Negative Regulation of MAVS-Mediated Innate Immune Response by PSMA7

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The 20S proteasome is a ubiquitous protease complex composed of two large complexes: the 20S catalytic core complex and the 19S regulatory complex. The 19S complex is required for recruiting specific substrates to the proteasome complex by substrate association as an important cellular regulatory control.

The innate immune system can rapidly detect invading pathogenic microbes and eliminate them (1–3). Among the best characterized receptors in the innate immune system are the TLRs, NOD-like receptors (NLRs), and Retinoic Acid Induced Gene-1 (RIG-I)-like receptors (4–10). RIG-I contains tandem N-terminal caspase recruitment domains (CARDs) that interact with the CARD domains of MAVS to induce IFNs (11–19). Recently, another NOD-like receptor, NLRX1, has been identified, which is located in mitochondria and appears to act as a negative regulator of RIG-I by sequestering MAVS to prevent RIG-I signaling (20). However, the mechanism by which the IFN pathway is negatively regulated is still poorly understood.

Innate immunity to viruses involves receptors such as Retinoic Acid Induced Gene-1 (RIG-I), which senses viral RNA and triggers a signaling pathway involving the outer mitochondrial membrane protein mitochondrial antiviral signaling (MAVS). Recent work has identified that NLRX1, a member of another class of innate immune receptors, sequesters MAVS away from RIG-I and thereby prevents mitochondrial antiviral immunity. In this study, we demonstrate that the proteasome PSMA7 (α4) subunit associates with MAVS in vivo and in vitro. Expression of PSMA7 results in a potent inhibition of RIG-I and MAVS-mediated IFN-β promoter activity; conversely, depletion of PSMA7 with small interference RNA enhances virus-induced type I IFN production, with consequent reduction of virus replication. Furthermore, a striking reduction in the abundance of endogenous MAVS with overexpressed PSMA7 was found and virus infection leads to transient increase in the endogenous PSMA7 protein level. Cumulatively, these results suggest that PSMA7 is a negative regulator of the MAVS-mediated innate immunity that probably serves to attenuate the establishment of an antiviral state during viral infection, highlighting the biological significance of PSMA7-MAVS association as an important cellular regulatory control.

Materials and Methods

Cell culture, transfections, and small interfering RNA (siRNA)

HEK293, MCF-7, and murine embryonic fibroblasts cells were grown in DMEM (Invitrogen) supplemented with 10% heat-inactivated FBS (FBS-HyClone), 2 mM 4-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Transient transfections were performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

For stable down-regulation of PSMA7 or PSMA4, cells were transfected with pU6-PSMA7-siRNA or pU6-PSMA4-siRNA using Lipofectamine 2000 (Invitrogen) and selected in the presence of G418 3 days after transfection. G418-resistant single cell clones were amplified and screened by Western blot with anti-Flag Ab (Sigma-Aldrich). Two independent clones for each construct were harvested and used for additional experiments. The selection of the coding sequences for siRNA was based on previous guidelines. Each sequence was analyzed using the NCBI-BLAST database to verify that there was no homology greater than 15/21 residues with other genes. The sequences for the siRNA-encoding regions for PSMA7 and PSMA4 were obtained from GenBank Accession No. DQ890589 (nucleotides 324–342, http://www.

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4 Abbreviations used in this paper: NLR, NOD-like receptor; CARD, caspase recruitment domain; siRNA, small interfering RNA; MOI, multiplicity of infection; VSV, vesicular stomatitis virus; UPS, ubiquitin-proteasome system; RIG-I, Retinoic Acid Induced Gene-1; MAVS, mitochondrial antiviral signaling; HA, hemagglutinin.

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ncbi.nlm.nih.gov/nuccore/1241268667 report = GenBank) and GenBank Accession No. DQ895750 (nucleotides 218–236, http://www.ncbi.nlm.nih.gov/nuccore/1239981487 report = GenBank). siRNA oligos directed at the same nucleotides were synthesized and were ligated into pL6 vector (Invitrogen) using BamH1 and EcoRI.

