Mini review

ROLE OF INFLAMMASOMES AND THEIR REGULATORS IN PROSTATE CANCER INITIATION, PROGRESSION AND METASTASIS

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Abstract: Prostate cancer is one of the main cancers that affect men, especially older men. Though there has been considerable progress in understanding the progression of prostate cancer, the drivers of its development need to be studied more comprehensively. The emergence of resistant forms has also increased the clinical challenges involved in the treatment of prostate cancer. Recent evidence has suggested that inflammation might play an important role at various stages of cancer development. This review focuses on inflammasome research that is relevant to prostate cancer and indicates future avenues of study into its effective prevention and treatment through inflammasome regulation. With regard to prostate cancer, such research is still in its early stages. Further study is certainly necessary to gain a broader understanding of prostate cancer development and to create successful therapy solutions.

Key words: Inflammation, Adaptor protein ASC, Caspase-1, Prostate cancer, IFI16, caspase-1, IL-1β, IL-18, NLR

INTRODUCTION

Prostate cancer is an uncontrolled growth of prostate gland cells. It usually affects older men, with the highest incidence at around 65 years of age. It has

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Abbreviations used: AIM2 – absent in melanoma 2; ASC – apoptosis-associated speck-like protein containing caspase recruitment domain; IFN – interferon; IFI16 – interferon gamma-inducible protein-16; IL – interleukin; IL-1R1 – interleukin-1 receptor 1; MyD88 – myeloid differentiation factor 88; NF-κB – nuclear factor of kappa light polypeptide gene enhancer in B-cells; NLR – NOD-like leucine-rich repeat-containing receptor; NOD – nucleotide oligomerization domain; PIA – proliferative inflammatory atrophy; PPR – pathogen-associated pattern recognition receptor; PSA – prostate-specific antigen; SNP – single nucleotide polymorphisms
been estimated that one in every 36 men will die of prostate cancer, and that it is one of the most prevalent cancers among men [1]. Castration-resistant prostate cancer (CRPC) is the most clinically challenging form and accounts for many deaths [1, 2].

The role of inflammation as an independent risk factor for prostate cancer development is rather a debatable issue. Studies have shown that there is considerable evidence for inflammatory conditions being involved in the initiation and progression of prostate cancer [3-8]. Furthermore, a 5-year follow-up study based on prostate needle biopsies established that chronic inflammation accounts for nearly 20% of prostate cancer development [9]. Another needle biopsy study revealed that there were less frequent associations between inflammatory atrophy and adenocarcinoma [10]. Nonetheless, inflammatory atrophy was found in about 40% of the adenocarcinoma cores in that study. Other studies report no significant association between prostatic inflammatory atrophy (PIA) and prostate cancer development [11, 12].

PIA can be diffuse or focal, and the detection of small focal changes occurring at the peripheral zone is challenging in needle biopsies [13]. This variability could also be due to the presence of other confounding factors, such as genetic, nutritional and hormonal factors, and previous usage of antibiotics and anti-inflammatory drugs. Moreover, intermittent low-grade inflammation poses a serious threat, as it is extremely difficult to detect via biopsy. Such low-grade inflammation may drive stem cells to become cancer stem cells.

A variety of factors (certain chemical substances and environmental, dietary, bacterial and viral agents) were observed to cause inflammation in the prostate gland [3]. Apart from these extrinsic risk factors, certain genetic factors, especially those observed in the inflammatory genes, were found to increase the risk of prostate cancer [6, 7]. The roles of inflammasomes and inflammation-causing factors are increasingly seen as crucial components in the pathophysiology of prostate cancer. A subset of prostate cancer cases could benefit from studies aimed at gaining deeper insight into the role of inflammasomes in prostate cancer.

**Inflammasomes**

Inflammasomes consist of pattern-recognition sensors (NLR family, AIM2, IFI16); adaptors (ASC); pro-caspase-1; and pro-inflammatory cytokines (Pro-IL-1β and IL-18). Inflammasome activation leads to the conversion of inactive inflammation mediators to active ones (IL-1β and IL-18). Their subsequent secretion to the cell exterior modulates cell function in an autocrine or paracrine fashion [14, 15], as shown in Fig. 1. The presence of external IL-1β can initiate self-reinforcing feedback loops to further perpetuate its existence through the IL-1R-MyD88-NF-κB pathway in the presence of inflammasome activators [16].
Fig. 1. Schematic diagram showing the events of inflammasome activation. Different pathogen-associated molecules are recognized by different PPRs. This leads to association with ASC, which acts as an adaptor to further recruit pro-caspase-1. Pro-caspase-1 molecules cleave among themselves to produce active caspase-1 complex, which then matures the pro-IL-1β. Matured IL-1β is released outside the cell, where it binds to the IL-1β receptor to further activate NF-κB signaling and give self-perpetuating cytokine production.

