AIM2, an IFN-Inducible Cytosolic DNA Sensor, in the Development of Benign Prostate Hyperplasia and Prostate Cancer

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Abstract

Close links have been noted between chronic inflammation of the prostate and the development of human prostatic diseases such as benign prostate hyperplasia (BPH) and prostate cancer. However, the molecular mechanisms that contribute to prostatic inflammation remain largely unexplored. Recent studies have indicated that the IFN-inducible AIM2 protein is a cytosolic DNA sensor in macrophages and keratinocytes. Upon sensing DNA, AIM2 recruits the adaptor ASC and pro-CASP1 to assemble the AIM2 inflammasome. Activation of the AIM2 inflammasome cleaves pro-interleukin (IL)-1β and pro-IL-18 and promotes the secretion of IL-1β and IL-18 proinflammatory cytokines. Given that human prostatic infections are associated with chronic inflammation, the development of BPH is associated with an accumulation of senescent cells with a proinflammatory phenotype, and the development of prostate cancer is associated with the loss of IFN signaling, the role of AIM2 in mediating the formation of prostatic diseases was investigated. It was determined that IFNs (α, β, or γ) induced AIM2 expression in human prostate epithelial cells and cytosolic DNA activated the AIM2 inflammasome. Steady-state levels of the AIM2 mRNA were higher in BPH than in normal prostate tissue. However, the levels of AIM2 mRNA were significantly lower in clinical tumor specimens. Accordingly, constitutive levels of AIM2 mRNA and protein were lower in a subset of prostate cancer cells as compared with BPH cells. Further, the cytosolic DNA activated the AIM2 inflammasome in the androgen receptor-negative PC3 prostate cancer cell line, suggesting that AIM2-mediated events are independent of androgen receptor status.

Implications: The AIM2 inflammasome has a fundamental role in the generation of human prostatic diseases. Mol Cancer Res; 11(10); 1193–202. ©2013 AACR.

Introduction

The etiologic agents that contribute to the development of human prostatic diseases such as benign prostate hyperplasia (BPH) and prostate cancer remain largely unknown (1–4). Studies have suggested close links among certain infections of the prostate, chronic inflammation, and the development of inflammation-associated prostatic diseases (5–10). It has been proposed that certain infections of the prostate contribute to the development of chronic inflammation and regenerative “risk factor” lesions, referred to as proliferative inflammatory atrophy, contribute to the development of prostate cancer (3–5). Accordingly, studies have found an increased relative risk of prostate cancer in men with a prior history of certain sexually transmitted infections or prostatitis, which are often associated with intraprostatic inflammation (6, 7). Furthermore, studies have indicated possible links between certain prostatic infections and inflammation (6, 7, 11, 12).

Prostatic infections, which can induce the production of type-I IFN-α/β) and the expression of the IFN-inducible genes (ISG; refs. 13, 14), reduce telomere length (15), resulting in an accumulation of senescent prostate epithelial cells (PrEC) in BPH (16). Accordingly, expression of a set of ISGs is upregulated during the onset of prostate epithelial cellular senescence (17–19). Furthermore, levels of the IFN-β increase in human diploid fibroblasts (HDF) when HDFs approach cellular senescence (20) and the expression of IFN-inducible proteins is upregulated in senescent PrECs (21) and HDFs (22). Senescent HDFs (and other cell types) are known to secrete proinflammatory cytokines (23). This phenotype of senescent cells has been termed senescence-associated secretory phenotype (SASP). The SASP, which depends on activation of NF-κB (24), activates the immune surveillance and exhibits protumorigenic properties (23). Furthermore, the cultures of senescent HDFs contain dead
cells, exhibit apoptosis, and the senescent HDFs release "exosomes" (microvesicles), which when fused with other cells, deliver dsDNA (a "danger signal") into the cytoplasm of neighboring cells (25, 26).

A complex of cytosolic proteins, which is termed "inflammasome", is activated in innate immune cells (macrophages and dendritic cells) in response to sensing "danger signals" by receptors (27–29). These receptors include members of the nucleotide binding and oligomerization domain (NOD)-like receptor (NLR) family such as NLRP3 and certain members of the IFN-inducible p200-family such as murine Aim2 and human AIM2 (29–31). The NLRP3 receptor can sense cytoplasmic pathogens, self-derived molecules, and environmental materials (29). Upon sensing these molecules, activated NLRP3 recruits an adaptor protein ASC and pro-caspase-1 to form the NLRP3 inflammasome. Activation of an inflammasome proteolytically cleaves pro-IL-1β and pro-IL-18 to the mature forms and potentiates their secretion from cells (27, 28). In addition, the activation of inflammasomes in macrophages induces a rapid highly inflammatory cell death called pyroptosis (29–31).

