STING Is Involved in Antiviral Immune Response against VZV Infection via the Induction of Type I and III IFN Pathways

Ji-Ae Kim¹, Seul-Ki Park¹, Seong-Wook Seo¹, Chan-Hee Lee² and Ok Sarah Shin¹

Varicella zoster virus (VZV) is a human-restricted z-herpesvirus that exhibits tropism for the skin. The VZV host receptors and downstream signaling pathways responsible for the antiviral innate immune response in the skin are not completely understood. Here, we show that STING mediates an important host defense against VZV infection in dermal cells including human dermal fibroblasts and HaCaT keratinocytes. Inhibition of STING using small interfering-RNA or short hairpin RNA-mediated gene disruption resulted in enhanced viral replication but diminished IRF3 phosphorylation and induction of IFNs and proinflammatory cytokines. Pretreatment with STING agonists resulted in reduced VZV glycoprotein E expression and viral replication. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.


INTRODUCTION

Varicella zoster virus (VZV) is a human-specific herpesvirus that causes chickenpox in humans; VZV can subsequently establish latency in the sensory ganglia, from where it reactivates in the form of herpes zoster. The skin is a major site where the signature varicella and zoster lesions appear as cutaneous vesicles; thus, skin-mediated innate immunity against VZV is essential for early control of VZV infection (Zerboni et al., 2014). Although vaccines for varicella and zoster prevention are currently available, the effectiveness of VZV. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.

 Varicella zoster virus (VZV) is a human-restricted virus that exhibits tropism for the skin. The VZV host receptors and downstream signaling pathways responsible for the antiviral innate immune response in the skin are not completely understood. Here, we show that STING mediates an important host defense against VZV infection in dermal cells including human dermal fibroblasts and HaCaT keratinocytes. Inhibition of STING using small interfering-RNA or short hairpin RNA-mediated gene disruption resulted in enhanced viral replication but diminished IRF3 phosphorylation and induction of IFNs and proinflammatory cytokines. Pretreatment with STING agonists resulted in reduced VZV glycoprotein E expression and viral replication. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.

INTRODUCTION

Varicella zoster virus (VZV) is a human-specific herpesvirus that causes chickenpox in humans; VZV can subsequently establish latency in the sensory ganglia, from where it reactivates in the form of herpes zoster. The skin is a major site where the signature varicella and zoster lesions appear as cutaneous vesicles; thus, skin-mediated innate immunity against VZV is essential for early control of VZV infection (Zerboni et al., 2014). Although vaccines for varicella and zoster prevention are currently available, the effectiveness of VZV. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.

Varicella zoster virus (VZV) is a human-restricted virus that exhibits tropism for the skin. The VZV host receptors and downstream signaling pathways responsible for the antiviral innate immune response in the skin are not completely understood. Here, we show that STING mediates an important host defense against VZV infection in dermal cells including human dermal fibroblasts and HaCaT keratinocytes. Inhibition of STING using small interfering-RNA or short hairpin RNA-mediated gene disruption resulted in enhanced viral replication but diminished IRF3 phosphorylation and induction of IFNs and proinflammatory cytokines. Pretreatment with STING agonists resulted in reduced VZV glycoprotein E expression and viral replication. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.

INTRODUCTION

Varicella zoster virus (VZV) is a human-specific herpesvirus that causes chickenpox in humans; VZV can subsequently establish latency in the sensory ganglia, from where it reactivates in the form of herpes zoster. The skin is a major site where the signature varicella and zoster lesions appear as cutaneous vesicles; thus, skin-mediated innate immunity against VZV is essential for early control of VZV infection (Zerboni et al., 2014). Although vaccines for varicella and zoster prevention are currently available, the effectiveness of VZV. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.