Transient knockdown of PSMA7 or PSMA4 was generated by PSMA7 siRNA or PSMA4 siRNA oligonucleotide (Santa Cruz Biotechnology) transfection following the manufacturer’s instructions. Scrambled siRNA oligos were used as knockdown control.

Plasmids

Flag-MAVS plasmid was provided by Dr. Zhijian Chen (University of Texas Southwestern Medical Center, Dallas, Texas). Truncated forms of MAVS lacking the CARD-like domain (residues 10–77), the proline-rich region (residues 103–152) or the transmembrane domain (residues 514–535) were cloned into pcdNA3 using overlap extension PCR. Flag-tagged PSMA7 and its mutants were provided by Cheng Cao (Beijing Institute of Biotechnology, China). Plasmids encoding GST fusion proteins were prepared by cloning PCR-amplified fragments into pGEX4T-2 (Amersham Biosciences). GFP-MAVS and RFP-PSMA7 were prepared by cloning PCR-amplified fragments into pDSRed and pEGFP (Biosciences Clontech).

Immunoprecipitation and immunoblot analysis

Cell lysates were prepared in lysis buffer (50 mM Tris-HCl (pH 7.5)/1 mM PMSF/1 mM DTT/10 mM sodium fluoride/10 mg/ml aprotinin/10 mg/ml leupeptin/10 mg/ml pepstatin A containing 1% Nonidet P-40). Soluble proteins were subjected to immunoprecipitation with anti-Flag (Sigma-Aldrich), anti-Myc (Santa Cruz Biotechnology), or anti-mouse IgG Ab (Sigma-Aldrich). An aliquot of the total lysates (5%, v/v) was included as a control. Immunoblot analysis was performed with anti-Myc (Santa Cruz Biotechnology), anti-Flag (Sigma-Aldrich), anti-GFP (Santa Cruz Biotechnology), anti-PSMA7 (Santa Cruz Biotechnology), anti-MAVS (Abcam), or anti-β-Tubulin (Sigma-Aldrich) Ab. The Ag-Ab complexes were visualized by chemiluminescence (PerkinElmer). When necessary, figures were cropped using Adobe Photoshop software (Adobe). Band density was analyzed using Image-Quant software (Amersham Biosciences).

Immunofluorescence assay

Cells were washed briefly in PBS, fixed in 4% paraformaldehyde in PBS for 10 min, and the nuclei were stained for 10 min with 4′,6-diamidino-2-phenylindole dihydrochloride. After a final wash in PBS, samples were preserved in glycerol and images were captured using a digital camera under a confocal microscope (Zeiss LSM510).

Protein binding assays

In GST pull-down experiments, cell lysates were incubated for 2 h at 4°C with 5 μg purified GST or GST fusion proteins bound to glutathione beads. The absorbates were washed with lysis buffer and then subjected to SDS-PAGE and immunoblotting analysis. An aliquot of the total lysates (5%, v/v) was included as a loading control on the SDS-PAGE.

Luciferase reporter assays

HEK293 cells were transfected with 0.2 μg of the Luciferase reporter pNF-κB-LUC, IFN-β-LUC, or IFR3-LUC plus 0.02 μg of the internal control reporter pCMV-LacZ, with or without various amounts of MAVS, RIG1-N (expression vector, poly (dAT: dAT) or poly (I: C). Transfected cells were collected and Luciferase activity was assessed. All experiments were repeated at least three times.

RNA analysis

First-strand cDNA was generated from total RNA using random priming and moloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time PCR was performed using Quantitect SYBR Green PCR Master Mix (Qiagen) in triplicate experiments and analyzed on an ABI Prism 7700 analyzer (Applied Biosystems). All real-time values were normalized to 18s ribosomal RNA. IFN-β using the following primers: 3′: IFN-β S, 5′-CAGGACAGCCTTCTCCATGA-3′; IFN-β AS, 5′-AGCCAGTGCTC GATGAATCT.