Activation of inflammasomes has been known in myeloid cells such as monocytes and macrophages (32). Interestingly, activation of an inflammasome has also been reported in nonmyeloid cells (33, 34), which are the preferred host cells for many intracellular pathogens. For example, an infection of cervical epithelial cells by Chlamydia trachomatis, an intracellular pathogen, leads to activation of caspase-1 through the NLRP3 inflammasome (33).

The human AIM2 protein from the IFN-inducible p200-protein family senses cytoplasmic dsDNA (hereafter referred as DNA) in myeloid cells (35–37) and "primed" (treated with IFN-γ and TNF-α) human keratinocytes (34). Upon sensing DNA, the AIM2 protein recruits an adaptor protein ASC to form the AIM2 inflammasome (31, 35–37). The activated AIM2 inflammasome in macrophages through activation of caspase-1 promotes the proteolytic cleavage and secretion of proinflammatory cytokines (IL-1β and IL-18). Given that human prostatic infections are associated with chronic inflammation (6, 7), the development of BPH is associated with an accumulation of senescent cells with a proinflammatory phenotype (16, 23), and the development of prostate cancer is associated with the loss of type I IFN signaling (18, 21), we investigated the role of AIM2 protein in human PrECs and prostatic diseases. Here, we report that cytosolic DNA can activate the AIM2 inflammasome in human PrECs. In addition, we found that the expression of AIM2 mRNA was higher in BPH than normal prostate, whereas levels of AIM2 mRNA were significantly lower in prostate tumors and certain cancer cell lines that were tested. Our observations show a role for the AIM2 inflammasome in the development of human prostatic diseases.

Materials and Methods

Reagents

Complete mini EDTA-free protease inhibitor cocktail was purchased from Roche Applied Science. Universal recombinant IFN-α, recombinant human IFN-β, recombinant human IFN-γ, and human TNF-α were purchased from R&D Systems. Caspase inhibitor VI (Z-VAD-FMK) was purchased from EMD Millipore. Poly(dA:dT)/LyoVec (synthetic double-stranded DNA in complex with LyoVec transfection agent) was purchased from InvivoGen.

Cell lines, culture conditions, and treatments

Human normal PrECs prostate cancer cell lines were cultured at 37°C in a humidified cell culture incubator containing 5% CO2. Human normal PrECs in culture (at passage 2) were purchased from Lonza and were maintained in culture as suggested by the supplier. Human prostate cancer cell lines (RWPE-1, RWPE-2, DU-145, PC-3, and LNCaP) were originally purchased from the American Type Culture Collection (ATCC) and maintained in culture as suggested by the supplier. BPH-1 cell line was generously provided by Dr. Simon Hayward (Vanderbilt University Medical Center, Nashville, TN; ref. 38). Cells were maintained in Dulbecco’s modified Eagle medium (high glucose) culture medium (Invitrogen Life Technologies) supplemented with 10% (v/v) FBS and antibiotics (Invitrogen). When indicated, cells were treated with either IFN-α (1,000 units/ml), IFN-β (1,000 u/ml), or IFN-γ (10 ng/ml) for the indicated time (hours). When indicated, PrECs and PC-3 cells in culture were “primed” by incubating with IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) for the indicated time as described previously (34).

Antibodies

Rabbit polyclonal antibodies that were raised against the keyhole limpet hemocyanin-conjugated hAim2 C-terminal peptide (GVHSTIKV1KAKKKT) have been described previously (20). Antibodies specific to detect ASC (sc-22514) and IL-18 (sc-7954) in immunoblotting were purchased from Santa Cruz Biotechnology. Antibodies for β-actin (cat #4967); IkBα ( #9247); histone 3 ( #9715); caspase 1 ( #8866); and IL-1β ( #2022) were purchased from Cell Signaling Technology. Horseradish peroxidase-conjugated secondary anti-mouse antibody (NA-931) and anti-rabbit (NA-934) antibodies were from GE Healthcare Biosciences.