INTRODUCTION

Varicella zoster virus (VZV) is a human-specific herpesvirus that causes chickenpox in humans; VZV can subsequently establish latency in the sensory ganglia, from where it reactivates in the form of herpes zoster. The skin is a major site where the signature varicella and zoster lesions appear as cutaneous vesicles; thus, skin-mediated innate immunity against VZV is essential for early control of VZV infection (Zerboni et al., 2014). Although vaccines for varicella and zoster prevention are currently available, the effectiveness of VZV. Additionally, using RNA sequencing to analyze dual host and VZV transcriptomes, we identified several host immune genes significantly induced by VZV infection. Furthermore, significant up-regulation of IFN-λ secretion was observed after VZV infection, partly through a STING-dependent pathway; IFN-λ was shown to be crucial for antiviral defense against VZV in human dermal cells. In conclusion, our data provide an important insight into STING-mediated induction of type I and III IFNs and subsequent antiviral signaling pathways that regulate VZV replication in human dermal cells.
induction of both type I and III IFNs. In addition, we used RNA sequencing (RNA-seq) technology to comprehensively analyze dual host and VZV transcriptomes, which allowed for the identification of distinct and dynamic changes of both transcriptomes depending on cell type and postinfection time point. Our findings provide valuable insights into host defense mechanisms in dermal cells; STING agonists and IFN-αs could have important implications for VZV treatment.

RESULTS
STING pathway modulates VZV replication in human dermal cells
To investigate the role of STING in human dermal fibroblasts (HDFs) and HaCaT keratinocytes, STING small interfering RNA (siRNA) was transiently transfected before infection with VZV. STING-siRNA transfected HaCaTs showed increased protein expression of VZV immediate early gene (IE62) and late gene VZV glycoprotein E (gE) compared with control siRNA-transfected cells (Figure 1a). Similar results were observed in HDFs; STING knockdown led to increased gE expression and viral replication (see Supplementary Figure S1a, S1b online). Additionally, the production of proinflammatory cytokines and IFNs was suppressed upon STING silencing, suggesting that STING knockdown can impair the antiviral and inflammatory response in HDFs (see Supplementary Figure S1c).

Next, we established stable MRC-5 fibroblasts depleted for STING using lentivirus-expressing short hairpin RNAs (shRNAs). Stable cells expressing a scrambled control shRNA were used as the control. Introduction of the STING shRNA resulted in significant down-regulation of STING expression, compared with the scrambled control shRNA cell line (Figure 1b). Significant up-regulation of the VZV IE62 protein was observed in STING shRNA cells, while a 2-fold increase in viral titers occurred in STING-depleted cells, compared with scrambled control shRNA cells (Figure 1b, 1c). STING abrogation led to the significant decrease in the transcript levels of IFN-λ1, IFN-β, and the IFN-γ-inducible protein, IP-10 (see Supplementary Figure S1d). The effect of TBK1 and IRF3 knockdown on VZV replication was also examined. Although TBK1 knockdown increased VZV gE expression, IRF3 knockdown led to increased IE62 protein expression (Figure 1d, 1e). Additionally, TBK1 or IRF3 siRNA-treated cells led to decreased IFN production (see Supplementary Figure S2 online). In contrast, we observed that overexpression of STING in HaCaTs and HDFs significantly reduced IE62 and gE expression, respectively (see Supplementary Figure S3a, S3b online). Moreover, STING overexpression resulted in increased IFN-β secretion, suggesting IFN-mediated antiviral response (see Supplementary Figure S3c, S3d). These data indicate that STING plays an antiviral role against VZV infection in human dermal cells.
STING agonists enhance VZV-stimulated IFN production and play an essential antiviral function

2’3’ and 3’3’ cGAMPs are produced in mammalian and bacterial cells, respectively; however, both cGAMPs act as STING agonists to induce IFN pathways (Zhang et al., 2013). We first tested whether a STING mutation in HEK293T cells potently affects IFN-β promoter-driven luciferase reporter activity in response to VZV genomic DNA and cGAMPs. Transfection of the wild-type STING plasmid induced higher IFN-β-luciferase activity than the empty vector control, whereas transfection of the STING mutant (h232) led to a decreased response to cGAMP and VZV genomic DNA co-treatment (Figure 2a).

STING agonists enhance VZV-stimulated IFN production and play an essential antiviral function

Moreover, we assessed whether major VZV open reading frames (ORFs), such as ORF68 (gE) and ORF47, could modulate IFN activity in response to STING agonists or synthetic polydeoxyadenylic-polydeoxythymidylic acid. The transfection efficiency of the ORF68 and ORF47 plasmids was first confirmed in HEK293T cells (see Supplementary Figure S5a online). As shown in Figure 2d, transfection of VZV ORF47 or ORF68 led to a significant reduction in STING-mediated IFN-β luciferase activity. Thus, these data suggest that VZV ORF47 and ORF68 can disrupt STING-mediated IFN production.