Subcellular fractionation

Cells were washed 36 h after transfection in hypertonic buffer (10 mM Tris HCl (pH 7.5)/10 mM KC1/1.5 mM MgCl2/protease inhibitors) and then homogenized in the same buffer by bouncing 20 times. The homogenate was centrifuged at 500 × g for 5 min to remove nuclei and unbroken cells. The supernatant was centrifuged again at 5,000 × g for 10 min to generate membrane pellets containing mostly mitochondria and cytosolic supernatant.

Viral infections

MCF-7 cells were plated in 96-well plates at a density of 1 × 104 cells and incubated overnight. Viral infection was performed when 60% cell confluence was reached. Culture medium was replaced by serum-free DMEM, and VSV was added into the medium at concentration of 80 hemagglutinating units per ml and 5 multiplicity of infection (MOI), respectively. After 1 h incubation, extracellular virus was removed by washing cells two times with serum-containing medium. Cells and supernatant were harvested at indicated times post infection.

In vivo ubiquitination assays

Cells were cotransfected with plasmids expressing increasing amounts of Myc-PSMA7. Flag-MAVS, and hemagglutinin (HA)-tagged ubiquitin. Cells were grown in medium containing MG132 (20 μM) for 6 h. Anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-HA Ab, whole cell lysates were subjected to immunoblotting with anti-Myc and anti-Flag Ab, anti-β-Tubulin was used as equal loading control.

Vesicular stomatitis virus (VSV) infection and plaque assay

MEFs cells were infected with VSV at a MOI of 0.002 for 20 h. Virus yield was measured in culture supernatants collected from VSV-infected MEFs by standard plaque assay. In brief, cells were infected with serial dilutions of recovered viruses for 1 h and were overlaid with DMEM containing 0.5% low melting agarose. After 24 h of incubation, plates were stained with crystal violet and plaques were counted.

Statistical analysis

Analyses were done using the statistical software SAS/STAT (SAS Institute). Data analyses over time were undertaken by repeated measures analysis using SAS/STAT. A value of p < 0.05 was considered the threshold value for statistical significance.

Results

PSMA7 interacts with MAVS in vivo and in vitro

Owing to the crucial role of MAVS in the RIG1-MAV5 mediated antiviral response, a search for MAVS-interacting molecules was performed to uncover regulatory components of the pathway. Yeast two-hybrid analysis indicate that PSMA7 associates with MAVS (data not shown). To confirm the yeast two-hybrid analysis, Flag-tagged MAVS and Myc-tagged PSMA7 were transfected into HEK293 cells, and a coimmunoprecipitation experiment was performed (Fig. 1A). Myc-tagged PSMA7 was detected in the anti-Flag immunoprecipitation from cells cotransfected with Flag-MAVS, but not with a negative control Flag-tagged protein. This observation substantiates the yeast two-hybrid analysis and establishes an interaction between MAVS and PSMA7. The specificity of the interactions between MAVS and PSMA7 was also confirmed by coimmunoprecipitation analysis using normal serum (IgG). Importantly, endogenous MAVS was found to be specifically coimmunoprecipitated with endogenous PSMA7 (Fig. 1B). Moreover, immunofluorescence analysis of HEK293 cells transfected with GFP-MAVS and RFP-PSMA7 showed that the staining patterns of MAVS overlapped partially with PSMA7 (Fig. 1C). Because MAVS is a mitochondrial membrane protein, we next examined whether PSMA7 and MAVS colocalized in the same membrane compartment. HEK293 cells were homogenized in an isotonic buffer that preserved mitochondria and other organelles, and the cell lysates were then subjected to differential centrifugation to separate mitochondria and cytosol. Immunoblotting with an Ab against anti-MAVS and anti-PSMA7 showed that the both MAVS and some portion of PSMA7 located in mitochondria compartment, indicating that both proteins localize to mitochondria (Fig. 1D).