Immunoblotting

Cells from subconfluent cultures were lysed with radioimmunoprecipitation assay (RIPA) buffer (50 mmol/L Tris-Cl (pH 7.4), 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS). The buffer was supplemented with complete mini EDTA-free protease inhibitor cocktail. Lysates were centrifuged at 12,000 rpm for 5 minutes at 4°C. Cell lysates containing equal amounts of protein (~50 µg) were separated by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and immunoblotted according to the manufacturer’s guidelines.

RNA isolation and PCR

Total RNA was isolated from the cultured cells with TRIzol reagent (Invitrogen). cDNA synthesis, reverse transcription polymerase chain reaction (RT-PCR), and
quantitative real-time TaqMan PCR were conducted to detect the expression of the AIM2 gene as described previously (20). The following primers were used for RT-PCR: human AIM2 (forward: 5′-ATGTTG AACCCGTCAGAAG-3′; backward: 5′-CATCATTCTGA TGGCTGCA-3′); human ASC (forward: 5′-GCTTGATCTTACAGCAGCAC-3′; backward: 5′-GCTTCCGATCTTGCTTG-3′); human CASP-1 (forward: 5′-TCCAAATATGGCAAGATGGC-3′; backward: 5′-GCTGTAACCACAGATTGTGATGAC-3′); human IL-1β (forward: 5′-CTCG CCGATGAAATGATG CGT-3′; backward: 5′-GTCGGAGATTCTAGCCTGAGAT-3′); and actin (forward: 5′-GCTCCTGCTGCTGACA ACGGCC-3′; backward: 5′-CATGATCTGGGTCTTCT-3′). The following TaqMan gene-specific assays were purchased from the Applied Biosystems and used as suggested by the supplier: AIM2 (Hs 00175457_m1) and the endogenous control β-actin (assay Id# Hs99999903_ml). TissueScan Cancer and Normal Tissue human prostate cDNA Arrays were purchased from OriGene. The arrays also included the information (age, tumor grade, and minimum stage of the tumor) about the samples. The PCR plates containing cDNAs from the normal or prostate tumors (Gleason score = 6–10) for quantitative polymerase chain reaction (qPCR) were subjected to RT-PCR as suggested by the supplier. In brief, the quantitative RT-PCR technology (Applied Biosystems) was used to compare the expression of the AIM2 gene. The PCR cycling program consisted of denaturing at 95°C for 10 minutes and 40 cycles at 95°C for 15 seconds, and annealing and elongation at 60°C for 1 minute. The signal in samples was normalized using the housekeeping genes as suggested by the supplier.

Transfections
Subconfluent cultures of PrECs in 60 mm plates were either left untreated (control) or were “primed” as described previously. Control or “primed” cultures of cells were either incubated with LyoVec (control) or poly(dA:dT)/LyoVec (5 μg/ml) for the indicated time. At the end of incubations, cells were harvested to isolate RNA or to prepare total cell lysates.

Nucleofections
PC-3 cells (~2 × 10^6 cells/nucleofection) were nucleofected as described previously (39). In brief, cells were nucleofected with 2 μg of plasmid DNA (pCMV-GFP) using the Nucleofector-II device (Amaxa Biosystems, Nathermannalle 1) and the nucleofection kit-V (program U-001). These nucleofection conditions resulted in approximately 60% cell survival after 24 hours of incubations of cells. After nucleofections, cells were harvested at the indicated times.

Reporter assays
We have cloned the 5′-regulatory region (~1.0 Kb) of the human AIM2 gene into a basic pGL3 reporter plasmid (plasmid designated as AIM2-luc), which does not contain any enhancer and promoter sequences. Therefore, the transcription of the reporter gene in the AIM2-luc-reporter is driven by the AIM2 promoter sequence. Subconfluent cultures of PC-3 cells in a 6-well plate were transfected with the AIM2-luc reporter plasmid (1.8 μg) along with the pRL-TK reporter plasmid (0.2 μg) as an internal control, using FuGene6 Transfection Reagent (Roche). Thirty-six to forty hours after transfections, the firefly luciferase and Renilla luciferase activities were assayed using dual-luciferase reporter assay kit (Promega). The relative firefly luciferase activity is expressed as the ratio of the firefly luciferase and Renilla luciferase.