RIG-I is not essential for VZV replication control

The subcellular localization of STING and VZV gE were analyzed by confocal microscopy and anti-calnexin antibody was used to stain endoplasmic reticulum membrane. The localization pattern of STING overlapped with VZV gE or calnexin and STING partly overlapped with MitoTracker, whereas the subcellular location of MAVS and STING barely overlapped (Figure 3a, Supplementary Figure S5b). We next determined the role of RIG-I, a key cytoplasmic pathogen recognition receptor that has been suggested to sense RNA viruses and several DNA viruses (Gack, 2014) in response to VZV infection. RIG-I-specific siRNA was introduced in MRC-5 cells, and a plaque assay was performed. As
expected, knockdown of STING resulted in a significant increase in viral replication, whereas knockdown of RIG-I did not affect viral titers (Figure 3b). Similarly, transfection of RIG-I siRNA in HaCaTs did not affect the protein expression of gE, although it did lead to a downstream decrease in TBK1/IRF3 phosphorylation (Figure 3c). When RIG-I was overexpressed in HDFs, significant suppression of virus replication was observed, similar to the overexpression of wild-type STING (Figure 3d). Given that both RIG-I and STING activation promotes the same downstream signaling pathways, it is possible that RIG-I–induced IFN response and IFN-stimulated genes production may have contributed to control VZV replication. The transfection of the STING variant (h232) no longer reduced viral titers, which may be a result of a failure to induce STING-mediated IFNs.

RNA-seq data shows differential and dynamic transcriptome responses to VZV infection

Our previous RNA-seq analysis study suggested that VZV infection in HDFs results in distinct up-regulation of the antiviral immune response (Kim et al., 2015). To obtain global and dynamic gene expression profiles of the VZV-induced immune response, HDFs and HaCaTs were infected with mock or VZV for 24, 48, and 72 hours, and the dual host/virus transcriptome was analyzed by RNA-seq. VZV-induced host transcriptomic changes in HaCaTs are schematically presented as differentially expressed gene (DEG) plots in Supplementary Figure S6a online. Heatmap analysis showed 153 DEGs specifically up-regulated (>8-fold) by VZV infection (Q-value threshold < 0.01; see Supplementary Figure S6b). The top 10 up-regulated DEGs are presented in Supplementary Table S1 (online) and include virus sensors/antiviral genes such as AIM2, DDX60, and Mx2. Gene ontology analysis also suggested that immune system and viral process-associated DEGs were significantly overrepresented (Figure S6c).

A VZV ORF heatmap was generated to compare the expression levels of VZV ORFs in fibroblasts versus keratinocytes. Similar to other herpesvirus family members, VZV gene expression occurs in a temporally regulated cascade: immediate early, early, and late genes. As shown in Figure 4a, the fragment per kilobase of exon per million values of most ORFs were similar in HDFs and HaCaTs; however, the expression of late viral genes, such as ORF31, -50, -67, and -68, was more highly elevated than that of immediate early genes at all time points. Furthermore, the expression levels of late viral genes were higher in HDFs than in HaCaTs. To validate the DEGs identified from the RNA-seq data, we used quantitative real-time reverse transcriptase–PCR (qRT-PCR) assays to analyze the expression levels of selected up-regulated genes. In accordance with the RNA-seq data, VZV-infected HaCaTs exhibited a statistically significant increase in the mRNA expression levels of AIM2, DDX60, and Mx2; however, the expression of late viral genes, such as ORF31, -50, -67, and -68, was more highly elevated than that of immediate early genes at all time points. Furthermore, the expression levels of late viral genes were higher in HDFs than in HaCaTs. To validate the DEGs identified from the RNA-seq data, we used quantitative real-time reverse transcriptase–PCR (qRT-PCR) assays to analyze the expression levels of selected up-regulated genes. In accordance with the RNA-seq data, VZV-infected HaCaTs exhibited a statistically significant increase in the mRNA expression levels of AIM2, DDX60, DDX58 (RIG-I), IFIHI (MDA5), Mx2, SLFN12, and MMP13 compared with mock control cells at 48 hours after infection (Figure 4b). To characterize whether AIM2 would result in a difference in the viral replication efficiency, we used AIM2 siRNAs to transfect HaCaTs, and
the transcript level of VZV ORF29 was significantly up-regulated upon AIM2 knockdown, whereas the downstream molecules IL-1β and ASC were down-regulated (Figure 4c). Western blot showed an enhanced IE62 expression by AIM2 knockdown, indicating a potential role of the AIM2 inflammasome in antiviral signaling (Figure 4d).