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To demonstrate the interaction of PSMA7 and MAVS in vitro, lysates from HEK293 cells expressing Flag-MAVS were incubated with GST or GST-PSMA7 fusion protein. Analysis of the absorbates by immunoblotting with anti-Flag showed that MAVS bound GST-PSMA7, but not GST (Fig. 1E), thus demonstrating an in vitro interaction between PSMA7 and MAVS.

To further map the PSMA7 binding sites in MAVS, three mutants containing truncated forms of MAVS lacking either the CARD-like domain (residues 10–77), the proline-rich region (residues 103–152), or the transmembrane domain (residues 514–535) were constructed and overexpressed together with PSMA7. Fig. 1F showed the N-terminal CARD domain and C-terminal TM domain of MAVS are required for MAVS signaling and for the interaction with PSMA7.

**FIGURE 1.** PSMA7 associates with MAVS. A, HEK293 cells were cotransfected with Flag-MAVS and Myc-PSMA7 expression plasmids or Flag-vector, and anti-Flag or IgG immunoprecipitates were analyzed by immunoblotting with anti-Myc or anti-Flag Ab. B, Lysates from HEK293 cells were subjected to immunoprecipitation with anti-PSMA7 or IgG, fractionated by SDS-PAGE, and subsequently analyzed by immunoblotting with anti-MAVS Abs. C, HEK293 cells were cotransfected with GFP-MAVS and RFP-PSMA7 expression plasmid with or without VSV infection; immunofluorescence was monitored. DNA was stained with 4′,6-diamidino-2-phenylindole dihydrochloride (blue). D, Mitochondria and cytoplasm fractions were analyzed by immunoblotting with anti-MAVS, anti-cytochrome C, anti-PSMA7, or anti-PSMA4 Ab. E, HEK293 cells were transfected with Flag-MAVS-expressing plasmid. The GST-PSMA7 fusion protein absorbates from cell lysates were analyzed by immunoblotting with anti-Flag Ab (top). Loading of the GST proteins was assessed by Coomassie blue staining (bottom). F, HEK293 cells were cotransfected with Myc-PSMA7 and Flag-MAVS expression plasmids or Flag-MAVS mutants; anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-Myc or anti-Flag Ab.

**PSMA7 inhibits MAVS-mediated induction of IFN-β**

To test whether this interaction has functional relevance, the effects of PSMA7 on MAVS-mediated type-I IFN activation were explored directly. Increasing amounts of expression vectors for PSMA7 were cotransfected with the expression construct for MAVS into HEK293 cells together with an IFN-β Luciferase reporter as well as pCMV-LacZ as an internal control. Thirty-six hours after transfection, the luciferase activity was measured and normalized based on β-galactosidase activity. As is shown in Fig. 2A, overexpression of PSMA7 in HEK293 cells potently activates the IFN-β promoter, while as low as 0.4 μg of PSMA7 was sufficient to exert a potent repression of IFN-β response. The extent of repression increased with increasing amounts of expressed PSMA7, suggesting that PSMA7 inhibited the induction of IFN-β by MAVS in a dose-dependent manner.
Both pathways that activate NF-κB and IRF3 lead to IFN-β transcription. Similar repressions of MAVS-induced activation of NF-κB and IRF3 reporters by PSMA7 were also observed (data not shown).