Cell fractionations
Cytosolic and nuclear fractions from cultured cells were prepared as described previously (20). Briefly, cell pellets were incubated on ice for 10 minutes in cell fractionation buffer (20 mmol/L Tris-HCl, pH 7.5; 0.65% NP-40, 150 mmol/L NaCl) that was supplemented with protease and phosphatase inhibitors. The cytosolic fractions were recovered by centrifuging cell lysates at 2,000 rpm for 3 minutes in a refrigerated microcentrifuge. The nuclear pellet was washed with the cell fractionation buffer and the pellet was suspended into the RIPA buffer followed by brief sonication. The lysates were centrifuged at 12,000 rpm for 10 minutes at 4°C and supernatants were collected.

Statistical analyses
The measurement values are presented as means ± SEM. The statistical significance of differences in the measured mean frequencies between the two groups was calculated using the Student two-tailed t test.

Results
Expression of AIM2 inflammasome proteins is detectable in human PrECs and the IFN treatment of cells increases the expression levels
Expression of AIM2 protein is induced by treatment of cells with IFNs in a variety of cell types (31, 40). Therefore,
to investigate the role of the AIM2 inflammasome in human PrECs, we examined basal and the IFN-induced levels of AIM2 inflammasome proteins in young PrECs. As shown in Fig. 1A, the basal levels of AIM2 protein, which is detected as two closely migrating protein bands (20), adapter protein ASC, pro-caspase-1 (p45), and pro-IL-1β (p31) were detectable in PrECs. Interestingly, the IFN (α, β, or γ) treatment of cells for 24 hours differentially increased the levels of the AIM2, pro-caspase-1, and pro-IL-1β proteins. Notably, the treatment also increased the levels of the activated caspase-1 (p20) to different extents. Consistent with the above observations, treatment of PrECs with either IFN-α, β, or γ also increased the steady-state levels of AIM2 mRNA to different extents (Fig. 1B). The maximum increase was noted after IFN-γ treatment. Notably, the treatment of PrECs with IFN-α or β, but not IFN-γ, decreased steady-state levels of IL-1β mRNA. Together, these observations revealed that human normal PrECs in culture express low, but detectable, basal levels of AIM2 inflammasome proteins and the activation of IFN signaling in cells by type I (IFN-α or β) or type II IFN (IFN-γ) differentially regulates the steady-state levels of the AIM2 and other inflammasome proteins.

Cytosolic DNA activates the AIM2 inflammasome in PrECs

Cytosolic DNA activates the AIM2 inflammasome in "primed" (primed with IFN-γ and TNF-α treatment) human keratinocytes (34). The "priming" of keratinocytes increases basal levels of both AIM2 and pro-IL-1β (34). Therefore, to investigate whether AIM2 inflammasome can be activated by cytosolic DNA in PrECs, we transfected synthetic DNA [poly(dA:dT)] into "primed" human PrECs. Surprisingly, transfection of cells with the synthetic DNA decreased cellular levels of AIM2 protein (Fig. 2A, compare lanes 2 with lane 1). However, pretreatment of cells with z-VAD (a broad-spectrum inhibitor of caspase; 41) decreased the extent of decrease in AIM2 protein levels after transfection of synthetic DNA (compare lane 3 with lane 2). Notably, transfection of the synthetic DNA into PrECs decreased the levels of pro-caspase-1 (p45; ref. Fig. 2A), thus, indicating a proteolytic cleavage of the pro-caspase-1 and activation of the AIM2 inflammasome (34). Correspondingly, treatment of cells with z-VAD increased cellular levels of procaspase-1 (p45) and cleaved caspase-1 (p20 and p10; compare lane 3 with 2), indicating an inhibition of proteolytic cleavage of pro-caspase-1 (p45) and the secretion of activated caspase-1 (p20 and p10; compare lane 3 with 2). Again, the treatment of cells with z-VAD inhibited a decrease in the cellular levels of IL-18 and an increase in levels of IL-18 in the medium. Accordingly, the introduction of synthetic DNA into cells reduced the cellular levels of IL-18, whereas the secreted levels of IL-18 increased in the medium (compare lane 2 with 1). Therefore, to investigate whether AIM2 inflammasome can be activated by cytosolic DNA in PrECs, we transfected synthetic DNA [poly(dA:dT)] into "primed" human PrECs. Surprisingly, transfection of cells with the synthetic DNA decreased cellular levels of AIM2 protein (Fig. 2A, compare lanes 2 with lane 1). However, pretreatment of cells with z-VAD (a broad-spectrum inhibitor of caspase; 41) decreased the extent of decrease in AIM2 protein levels after transfection of synthetic DNA (compare lane 3 with lane 2). Notably, transfection of the synthetic DNA into PrECs decreased the levels of pro-caspase-1 (p45; ref. Fig. 2A), thus, indicating a proteolytic cleavage of the pro-caspase-1 and activation of the AIM2 inflammasome (34). Correspondingly, treatment of cells with z-VAD increased cellular levels of procaspase-1 (p45) and cleaved caspase-1 (p20 and p10; compare lane 3 with 2), indicating an inhibition of proteolytic cleavage of pro-caspase-1 (p45) and the secretion of activated caspase-1 (p20 and p10; compare lane 3 with 2). Notably, transfection of the synthetic DNA into PrECs decreased the levels of pro-caspase-1 (p45; ref. Fig. 2A), thus, indicating a proteolytic cleavage of the pro-caspase-1 and activation of the AIM2 inflammasome (34).