Inflammasomes can be assembled in response to a variety of pathogen-associated molecules. Upon activation, they may heighten the cellular defense mechanisms. However, cases of chronic stimulation may lead to persistent unresolved inflammation and tissue damage, and may provide a favorable environment for uncontrolled growth. Here, we discuss the association of various components of inflammasomes with the initiation, progression and metastasis of prostate cancer. Furthermore, we highlight the possible therapeutic targets that might aid in the treatment or early prevention of prostate cancer.

THE ROLE OF INFLAMMASOME COMPONENTS AND REGULATORS IN PROSTATE CANCER PATHOPHYSIOLOGY

Caspase-1
Mutually contrasting observations have been made with regard to the involvement of caspase-1 in the progression of prostate cancer. It has been shown that induction of caspase-1 is involved in apoptosis of LNCaP cells [17]. Furthermore, caspase-1 protein levels were downregulated in prostate cancer specimens by an unknown mechanism without much alteration in the mRNA
levels [18]. These results suggest that caspase-1 downregulation is involved in prostate cancer cell survival. However, it has also been observed that stimulation of prostate cancer cells with dihydrotestosterone induces caspase-1 [19], and that treatment of prostate cancer cells with anti-androgens suppresses caspase-1 [20]. Given that androgen deprivation is involved in the regression or growth inhibition of early stage prostate cancers, it is plausible that the presence of androgens might promote aggressive forms of prostate cancer by inducing higher caspase-1 levels to propagate inflammation.

Thus far, caspase-1 can contribute to both the induction of apoptosis and the inflammasome-mediated activation of the pro-inflammatory cytokines, IL-1β and IL-18. Perhaps such functions of caspase-1 are context mediated and may depend on the relative abundance of other proteins such as Bcl2, ASC and pro-IL-1β, and the presence of inflammasome activators. From an inflammation perspective, the activation of caspase-1 could be a risk factor for prostate cancer initiation. Identification of inflammasome-independent mechanisms of caspase-1 activation would help resolve the function of caspase-1 in prostate cancer.

Adenoviruses expressing inducible caspase-1 have been used to treat prostate tumors in mouse models in combination with other molecules [21]. However, the molecular mechanisms of caspase-1-induced apoptosis have not been well characterized. In one report, it was shown that the expression of caspase-1 results in the activation of caspase-3 and sensitizes prostate cancer cells to ionizing radiation [22]. There is a need to distinguish between the functions of caspase-1 as an accelerator of inflammation and those as a mediator of apoptosis. In addition, there could be cell-dependent functional differences with regard to the caspase-1-induced pathways [23].

**ASC**

Downregulation of ASC gene expression due to hypermethylation was reported in prostate cancer cell lines and prostate cancer specimens [24, 25]. Interestingly, the ASC promoter methylation that is predictive of aggressiveness was observed in the tissues surrounding prostate cancer lesions. However, the consequences of ASC methylation on its expression remain unknown. Given that ASC plays a crucial role in the activation of caspase-1 and that ASC can serve many pattern recognition sensors in assembling inflammasomes, ASC targeting would aid in the prevention of prostate cancer. In this context, it is necessary to distinguish ASC function in inflammasome formation and other non-caspase-1-dependent apoptotic functions [23]. Mice bearing a prostate-specific ASC gene deletion would be useful for unraveling the function of ASC in prostate biology in general and in prostate cancer pathology in particular.

**IL-1β and IL-18**

Polymorphisms and genetic variations (SNPs) in the IL-1β gene were shown to be associated with prostate cancer and its recurrence [7]. The impact of such SNP variation is unknown. The evaluation of genetic variants of IL-1β through
functional assays would help in understanding the influence of these variants on IL-1β production and consequent effects on the course of inflammation and prostate cancer. Observations from association studies revealed that non-steroidal anti-inflammatory drugs (NSAIDs) can help to reduce the risk of prostate cancer occurrence [8]. The ability of NSAIDs to inhibit the COX-2 pathway was found to reduce tumor progression and metastasis [8]. Given that IL-1β is a potent inducer of COX2 in many cancer cells [26, 27], IL-1β inhibition could control prostate cancer progression. It would be interesting to know whether IL-1β can induce COX2 in normal and cancerous prostate cells. Furthermore, IL-1β was shown to induce matrix metalloproteinases and pro-angiogenic factors in different cells, including cancer cells, and thus it may contribute to altering tissue microenvironment and homeostasis [28, 29]. These features of IL-1β might also positively cooperate with tumor development, progression and metastasis in the prostate gland.

