorectal cancer. NLRP3/IL-18-mediated downregulation may also provide protection against intestinal tissue damage during peaks of the inflammatory process. However, sample limitations in the present study may affect these conclusions, which need to be confirmed by a large sample study.

Tumorigenesis is strongly correlated with interactions between cancer cells and their TME (37). The major components of the TME are the extracellular matrix, fibroblasts, myofibroblasts, mesenchymal stem cells, neuroendocrine cells, fatty cells, immune and inflammatory cells, and blood and lymphatic networks (38). Galectin-1 overexpression in cancer-associated fibroblasts is associated with poor prognosis in certain types of cancer, including prostate cancer (39). Neuroendocrine cells serve a key role in the development of prostate cancer cells by influencing their proliferation and invasiveness (39). Abnormal AR reactions in the epithelium and stroma may lead to tumor occurrence (40). Immune cells such as regulatory T cells, T helper 17 cells and macrophages are also involved in prostate cancer progression. However, cytokines secreted by cells in the TME, such as IL-1β, IL-6, and RANKL have been reported to have pleiotropic effects on prostate cancer cells (37). These findings suggest that the TME serves an essential role in the occurrence and progression of prostate cancer.

The present study demonstrated that the NLRP1 and NLRC4 inflammasome-mediated inflammatory response may be involved in the occurrence of prostate cancer, as a significant increase in the expression of inflammasomes in tumor tissues compared with non-tumor tissues was demonstrated. In addition, expression of the NLRP1 and NLRC4 inflammasomes positively correlated with the Gleason score of prostate cancer, which indicated that NLRP1 and NLRC4 expression was closely related to the risk assessment score of prostate cancer and could predict the prognosis of patients with prostate cancer. Expression of NLRP1 and NLRC4 was significantly increased in intermediate and advanced prostate cancer tissues compared with early-stage prostate cancer tissues, which suggested that these inflammasomes also serve a role in prostate cancer progression and metastasis. Therefore, expression of the NLRP1 and NLRC4 inflammasomes and their downstream products IL-1β and IL-18 were involved in the construction of TME, thus promoting the occurrence and development of prostate cancer. This result demonstrated potential clinical value for predicting the progression and prognosis evaluation of prostate cancer.

Inflammasomes promote the occurrence and development of inflammation, which can lead to the development of prostate cancer. Future research into inflammasome inhibitors could give rise to potential candidates for the treatment of prostate cancer. The main types of inflammasome inhibitors currently in use function by blocking IL-1β activation (40). MCC950, CY-09, trails and OLT1177 can reduce the activity of caspase-1 and the production of IL-1β in mouse experiments, thus inhibiting inflammation (41,42). Although research into the efficacy of these compounds in humans is currently lacking. However, the present study provides a valuable direction for the diagnosis and treatment of prostate diseases.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XZ conceptualized the study, gave final approval of the version to be published, and agreed to be accountable for the work in ensuring that questions related to the integrity of any part of the work are appropriately investigated and resolved (according to the ICMJE). KL wrote and edited the manuscript, made substantial contributions to the design of the study, and the acquisition and analysis of data. JH performed the data