**IFN-λ is indispensable for VZV replication control**

Although IFN-λ is the main IFN produced in keratinocytes, the role of IFN-λ during VZV infection is yet to be determined. First, we analyzed the effect of IFN signaling and function in human dermal cells. Recombinant human IFNs (10 ng/ml) were added to cells, and qRT-PCR was performed.
Similar to the results shown in Figure 4b, genes encoding proteins involved in intrinsic viral sensing and defense, including AIM2, DDX60, DDX58, IFI1H1, and Mx2, were highly up-regulated upon recombinant IFN treatment (see Supplementary Figure S7a online). Previous studies indicate that IFN-λ is regulated by a unique pathway that involves IRF1 and JAK2 (Odendall et al., 2014). IFN-λ treatment led to time-dependent phosphorylation of STAT1 in HaCaTs (see Supplementary Figure S7b). Furthermore, specific up-regulation of IRF1 and JAK2 after IFN-λ treatment was also detected, suggesting that IFN-λ signaling is functional. Having established the responsiveness of IFN-λ in HaCaTs, we next measured IFN-λ protein levels after VZV infection. Time-dependent enhanced secretion of IFN-λ1/3 and IFN-λ2 after VZV infection occurred in HaCaTs, whereas the secretion levels in fibroblasts were lower and remained unaltered by VZV infection (see Supplementary Figure S8 online).

Next, we evaluated whether STING deficiency could modulate IFN-λ production. The secretion levels of both IFN-λ1/3 and IFN-λ2 were partly suppressed in STING siRNA-transfected VZV-infected cells (Figure 5a). The ability of IFN-λ to inhibit VZV replication was also examined. First, a 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay was performed to rule out cytotoxicity in HaCaTs and HDFs (see Supplementary Figure S9 online). Viral titers were measured in IFN-λ–treated HDFs or malignant melanoma cells (MeWo). As shown in Figure 5b, both IFN-λ1 and IFN-λ2 significantly suppressed viral replication in HDF cells by 65.1% and 43.2%, respectively, whereas in MeWo, virus titers were suppressed 27.6% by IFN-λ1 and 22.3% by IFN-λ2 treatment.

In addition to plaque assays, gE expression was analyzed by confocal microscopy and Western blotting. IFN-λ treatment dramatically impaired gE expression (Figure 5c). Because IFN-λ severely limits VZV replication, we examined the ability of IFN-λ treatment to modulate the induction of IFN-stimulated genes after VZV infection. Both IFN-λ1 and IFN-λ2 significantly enhanced the expression of MxA and 2’5’-oligoadenylate synthetase as compared with control-treated cells; whereas VZV ORF63 gene expression decreased significantly after IFN-λ treatment (Figure 5d). Furthermore, we sought to determine whether IFN combination treatment could promote a synergistic antiviral effect against VZV. A combination of IFN-λ together with IFN-λ1 and IFN-λ2 exhibited maximum synergy compared with single IFN treatment; IE62 expression levels decreased by 63%, based on Western blotting (Figure 5e). Taken together, these data indicate that IFN-λ is a potent antiviral factor in human dermal cells.