Figure 2. Cytosolic dsDNA activates the AIM2 inflammasome in human PrECs. A, human PrECs at early passage (passage 3) were "primed" by treating with IFN-γ (10 ng/mL) for 14 hours and then treated with TNF-α (10 ng/mL) for 3 hours. The "primed" cells were either left untreated (lane 1) or incubated with poly(dA:dT)/LyoVec (5 μg/mL) without any further treatment (lane 2) or with Z-VAD-FMK (10 μmol/L lane 3) for 6 hours. At the end of the incubations, cells were lysed and cell lysates containing equal amounts of proteins were analyzed by immunoblotting. B, cells of human benign prostate hyperplasia cell line BPH-1 were either left without any treatment (lanes 1 and 2) or "primed" with IFN-γ and TNF-α treatment (lanes 3 and 4). Control and "primed" cells were either left untreated (lanes 1 and 2) or incubated with poly(dA:dT)/LyoVec (5 μg/mL) for 6 hours. At the end of the incubations, cells were harvested and lysates containing equal amounts of proteins were analyzed for the indicated proteins by immunoblotting. Caspase, CASP.
Furthermore, transfection of synthetic DNA into untreated (control) or "primed" benign prostate hyperplasia BPH-1 cells also activated the AIM2 inflammasome as indicated by a decrease in cellular levels of the activated caspase-1 (p20) and corresponding increases in cellular levels of IL-18 (Fig. 2B). Together, these observations revealed that the cytosolic DNA can activate the AIM2 inflammasome in human normal PrECs and in an immortalized BPH cell line.

AIM2 expression in BPH and prostate cancer

Senescent cells, which secrete proinflammatory cytokines (23), accumulate in aging-associated BPH (16, 42). Moreover, defects in IFN signaling that result in the loss of the expression of the IFN-inducible proteins have been associated with the development of human prostate cancer (17, 21). Given that the expression of the AIM2 protein increases in senescent human fibroblasts (20), we compared the expression levels of AIM2 and other inflammasome proteins between young proliferating PrECs (passage 3) and senescent PrECs (passage 8; ref. 21). As shown in Fig. 3A, levels of the AIM2 protein and other inflammasome proteins such as ASC, pro-caspase-1 (p45), caspase-1 (p20), and pro-IL-1β (p31) were appreciably higher in the senescent PrECs than the young proliferating cells. These observations prompted us to investigate the potential role of AIM2 protein in human prostatic diseases such as BPH and prostate cancer. For this purpose, we compared steady-state levels of the AIM2 mRNA between normal prostate, BPH, and prostate tumors. As shown in Fig. 3B, steady-state levels of the AIM2 mRNA were measurably higher in BPH tissues as compared with the normal prostate. However, levels of AIM2 mRNA were significantly lower in the prostate adenocarcinoma samples (Gleason scores 3–5 + 3–5 = 6–9/10; Fig. 3C).

The above observations that levels of AIM2 mRNA were lower in prostate tumors prompted us to investigate the levels of AIM2 mRNA and protein in prostate epithelial cell lines (RWPE-1 and RWPE-2) that differ with respect to their ability to form tumors in nude mice: the RWPE-1 cell line is an immortalized cell line, which does not grow in soft agar and does not form tumors when injected in the nude mice, whereas RWPE-2 cell line is transformed (with activated Ki-Ras oncogene) and forms tumors in nude mice (43). As shown in Fig. 4A, treatment of RWPE-1 and RWPE-2 cells with either IFN-α or IFN-γ activated an IFN signaling (as detected by increases in levels of STAT1 transcription factor). Levels of STAT1 protein were higher in cells that were treated with IFN-γ than IFN-α (compare lane 3 with either lane 2 or 1). Notably, treatment of RWPE-1 cells with IFN-γ, but not IFN-α, measurably increased levels of AIM2 mRNA (compare lane 3 with either lane 2 or 1). In contrast, treatment of RWPE-2 cells with IFN-α or IFN-γ did not appreciably increase levels of AIM2 protein. Accordingly, steady-state levels of AIM2 mRNA were detectable in RWPE-1, but not in the RWPE-2, cells (Fig. 4B). Furthermore, treatment of RWPE-1 cells with IFN-γ increased the levels of the AIM2 mRNA approximately 6-fold, whereas the
treatment of RWPE-2 cells only moderately increased the mRNA levels (Fig. 4B).