Overexpression of the tandem N-terminal CARD-like domains of RIG-I (designated as RIGI (N)) also induces IFN-β through MAVS. As is shown in Fig. 2A, PSMA7 significantly reduces the ability of RIG-I to activate IFN-β transcription. Signaling in response to the synthetic viral dsDNA analog poly(dAT:dAT) and synthetic viral dsRNA analog poly(I:C) are mediated both at the cell membrane through TLR3 and in the cytoplasm by means of direct binding to the RLH molecules to activate MAVS. We delivered poly(dAT:dAT) and poly(I:C) into the cytoplasm by transfection; PSMA7 also inhibited induction of IFN-β by poly(dAT:dAT) and poly(I:C) in a dose-dependent manner (Fig. 2C and data not shown). As a control for specificity, Jnk luciferase reporter activated by racL19 was not affected by PSMA7 (Fig. 2D). Other proteasome complex subunit proteins, PSMB2 and PSMA4, did not affect IFN-β luciferase (Fig. 2E and data not shown), suggesting the specific negative role of PSMA7 in IFN-β induction. Furthermore, IFN-β mRNA levels reduced sharply in poly(I:C) transfected cells containing the PSMA7 expression plasmid (Fig. 2F). Collectively, these data indicate that PSMA7 functions as an inhibitor of RLH-mediated MAVS antiviral signaling.

**PSMA7 siRNA enhances VSV induced-IFN-β production**

We next investigated the function of endogenous PSMA7 in dsRNA induced IFN-β production. PSMA7 and PSMA4-specific siRNA oligonucleotides, which reduced PSMA7 and PSMA4 protein by ~80% and 90%, were used (Fig. 4B). Consistent with the inhibitory effects of PSMA7 on the IFN-β promoter, expression of PSMA7-specific siRNA, but not control siRNA or PSMA4 siRNA, enhanced IFN-β promoter activity substantially by 16 h posttransfection with poly(I:C) (Fig. 3A). We then assessed whether endogenous PSMA7 was sufficient to induce the same regulation upon virus infection. IFN-β mRNA and protein levels were quantified in PSMA7 siRNA cells infected with VSV, which activates RIG-I and MAVS for type-1 IFN production. IFN-β mRNA induction by VSV was increased ~3-fold in cells with PSMA7 siRNA compared with control siRNA or PSMA4 siRNA (Fig. 3B). Consistent with the transcriptional data, VSV-induced IFN-β protein level was also greater in the infected PSMA7 siRNA cells (Fig. 3C). The effect of PSMA7 on IFN-β
mRNA induction is not restricted to a single cell type, because PSMA7 siRNA also led to increased IFN-β mRNA induction by VSV in MCF-7 cells and MEFs cells (Fig. 3D).

We next sought to determine whether PSMA7 regulated replication of VSV, as MAVS-mediated IFN signaling is critical in restricting replication of these RNA viruses. To this end, two PSMA7 expressing stable clones of MEFs cells with two empty vector expressing clones and two PSMA7 siRNA knockdown clones with two control siRNA expressing clones were obtained. Consistent with the suppressive effects of MAVS on RIG-I-mediated IFN signaling (Fig. 2), exogenous expression of PSMA7 increased the production of infectious VSV in the culture supernatants of infected MEFs, whereas interference of PSMA7 expression decreased it (Fig. 3, E and F). These results provide biological evidence that PSMA7 acts as a negative regulator of RIG-I-mediated type I-IFN signaling and thereby modulates the innate antiviral cellular response.

**PSMA7 destabilizes MAVS**

To explore the mechanism for the inhibitory effect of PSMA7 on antiviral signaling, we examined the effect of PSMA7 on endogenous MAVS abundance. When expression plasmids encoding PSMA7 were transfected into MCF-7 cells, remarkably, a striking reduction in the abundance of endogenous MAVS with overexpressed PSMA7 was found and this reduction of MAVS by PSMA7 was also dose dependent (Fig. 4A). As a control, increasing amounts of PSMA4 expression vector did not change the endogenous MAVS level (data not shown), indicating that the MAVS down-regulation is a specific effect elicited by PSMA7. We next investigated the function of endogenous PSMA7 in MAVS regulation. Expression of PSMA7-specific siRNA, but not control siRNA or PSMA4 siRNA, increased endogenous MAVS abundance in MCF-7 cell line (Fig. 4B). Quantitative RT-PCR revealed no change in endogenous MAVS mRNA
level, suggesting that PSMA7 down-regulates MAVS by posttranscriptional modification (Fig. 4C).