**DISCUSSION**

Here, we describe VZV-triggered host signaling pathways leading to the induction of an antiviral state in human dermal cells, the most important viral target cells in an infected human (Figure 6). Of the 71 known or predicted VZV ORFs, ORF68 encodes the most abundant viral glycoprotein and is
Figure 6. Proposed schematics for our data show STING-mediated antiviral signaling. VZV DNA can be sensed by cytosolic DNA sensors, such as cGAS or AIM2. cGAS-mediated sensing can lead to production of cGAMP and subsequent STING/TBK1/IRF3 signaling pathway. This event can stimulate gene transcription of type I and III IFNs. Upon binding to their cognate receptors, type I and III IFNs induce the JAK/STAT pathway, leading to phosphorylation of STAT1 and STAT2 to induce ISG expression. Both type I and III IFNs can act as antiviral factors capable of blocking VZV replication in dermal compartments. cGAMP, cyclic guanosine monophosphate-adenosine monophosphate; cGAS, cyclic guanosine monophosphate-adenosine monophosphate synthase; dsDNA, double-stranded DNA; ISG, IFN-α/β-stimulated genes; JAK, Janus kinase; VZV, varicella zoster virus.

thus important for viral replication (Mo et al., 2002). Additionally, IE62 is a virion-associated transactivator required for initiating infection, which counteracts the activities of IRF3 (Sen et al., 2010), whereas VZV ORF47 induces atypical inhibitory phosphorylation of IRF3, further preventing IRF3 homodimerization and the induction of target genes (Heineman and Cohen, 1995; Vandevenne et al., 2011). In accordance with these data, our results show that the products of VZV ORF68 and ORF47 can specifically disrupt STING-mediated IFN-α activities, suggesting the possibility that VZV is able to suppress STING-mediated antiviral defense mechanisms.

Recent studies indicate that STING is essential for the activation of antiviral signaling pathways in response to herpes simplex virus-1 infection (Royer and Carr, 2016); however, the antiviral role of STING for VZV has not been clearly elucidated. Our findings show that STING pathways are important for the induction of IFNs and the regulation of viral replication, showing that shRNA-mediated silencing of STING and subsequent abrogation of downstream antiviral molecules in dermal cells lead to elevated viral titers. The STING agonist 2′3′ cGAMP is known to bind to STING with a higher affinity than 3′3′ cGAMP. Our results show the potent antiviral effect of both 2′3′ and 3′3′ cGAMPs against VZV replication, although 2′3′ exhibited stronger inhibition of plaque formation than 3′3′. STING has been shown to interact with STAT6 (independent of Janus kinase), and STAT6 is required for antiviral immunity in vivo (Chen et al., 2011). We also examined the antiviral role of STAT6, but STAT6 knockdown in HaCaTs did not lead to significant change in phospho-IRF3 or VZV IE62 expression, and furthermore, STAT6 expression did not appear to be dependent on STING (Figure 1a).

Although STING-mediated activation of the TBK1/IRF3/ type I IFN pathway has been well characterized, the ability of STING to induce the type III IFN response has yet to be examined, despite the importance of IFN-λ in epithelial responses (Wolk et al., 2013; Zahn et al., 2011). The critical defense role of IFN-λ in dermal compartments was highlighted by recent findings that IFN-λ can specifically induce MxA in human dermal fibroblasts (Alase et al., 2015). Our data also suggest that IFN-λ production was significantly up-regulated upon VZV infection in HaCaTs and that their expression is partly dependent on STING activation. Furthermore, IFN-λ treatment resulted in the reduction of viral titers and significant upregulation of Mxa and OAS gene expression (Figure 5). IFN-λ antiviral effect was less efficient in MeWo than in HDFs. This attenuated IFN- λ antiviral effect in MeWo could be caused by a lack of AIM2-mediated antiviral signaling in MeWo, which may have contributed to increased VZV susceptibility, given that AIM2 was originally identified as a unique gene absent in melanoma cells (DeYoung et al., 1997).