We also compared basal and the IFN-induced levels of AIM2 mRNA and protein in human prostate cancer cell lines (DU-145, PC-3, and LNCaP) and a BPH cell line (BPH-1). The basal levels of the AIM2 mRNA and protein were very low in DU-145, PC-3, and LNCaP human prostate cancer cell lines (Fig. 5A–D). However, treatment of DU-145 and PC-3, but not LNCaP, cells with IFN-α or IFN-γ differentially increased levels of the AIM2 mRNA and protein (Fig. 5A–D). Of note, the basal levels of the AIM2 protein were measurably higher in the BPH-1, a benign prostate hyperplasia cell line than normal PrECs (Fig. 5E). Together, these observations revealed that the basal levels of AIM2 mRNA and protein are relatively lower in prostate tumors and prostate cancer cell lines that were tested. However, AIM2 expression is differentially induced in DU-145 and PC-3, but not LNCaP, cells upon treatment with IFN-γ.

IFN-γ treatment of PC-3 cells induces the expression of AIM2 and the induced levels of the AIM2 are detected primarily in the cytoplasm.

Because IFN-γ treatment of human prostate cancer cell lines (such as DU-145 and PC-3) increased the levels of the AIM2 protein (Fig. 5), to investigate the role of AIM2 protein in prostate cancer cells, we first investigated how treatment of prostate cancer cells with IFN-γ increases levels of AIM2 protein and its subcellular localization. For this purpose, we chose PC-3 cancer cell line because we had used this cell line to investigate the localization of the IFI16 protein (a member of the p200 protein family; ref. 21). As shown in Fig. 6A, treatment of PC-3 cells with IFN-γ for the indicated time increased the levels of STAT1, an IFN-inducible transcription factor (13). In addition, the treatment also increased an activating phosphorylation of STAT1 on Tyr-701 (PY-STAT1), indicating activation of the IFN signaling in PC-3 cells. Interestingly, the activation of an IFN signaling in PC-3 cells was accompanied by increases in AIM2 protein levels. Furthermore, the activity of the AIM2-luc-reporter plasmid (in which the transcription of the reporter gene was driven by the 5′-regulatory region of the AIM2 gene) was induced approximately 3-fold upon treatment of PC-3 cells with IFN-γ (Fig. 6B). Together, these observations indicated that treatment of PC-3 prostate cancer cells with IFN-γ activated an IFN signaling in cells, which increased the steady-state levels of the AIM2 protein through transcriptional activation of the AIM2 gene.

In transfected cells that overexpress the human AIM2 protein, the protein is primarily detected in the cytoplasm.
observations revealed that IFN-γ fraction of BPH-1 cells (data not shown). Together, these detected in the cytoplasm as well as in the nuclear cytoplasm and nucleus (albeit more in the cytoplasm).

However, basal and IFN-γ AIM2 protein were primarily detected in the cytoplasm, whereas the IFI16 protein was detected both in the nuclear fraction served as the quality control for the cell fractionations.

(35–37). Given that the IFI16 protein, which can bind to the AIM2 protein (44), is detected both in the cytoplasm and nucleus (45), we investigated the subcellular localization of the endogenous AIM2 protein in IFN-γ-treated PC-3 cells. As shown in Fig. 6C, the induced levels of the AIM2 protein were primarily detected in the cytoplasm of PC-3 cells.