A negative correlation was proposed between the levels of testosterone and inflammatory cytokines, including IL-1β [30]. However, it was also shown that androgen deprivation therapy (ADT) could not reduce the higher levels of IL-1β in prostate cancer patients [31] and may be associated with the aggressiveness and recurrence of the disease. Since the presence of IL-1β can initiate self-amplifying feedback loops, removing the source of inflammation or inhibiting IL-1β through pharmacological means might potentiate ADT. Enumerating the relationship between higher levels of IL-1β and the aggressive disease or recurrence of the disease would give an accurate assessment of IL-1β involvement.

Systemic administration of IL-1β was reported to activate NF-κB in prostate cells and to facilitate propagation of inflammation through secretion of chemokines and attraction of immune cells [32]. Furthermore, infiltrating macrophages and monocytes that respond to various insults to prostate epithelial cells might contribute to the secretion of metalloproteinases [33], which in turn would alter the microenvironment and facilitate cancer progression and metastasis. These studies suggest that local and systemic inflammatory conditions that involve IL-1β production may increase the risk of prostate cancer development and may contribute to the genesis of non-responsive forms of cancer. Interferon (IFN) stimulation was shown to enhance the expression of IL-18 and caspase-1 along with more secretion of IL-18 [34]. These results are consistent with previous reports that IFNs are capable of increasing the levels of inflammasome components to modulate the production of inflammatory cytokines [35]. However, a recent study suggests that IFNs might also enhance the production of the IL-18 inhibitor [36]. Other factors, such as the levels of different cytokines and type and quantity of immune cell, might influence IFN-mediated production of IL-18 and its inhibitor (IL-18BP). Stage-specific inflammatory cytokine profiling would provide an accurate understanding of prostate cancer progression. These reports further corroborate the notion that
IFNs play an important role in the regulation of inflammasomes and the outcome of prostate cancer.

**Interferon-inducible genes (IFI16 and AIM2)**

Previous studies have shown that the interferon-inducible gene IFI16 is involved in the recognition of pathogen-associated molecules (cytosolic dsDNA) and is capable of inflammasome assembly and the maturation of IL-1β and IL-18 [37]. However, its role in prostate inflammation and the pathogenesis of prostate cancer remains unknown. It has been shown that IFI16 levels are downregulated in prostate cancer cell lines and that induction of this particular protein has been observed to produce the senescence phenotype [38]. Given that the chemokines associated with senescence-associated secretory phenotype (SASP) are involved in the generation of cancer stem-like cells [39], it is necessary to understand the function of SASP in prostate homeostasis, especially during old age. Complete profiling of senescence-associated chemokines along with inflammatory cytokines at different stages of prostate cancer might enable better interventions in the treatment of prostate cancer.

AIM2 is a recently identified cytosolic dsDNA sensor and a member of the interferon inducible p200 family. It was found to trigger inflammasome formation and the secretion of pro-inflammatory cytokines [40]. Given that IFI16 also interacts with AIM2 and can potentially modulate inflammasome formation [35, 37], it will be interesting to know the relative contribution of these proteins in the pathophysiology of prostate cancer.

**NLRP and NLRC inflammasomes**

Many bacteria and viruses that were shown to infect the prostate gland were also observed to cause inflammation, which might be conducive for derailed growth in the prostatic glandular epithelium [3]. Apart from infections, several chemical agents were also reported to cause inflammatory conditions in the prostate. The NLR family receptors are key components in the recognition of bacterial, viral and chemical substances. They activate inflammasomes to cause the maturation of IL-1β and IL-18 [41]. Characterization of the function of NLR family members in the initiation and progression of prostate cancer has potential for the development of new therapeutic strategies and interventions, especially for the effective management of hormone-resistant prostate cancer. Recent studies focused on the ubiquitous bacterium *Propionibacterium acnes* discovered that it could cause chronic prostatic inflammation [42, 43]. *P. acnes* was also found to be involved in the maturation of IL-1β and IL-18 in the neutrophils through the activation of caspase-1 [44]. It is plausible that this organism might activate caspase-1 and produce IL-1β in prostate epithelial cells as well and cause chronic inflammation.
POTENTIAL INFLAMMASOME ACTIVATORS IN THE PROSTATE GLAND AND THE CONSEQUENCES OF UNCONTROLLED INFLAMMASOME ACTIVATION

Agents that cause prostate inflammation or were shown to activate inflammasomes but were also found to infect prostate tissue are listed in Table 1. Given that the inflammasomes were activated in immune cells in the majority of cases, the cytokines produced by these inflamed cells might play a significant role in the initiation and progression of prostate cancer. Inflammation-mediated upregulation of chemotactic factors in prostate epithelial cells might also aggravate the immune cell infiltration.