analysis. ZK contributed to the interpretation of data for the manuscript, drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final version of the manuscript. XZ and KL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The project was approved by The Ethics Committee of The First People's Hospital of Pinghu (Pinghu, China; approval no. 002) and all patients provided written, informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Boehm BJ, Colopy SA, Jerde TJ, Loftus CJ and Bushman W: Acute bacterial inflammation of the mouse prostate. Prostate 72: 307-317, 2012.
2. Guo YL, Na YQ, Ye ZQ and Huang J: Guidelines for Diagnosis and Treatment of Urology and Andrology Diseases in China. Science Press, Beijing, China, pp439, 2019.
3. Xie Jinbo and Peng Bo: Research progress on the epidemiological characteristics and risk factors of benign prostatic hyperplasia. J Tongji Univ Med Edition 42: 6, 2021 (In Chinese).
4. Leela SCA, Paes Batista da Silva A, Verma S, Fu P, Shen DL, MacLennan G, Gupta S and Bissada NF: Presence of specific periodontal pathogens in prostate gland diagnosed with chronic inflammation and adenocarcinoma. Cureus 13: e17742, 2021.
5. Gurel B, Lucia MS, Thompson IM Jr, Goodman PJ, Tangen CM, Kristal AR, Parnes HL, Hoque A, Lippman SM, Sutcliffe S, *et al*: Chronic inflammation in benign prostate tissue is associated with high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev 23: 847-856, 2014.
6. Mishra V and Pathak C: Human Toll-Like Receptor 4 (hTLR4): Structural and functional dynamics in cancer. Int J Biol Macromol 122: 425-451, 2019.
7. Krysko DV, Agostinis P, Krysko O, Garg AD, Bachert C, Lambrecht BN and Vandenabeele P: Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation. Trends Immunol 32: 157-164, 2011.
8. Costa FRC, Leite JA, Rassi DM, da Silva JF, Elias-Oliveira J, Guimarães JB, Foss-Freitas MC, Câmara NOS, Pontillo A, Tostes RC, *et al*: NLRP1 acts as a negative regulator of Th17 cell programming in mice and humans with autoimmune diabetes. Cell Rep 35: 109176, 2021.
9. Alehashemi S and Goldbach-Mansky R: Human autoinflammatory diseases mediated by NLRP3-, Pyrin-, NLRP1-, and NLRC4-Inflammasome dysregulation updates on diagnosis, treatment, and the respective roles of IL-1 and IL-18. Front Immunol 11: 1840, 2020.
10. Moecking J, Laohamonthonkul P, Chalker K, White MJ, Harapas CR, Yu CH, Davidson S, Hrovat-Schaale K, Hu D, Eng C, *et al*: NLRP1 variant M1184V decreases inflammasome activation in the context of DPP9 inhibition and asthma severity. J Allergy Clin Immunol 147: 2134-2145.e20, 2021.
11. Ciążyńska M, Olejniczak-Staruch I, Sobolewska-Sztychny D, Narbutt J, Skibińska M and Lesiak A: The role of NLRP1, NLRP3, and AIM2 inflammasomes in psoriasis: Review. Int J Mol Sci 22: 5898, 2021.
12. Huang T, Yin H, Ning W, Wang X, Chen C, Lin W, Li J, Zhou Y, Peng Y, Wang M, *et al*: Expression of inflammasomes NLRP1, NLRP3 and AIM2 in different pathologic classification of lupus nephritis. Clin Exp Rheumatol 38: 680-690, 2020.
13. Gong Q, Robinson K, Xu C, Huynh PT, Chong KHC, Tan EYJ, Zhang J, Boo ZZ, Teo DET, Lay K, *et al*: Structural basis for distinct inflammasome complex assembly by human NLRP1 and CARD8. Nat Commun 12: 188, 2021.
14. Zhang J, Pei L, Zang D, Xue Y, Wang X, Chen Y, Li J, Yu J, Gao Q, Di W, *et al*: Gender differences of NLRP1 inflammasome in mouse model of Alzheimer's disease. Front Aging Neurosci 12: 512097, 2020.
15. Karki R and Kanneganti TD: Diverging inflammasome signals in tumorigenesis and potential targeting. Nat Rev Cancer 19: 197-214, 2019.
16. Gouravani M, Khalili N, Razi S, Keshavarz-Fathi M, Khalili N and Rezaei N: The NLRP3 inflammasome: A therapeutic target for inflammation-associated cancers. Expert Rev Clin Immunol 16: 175-187, 2020.
17. Sundaram B and Kanneganti TD: Advances in understanding activation and function of the NLRC4 inflammasome. Int J Mol Sci 22: 1048, 2021.
18. Broz P: Recognition of intracellular bacteria by inflammasomes. Microbiol Spectr 7: 2019.
19. Chear CT, Nallusamy R, Canna SW, Chan KC, Baharin MF, Hishamshah M, Ghani H, Ripen AM and Mohamad SB: A novel de novo NLRC4 mutation reinforces the likely pathogenicity of specific LRR domain mutation. Clin Immunol 211: 108328, 2020.
20. Yang J, Gu J, Hu Y, Wang N, Gao J and Wang P: Molecular cloning and characterization of HSP60 gene in domestic pigeons (Columba livia) and differential expression patterns under temperature stress. Cell Stress Chaperones 26: 115-127, 2021.
21. Barry MJ, Fowler FJ jr, O'Leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK and Cockett AT: The American Urological Association symptom index for benign prostatic hyperplasia The Measurement Committee of the American Urological Association. J Urol 148: 1549-1557; discussion 1564, 1992.
22. Chen YJ, Luo SN, Dong L, Liu TT, Shen XZ, Zhang NP and Liang L: Interferon regulatory factor family influences tumor immunity and prognosis of patients with colorectal cancer. J Transl Med 19: 379, 2021.
23. Zhou T, Cai Z, Ma N, Xie W, Gao C, Huang M, Bai Y, Ni Y and Tang Y: A novel ten-gene signature predicting prognosis in hepatocellular carcinoma. Front Cell Dev Biol 8: 629, 2020.
24. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal Aand Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
25. Cao W, Chen HD, Yu YW, Li N and Chen WQ: Changing profiles of cancer burden worldwide and in China: A secondary analysis of the global cancer statistics 2020. Chin Med J (Engl) 134: 783-791, 2021.
26. Puhr M, De Marzo A, Isaacs W, Lucia MS, Sfanos K, Yegnasubramanian S and Culig Z: Inflammation, microbiota, and prostate cancer. Eur Urol Focus 2: 374-382, 2016.
27. Ohashi K, Wang Z, Yang YM, Billet S, Tu W, Pimienta M, Cassel SL, Pandol SJ, Lu SC, Sutterwala FS, *et al*: NOD-like receptor C4 inflammasome regulates the growth of colon cancer liver metastasis in NAFLD. Hepatology 70: 1582-1599, 2019.
28. Schillaci O, Scimeca M, Trivigno D, Chiaravalloti A, Facchetti S, Anemona L, Bonfiglio R, Santeusanio G, Tancredi V, Bonanno E, *et al*: Prostate cancer and inflammation: A new molecular imaging challenge in the era of personalized medicine. Nucl Med Biol 68-69: 66-69, 2019.
29. Yu CH, Moecking J, Geyer M and Masters SL: Mechanisms of NLRP1-mediated autoinflammatory disease in humans and Mice. J Mol Biol 430: 142-152, 2018.
30. Fan Y, Yang L, Wei Q, Ding Y, Tang Z, Tan P, Lin T, Guo D and Qiu S: Toll-like receptor 10 (TLR10) exhibits suppressive effects on inflammation of prostate epithelial cells. Asian J Androl 21: 393-399, 2019.
31. Kashyap M, Pore S, Wang Z, Gingrich J, Yoshimura N and Tyagi P: Inflammasomes are important mediators of prostatic inflammation associated with BPH. J Inflamm (Lond) 12: 37, 2015.
32. Glinskii AB, Ma S, Ma J, Grant D, Lim CU, Guest I, Sell S, Buttyan R and Glinsky GV: Networks of intergenic long-range enhancers and snpRNAs drive castration-resistant phenotype of prostate cancer and contribute to pathogenesis of multiple common human disorders. Cell Cycle 10: 3571-3597, 2011.