To further delineate the mechanism for the PSMA7-mediated MAVS degradation, HEK293 cells were cotransfected with plasmids expressing increasing amount of Myc-PSMA7, and MAVS mRNA was analyzed by quantitative RT-PCR. Increasing amounts (indicated on the top) of Myc-PSMA7 were cotransfected with plasmids encoding Flag-MAVS and HA-tagged ubiquitin. Cells were grown in medium containing MG132 (20 μM) for 6 h. Anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-HA Ab. Whole cell lysates were subjected to immunoblotting with anti-Myc and anti-Flag Ab, and anti-β-Tubulin was used as equal loading control.

Regulation of PSMA7 during virus infection

Negative regulatory proteins can function as negative feedback molecules to attenuate the response or they can function as “brakes” that are removed to allow the response to be enhanced. To investigate which of the above mechanisms applies to PSMA7, we analyzed the PSMA7 expression level in response to VSV infection in MCF-7 cells. As shown in Fig. 5A, VSV infection markedly increased the amount of PSMA7 protein as early as 1 h after infection. Moreover, immunofluorescence analysis of HEK293 cells transfected with GFP-MAVS and RFP-PSMA7 followed by VSV infection showed that the staining patterns of PSMA7 and MAVS were intensified by virus infection (Fig. 1C). Notably, the increase of PSMA7 persisted and resumed back to its background level 24 h after VSV infection. As a control, the abundance of PSMA4 protein in response to VSV infection remained unchanged. Moreover, we determined whether the presence of PSMA7 disrupted the interaction between Rig1 and MAVS in response to virus infection. Results showed that VSV infection led to enhanced interaction of MAVS and Rig1, while PSMA7 introduction destroyed the increased binding between MAVS and RIG1 (Fig. 5B).

Cumulatively, these results suggest that PSMA7 is a negative regulator of MAVS-mediated innate immunity that probably serves to attenuate the establishment of an antiviral state during viral infection, highlighting the biological significance of PSMA7-MAVS association as an important cellular regulatory control. The transient inhibition of the activity of PSMA7 by pharmacological agents might therefore provide a strategy to enhance antiviral responses.

Discussion

Innate immunity to viruses involves receptors such as RIG-I, which senses viral RNA and triggers a signaling pathway involving the outer mitochondrial membrane protein MAVS. Recently, the regulation of MAVS has been cast into the limelight. NLRX1, a member of another class of innate immune receptors, sequesters MAVS away from RIG-I and thereby prevents downstream antiviral signaling emanating from the mitochondria via MAVS (20).

Regulating the stability of MAVS is another potential mechanism to modulate the innate immune response. In this study, we characterized PSMA7 as a novel and essential cofactor for MAVS function in RIG1 signaling. Several lines of findings support this argument. First, PSMA7 was shown to interact directly with MAVS both in vitro and in vivo. Second, overexpression of PSMA7 destabilized MAVS and severely impaired IFN-β activation. The abundance of endogenous MAVS was affected by overexpressed PSMA7, but not by another proteasome subunit PSMA4. Therefore, like HIF1α (17), PSMA7 targets MAVS for proteasome-dependent