Figure 6. IFN-γ treatment of PC-3 cells induces the expression of AIM2 and the induced levels of the AIM2 are detected primarily in the cytoplasm. A, subconfluent cultures of PC-3 cells were either left untreated (lane 1) or treated with IFN-γ for the indicated times (lanes 2–4). Total cell lysates containing equal amounts of proteins were analyzed by immunoblotting using antibodies specific to the indicated proteins. B, PC-3 cells were transfected with the AIM2-luc reporter plasmid (1.8 μg) along with pRL-TK (0.2 μg) plasmid using FuGene 6 transfection agent. After 24 hours of transfections, cells were either left untreated or treated with IFN-γ for 14 hours. Thirty-six to forty hours of transfections, the firefly and Renilla luciferase activities as were determined as described in Materials and Methods. Normalized relative firefly luciferase activity is shown. C, subconfluent cultures of PC-3 cells were either left without any treatment or treated with IFN-γ for 18 hours. Control and IFN-treated cells were fractionated into cytoplasmic (C) and nuclear (N) fractions. Fractions containing equal amounts of proteins were analyzed by immunoblotting using antibodies specific to the indicated proteins. Detection of IFI16 protein primarily in the cytoplasm, whereas histone H3 in the nuclear fraction served as the quality control for the cell fractionations.

Cytosolic DNA activates the AIM2 inflammasome in PC-3 cells

In our preliminary experiments, PC-3 cells expressed detectable levels of ASC adaptor protein (which is necessary to assemble an inflammasome) and pro-caspase-1. Therefore, our observations (Figs. 5 and 6) that the expression of AIM2 protein can be induced by activation of the IFN-γ signaling in PC-3 cells and AIM2 protein is primarily detected in the cytoplasmic fraction prompted us to investigate whether cytosolic DNA can activate the AIM2 inflammasome in PC-3 prostate cancer cells. As expected (34), “priming” of PC-3 cells increased levels of both AIM2 and pro-IL-1β (p31; Fig. 7). However, the “priming” decreased the levels of pro-caspase-1 and ASC moderately (compare lane 3 with 1). Furthermore, nucleofection of plasmid DNA (pCMV-GFP) into control and “primed” PC-3 cells measurably decreased the levels of cellular IL-1β (p17; compare lane 2 with 1 or lane 4 with 3). Notably, the decrease in the cellular levels of mature IL-1β (p17) was appreciably higher in “primed” cells as compared with control cells. This observation is consistent with higher steady-state levels of the AIM2 protein in “primed” cells as compared with control cells (compare lane 3 with lane 1). Although a decrease in cellular levels of the mature IL-1β (p17) is consistent with its secretion into the culture medium (44), we were unable to detect the secreted lower levels of IL-1β (p17) in the culture medium by immunoblotting (data not shown). Together, these observations revealed that nucleofected DNA can

Figure 7. Cytosolic DNA in PC-3 cells activates the AIM2 inflammasome. Subconfluent cultures of PC-3 cells were either left untreated or cells were “primed” by treating them with IFN-γ and TNF-α. Control and “primed” cells were nucleofected without DNA (lanes 1 and 3) or with 2 μg of plasmid DNA (pCMV-GFP) provided with the Amaxa Nucleofection Kit. Twenty-four hours after nucleofections, cells were lysed and cell lysates containing equal amounts of proteins were analyzed by immunoblotting using antibodies specific to the indicated proteins (C indicates cell-associated levels of IL-1β). Caspase, CASP.
activate the AIM2 inflammasome in control as well as "primed" PC-3 cells. In addition, our observations indicated that PC-3 cancer cells retain the ability to assemble the cytosolic DNA-responsive AIM2 inflammasome.

Discussion

We show that the cytosolic DNA, a "danger signal", can activate the AIM2 inflammasome in human normal PrECs and PC-3 prostate cancer cells. These observations are consistent with the idea that activation of the AIM2 inflammasome in human prostate by certain pathogens or self-derived DNA contributes to the prostatic inflammation (Fig. 8). In addition, our observations revealed that steady-state levels of AIM2 protein were appreciably higher in senescent PrECs (Fig. 3) and that AIM2 mRNA levels were higher in BPH tissues as compared with normal prostate tissues. Furthermore, our observations indicated that the steady-state levels of the AIM2 mRNA were significantly lower in prostate tumors (as compared with normal prostate) and certain prostate cancer cell lines that were tested. Together, these observations are consistent with the idea that accumulation of senescent PrECs in BPH contributes to an increased levels of the AIM2 protein in BPH, whereas reduced levels of the AIM2 protein in PrECs are associated with the development of prostate cancer.