33. Wen J, Xuan B, Liu Y, Wang L, He L, Meng X, Zhou T and Wang Y: Updating the NLRC4 inflammasome: From bacterial infections to autoimmunity and cancer. Front Immunol 12: 702527, 2021.
34. Li Y, Fu TM, Lu A, Witt K, Ruan J, Shen C and Wu H: Inaugural Article: Cryo-EM structures of ASC and NLRC4 CARD filaments reveal a unified mechanism of nucleation and activation of caspase-1. Proc Natl Acad Sci USA 115: 10845-10852, 2018.
35. Man SM, Tourlomousis P, Hopkins L, Monie TP, Fitzgerald KA and Bryant CE: Salmonella infection induces recruitment of caspase-8 to the inflammasome to modulate IL-1β production. J Immunol 191: 5239-5246, 2013.
36. Dupaul-Chicoine J, Arabzadeh A, Dagenais M, Douglas T, Champagne C, Morizot A, Rodrigue-Gervais IG, Breton V, Colpitts SL, Beauchemin N and Saleh M: The Nlrp3 inflammasome suppresses colorectal cancer metastatic growth in the liver by promoting natural killer cell tumoricidal activity. Immunity 43: 751-763, 2015.
37. Lv Y, Ruan G, Liu Y, Cui D, Zhao Y, Yan C, Lv M, Xu D, Mao Y, Cao J, *et al*: Aberrant expression of NLRP3,NLRC4 and NLRP6 inflammasomes in patients with primary im-mune thrombocytopenia. Thromb Res 176: 101-103, 2019.
38. Di Donato M, Giovannelli P, Cernera G, Di anti A, Marino I, Bilancio A, Galasso G, Auricchio F, Migliaccio A and Castoria G: Non-genomic androgen action regulates proliferative/migratory signaling in stromal cells. Front Endocrinol (Lausanne) 5: 225, 2014.
39. Van den Brule FA, Waltregny D and Castronovo V: Increased expression of galectin-1 in carcinoma-associated stroma predicts poor outcome in prostate carcinoma patients. J Pathol 193: 80-87, 2001.
40. Dai J, Lu Y, Roca H, Keller JM, Zhang J, McCauley LK and Keller ET: Immune mediators in the tumor microenvironment of prostate cancer. Chin J Cancer 36: 29, 2017.
41. Jiang H, He H, Chen Y, Huang W, Cheng J, Ye J, Wang A, Tao J, Wang C, Liu Q, *et al*: Identification of a selective and direct NLRP3 inhibitor to treat inflammatory disorders. J Exp Med 214: 3219-3238, 2017.
42. Huang Y, Jiang H, Chen Y, Wang X, Yang Y, Tao J, Deng X, Liang G, Zhang H, Jiang W and Zhou R: Tranilast directly targets NLRP3 to treat inflammasome-driven diseases. EMBO Mol Med 10: e8689, 2018.