FIGURE 4. PSMA7 promotes MAVS degradation. A, MCF-7 cells were transfected with plasmids expressing increasing amount of Myc-PSMA7. Whole cell lysates were analyzed by immunoblotting with anti-MAVS or anti-Myc Ab; anti-β-Tubulin was used as equal loading control. B, MCF-7 cells were transfected with siPSMA7 or siPSMA4 oligos. After 24 h, whole cell lysates were analyzed by immunoblotting with anti-MAVS, anti-PSMA7 or anti-PSMA4 Ab; anti-β-Tubulin was used as equal loading control. C, MCF-7 cells were transfected with plasmids expressing increasing amount of Myc-PSMA7, RNA was extracted, and MAVS mRNA was analyzed by quantitative RT-PCR. D, Increasing amounts (indicated on the top) of Myc-PSMA7 were cotransfected with plasmids encoding Flag-MAVS and HA-tagged ubiquitin. Cells were grown in medium containing MG132 (20 μM) for 6 h. Anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-HA Ab. Whole cell lysates were subjected to immunoblotting with anti-Myc and anti-Flag Ab, and anti-β-Tubulin was used as equal loading control.
and, in fact, dysregulation of the UPS has been linked to several pathway contribute to protein alterations associated with aging quality control function. Alterations in the proteasome proteolytic proteasome complex.

PSMA7, is also quickly degraded by the proteasome pathway, sug-
tective feedback molecule to attenuate the persistence of antiviral state, in response to virus infection indicates that PSMA7 serves as a neg-
hanced IFN-
singalizing, we suggest that the reduced contact of MAVS with the

FIGURE 5. Regulation of PSMA7 during infection. A, MCF-7 cells were treated with VSV for indicated times. Cells were then harvested at different times after VSV infection. Whole cell lysates were analyzed by immunoblotting with anti-PSMA7, anti-PSMA4, or anti-β-Tubulin Ab. B, MCF-7 cells were infected with VSV virus for 1 h and then cotransfected with Flag-MAVS and Myc-RIG1 with or without the expression vector encoding GFP-PSMA7. Anti-Flag immunoprecipitate was analyzed by immunoblotting with anti-Myc, anti-GFP, or anti-Flag Ab.

degradation. Third, the knockdown of endogenous PSMA7 increased the cellular amount of MAVS and IFN-β induction, possibly due to a reduced contact with proteasome complex or decreased proteasome activity. Because PSMA4 knockdown cells showed normal IFN-β signaling, we suggest that the reduced contact of MAVS with the proteasome complex in PSMA7 siRNA cells contributes mainly to the enhanced IFN-β signaling. Fourth, up-regulation of PSMA7 protein in response to virus infection indicates that PSMA7 serves as a neg-

There have been several reports about cellular regulatory proteins and viral proteins that interact with subunits of the protea-
some complex and participate in the proteasome-dependent regul-
ation (28, 32). Because proteasome subunits can be exchanged with exogenous subunits in vivo and in vitro (29–31), the regula-
tory effect of the exogenous PSMA7 on MAVS may be due to the PSMA7-mediated recruitment of MAVS to the proteasome complex. Human hepatitis B virus X protein, another binding target of PSMA7, is also quickly degraded by the proteasome pathway, sug-
gesting that PSMA7 may have a role in recruiting substrates to the proteasome complex.

The ubiquitin-proteasome system (UPS) displays an important quality control function. Alterations in the proteasome proteolytic pathway contribute to protein alterations associated with aging and, in fact, dysregulation of the UPS has been linked to several disease states including neurodegenerative diseases, malignancies, and inflammatory-related diseases (33–38). Strong preclinical data now exist, which supports the use of reversible proteasome inhib-
itors to treat a variety of disease states including cancer, autoim-
une and inflammatory diseases, myocardial infarction, and isch-
emic brain injury (39–42). PSMA7, a subunit of proteasome complex might play an important, yet undefined, key roles in UPS-linked disease. In this study, we have demonstrated that PSMA7 is involved in the stability of MAVS, which thereby provides negative regulation of the innate antiviral response against infection by RNA viruses. Thus, PSMA7 might function to regulate host innate immune signaling by destabilizing MAVS and targeting of PSMA7 through approaches such as siRNA could enhance antiviral responses, which has broad implications for the treatment of viral associated diseases.

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Disclosures
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