AIM2 gene is constitutively expressed in the spleen, small intestine, and peripheral leukocytes (49). Furthermore, IFN-γ treatment of human HL-60 cell line (52) or IFN-β treatment of human THP-1 monocytic cell line (44) increased steady-state levels of the AIM2 mRNA. Consistent with these observations, our observations indicated that the AIM2 expression is induced by IFN-α or IFN-γ treatment of human normal PrECs (Fig. 1) and certain prostate cancer cell lines that were tested (Figs. 4 and 5). Furthermore, the IFN-γ treatment of PC-3 cells, which activated the IFN-activatable transcription factor STAT1 (Fig. 6), also increased the levels of the AIM2 protein and stimulated the activity of the AIM2-luc reporter. However, it remains unclear how activation of IFN-γ signaling in PrECs and PC-3 cells increases the steady-state levels of the AIM2 mRNA and protein. Given that treatment of prostate cancer cell lines such as PC-3 with either IFN-α or IFN-γ increased steady-state levels of AIM2 mRNA (Fig. 5C) and only IFN-γ treatment of cells increased the AIM2 protein levels (Fig. 5A), suggest that additional mechanisms involving a post-transcriptional mechanisms may contribute to the IFN-induced levels of the AIM2 protein in PC-3 prostate cancer cells (and possibly other cancer cells). Further work is in
progress to investigate how IFN-α and IFN-γ transcriptionally activate the expression of the AIM2 gene in PrECs.

Caspase-1 activation is required for human prostate cancer cells to undergo apoptosis in response to TGF-β treatment (53). Notably, the majority of primary prostate cancer specimens do not express detectable levels of caspase-1 (54). Moreover, certain prostate cancer cell lines express reduced levels of caspase-1 (54). Accordingly, we found that levels of AIM2 mRNA were significantly lower in prostate tumors as compared with normal prostate (Fig. 3) and certain prostate cancer cell lines did not express detectable basal levels of the AIM2 protein (Figs 4 and 5). Together, these observations suggest that the pattern of caspase-1 and AIM2 expression in prostate tumors may have prognostic significance in the disease progression.

Methylation-mediated silencing of the gene encoding for the ASC, an adaptor protein that is required to assemble certain inflammasome (including the AIM2 inflammasome; refs. 35–37), has been associated with the initial development of the human prostate cancers (55). Furthermore, a complete or partial methylation of the ASC gene has been noted in certain human prostate cancer cell lines (including LNCaP, DU145, PC-3, MDAPCa2b, and LAPC4). Notably, the PC-3 cells were reported to express detectable levels of ASC mRNA (55). Consistent with these observations, we were able to detect the expression of ASC protein in PC-3 cells (Fig. 7) and treatment of cells with 5-aza-2-deoxycytidine, an inhibitor of methylation, increased the protein levels further (data not shown). Moreover, nucleofection of plasmid DNA into “primed” PC-3 cells activated the AIM2 inflammasome (Fig. 7). Therefore, these observations indicated that PC-3 cells can assemble the AIM2 inflammasome upon sensing the cytosolic DNA.

We have reported previously that increased levels of AIM2 protein in senescent human fibroblasts are associated with an increased production of IL-1β and an inflammatory phenotype (20). Notably, the accumulation of senescent prostate epithelial cells in elderly patients plays an important role in the development of BPH (16, 42) and increased expression levels of IL-1β are associated with the development of experimental BPH (56). Therefore, our observations that steady-state levels of AIM2 mRNA were relatively higher in BPH than normal prostate are consistent with the idea that increased levels of the AIM2 protein in senescent PrECs contribute to senescence-associated secretory phenotype that is associated with the development of BPH.

Inflammasomes contribute to tissue homeostasis, inflammation, and immune responses (both innate and adaptive; ref. 29). Therefore, it is likely that inflammasomes also influence the formation, progression, and therapeutic responses of the human prostate cancer. Consequently, our observations (Fig. 8) that the presence of cytosolic DNA (derived from pathogens, necrotic cells, or senescent cells-derived “exosomes”) in normal PrECs and PC-3 prostate cancer cell line can activate the AIM2 inflammasome will serve as a basis to identify the molecular mechanisms through which certain prostastic infections and/or sterile inflammation contribute to the development of human prostatic diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Ponomareva, H. Liu, X. Duan, E. Dickerson, R. Pandamanathan

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Ponomareva, R. Pandamanathan, D. Choubey

Writing, review, and/or revision of the manuscript: L. Ponomareva, H. Shen, D. Choubey

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Duan, E. Dickerson, H. Shen

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