Researchers have found critical links between cellular autophagy responses and inflammation. While defective autophagy was shown to release mitochondrial DNA and directly activate NLRP3-mediated inflammasomes [45], active autophagy could limit IL-1β secretion and shield from IL-1β-induced inflammation [46]. Since autophagy is so significant in the modulation of inflammation, it would be interesting to know if any defective autophagy is involved in uncontrolled inflammation and prostate cancer development. It has been shown that chronic ER stress other than the unfolded protein response (UPR) also activates NLRP3-mediated maturation of IL-1β [47]. It is interesting to identify the agents that cause ER stress as well as prostate cancer to better understand the function of inflammasomes in prostate cancer pathophysiology.

Table 1. Potential inflammasome triggers in prostate cells.

<table>
<thead>
<tr>
<th>Agents causing prostatitis [3] or reported infections in prostate tissue</th>
<th>Receptors for inflammasome assembly</th>
<th>Inflammasome activation</th>
<th>Investigation in prostate cells</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>NLRP3</td>
<td>IL-1β secretion and pyronecrosis in monocytes</td>
<td>Not done</td>
<td>[48]</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>NLRP3</td>
<td>IL-1β secretion in monocytes</td>
<td>Not done</td>
<td>[49]</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>Not known</td>
<td>Not known</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>Treponema pallidum</td>
<td>Not known</td>
<td>IL-1β secretion in monocytes</td>
<td>Not done</td>
<td>[50]</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>Not known</td>
<td>IL-1β secretion in neutrophils</td>
<td>Not done</td>
<td>[44]</td>
</tr>
<tr>
<td>Human papillomavirus (HPV)</td>
<td>Not known</td>
<td>Not known</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>Human herpes simplex virus (HSV)</td>
<td>IFI16 and NLRP3 (transient activation)</td>
<td>IL-1β secretion in fibroblasts</td>
<td>Not done</td>
<td>[51]</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>AIM2</td>
<td>IL-1β secretion</td>
<td>Not known</td>
<td>[52]</td>
</tr>
<tr>
<td>Crystalline uric acid</td>
<td>NLRP3 in macrophages</td>
<td>IL-1β secretion</td>
<td>Partial evidence</td>
<td>[53]</td>
</tr>
<tr>
<td>Corpora amylacea</td>
<td>Not known</td>
<td>Not known</td>
<td>Not known</td>
<td></td>
</tr>
</tbody>
</table>
Uncontrolled inflammasome activation
It has been observed that the uncontrolled activation of inflammasomes by uric acid leads to tissue destruction, inflammation and fibrosis, and that these symptoms can be relieved by the inhibition of IL-1β signaling in the mouse model of lung emphysema [55]. Furthermore, uncontrolled inflammation may interfere with normal immune function and help prolong the infection [56]. These features of uncontrolled inflammation might also occur in cases of prostate cancer and may result in aggressive or uncontrollable tumors. PIA-associated adenocarcinoma patients and unresponsive carcinoma patients might benefit from the inclusion of inflammatory inhibitors in their treatment regimen.

CONCLUSIONS
A recent meta analysis of the literature suggests that there is no conclusive evidence for pathogens having a causative role in prostate cancer [57]. However, improvements in diagnostic methods and screening of large-scale populations with full patient histories would give us a better insight into the impact of pathogens in this form of cancer [3]. It is plausible that in a subset of patients, pathogen-mediated, sub-clinical, persistent or recurrent low-grade inflammation could act as a driver of prostate cancer initiation and progression and might give rise to resistant aggressive forms. Moreover, the role of non-pathogen inflammasome activators should be carefully evaluated for their involvement. Inflammation in neighboring tissues might also be considered a risk factor for prostate cancer development. Given the limitations of biopsies in missing small focal lesions, especially at the periphery of the prostate gland, screening of large patient groups along with recurrent follow-ups would help to provide a better perspective of the role of inflammation in prostate cancer. Furthermore, concurrent inflammatory cytokine profiling and blood biochemical parameter profiling with the patient histories of antibiotic and anti-inflammatory drug use would provide a more rational assessment of the function of inflammasomes in prostate cancer pathophysiology.
The generation of appropriate mouse models with prostate-specific inflammatory gene deletion would aid in the dissection of inflammasome function in prostate cancer and the screening of drugs for efficacy. While many factors have the potential to produce additive effects, identifying the single most influential factor that changes the physiological balance in an irreversible way towards the development of prostate cancer would give us better options for its control and treatment. Certainly, inflammasomes and their regulators could be such causative factors in the pathogenesis of prostate cancer.

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REFERENCES