Previously, Jones et al. (2014) showed that the differentiation status of keratinocytes affects the replication pattern of the viral genome and protein expression using dual RNA-seq in VZV-infected keratinocytes. In line with this, our previous study using senescent HDFs and progeria cells highlighted the effect of cellular replicative senescence on VZV replication (Kim et al., 2015, 2016). Here, we aimed to evaluate the underlying features of dermal cell type-specific and post-infection time-specific mRNA profiles using high-throughput dual RNA-seq. As shown by the heatmap (Figure 4a), ORF68 (gE) was one of the most highly up-regulated transcripts in both HDFs and HaCaTs; its expression level was higher in HDFs than in HaCaTs. Additionally, our data highlight distinct and dynamic changes in the expression patterns of host genes after VZV infection in keratinocytes. Considering the robust induction of potential viral DNA sensors such as AIM2 and DDX60 after VZV infection, it is highly plausible that these sensors, together with STING pathways, function differently according to cell type or at temporally distinct stages of the IFN response after viral infection. Despite the compelling evidence suggesting the essential role of STING in response to viral DNA sensing, the detailed role of other viral sensors remains to be elucidated. This relatively unexplored area could provide important insights into VZV pathogenesis in dermal cells.

Although varicella is not a serious disease in immunocompetent children, it can cause severe morbidity in the elderly and in immunocompromised patients, similar to herpes zoster (Gnann, 2002). The outcomes of varicella and zoster have been improved by the introduction of well-tolerated, safe, and effective antiviral drugs such as...
acyclovir (Whitley, 1992). Our data suggest that a combinatorial treatment of IFN-β, λ1, and λ2 can mediate a more potent antiviral effect than treatment with these agents on their own in vitro. These synergistic effects are likely attributable to more potent amplification of IFN-stimulated genes expression by combined IFN-β, λ1, and λ2 compared with single IFNs. Further in vivo experiments examining the antiviral role of IFN-λ in animal models of VZV infection will likely facilitate an evaluation of IFN-λ as an alternative antiviral therapeutic agent. Taken together, these data provide important insights into understanding skin immunity defense mechanisms against VZV infection.

**MATERIALS AND METHODS**

**Cells and reagents**

Full details are provided in the Supplementary Materials online.

**VZV plaque assays**

VZV strain YC01 (GenBank Accession No. KJ808816) has been described previously (Won et al., 2014) and was cultured in human fetal fibroblasts. VZV strain YC01 was used to infect fibroblasts or keratinocytes at a multiplicity of infection of 0.01 for all experiments. Plaque assays were performed as previously described (Kim et al., 2015).

**qRT-PCR assays**

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), and qRT-PCR was performed accordingly (Kim et al., 2016). Full details are provided in the Supplementary Materials online.

**Plasmid transfection**

Wild-type RIG-I and RIG-I–dominant negative plasmids were kind gifts of Takashi Fujita (Kyoto University, Japan). Wild-type STING-pUNO-HA and STING-h232 isoform plasmid were from InvivoGen (San Diego, CA). For overexpression experiments, plasmids were transfected using lipofectamine 2000 (Invitrogen, Waltham, MA) or the HDF Avalanche transfection reagent (EZ Biosystems, College Park, MD) according to the manufacturer’s instructions.

**STING knockdown using lentivirus-delivered shRNA**

To knock down STING expression, we used lentiviral particles containing nontargeting (scrambled) or targeting STING shRNA (Sigma-Aldrich, St. Louis, MO). Stable cells were created using puromycin selection and maintained as a pool of cells. Cells were maintained in DMEM supplemented with 10% fetal bovine serum and 100 U/ml penicillin/streptomycin. STING knockdown was confirmed by immunoblot analysis of whole cell lysates.

**ELISAs**

To determine the concentration of interleukin IFN-β, IFN-λ1/3, and IFN-λ2 in the samples, we used a specific ELISA kit (R&D) according to the manufacturer’s instructions.

**Western blotting analysis**

Cells were lysed at the specified times after infection with RIPA buffer (Sigma-Aldrich) containing a protease inhibitor cocktail (Roche Applied Bioscience, Rotkreuz, Switzerland) and phosphatase inhibitors (Sigma-Aldrich). Samples were separated by 8–15% SDS-PAGE gels and then transferred to polyvinylidene fluoride membranes. The blots were probed with primary antibodies overnight at 4 °C. All antibodies except for anti-VZV gE, pIRF-3 (Abcam), IE62 (Santa Cruz), and anti-β-actin (Abgent) were acquired from Cell Signalling Technology (Danver, MA). Signals were determined by Fusion Solo Imaging System (Vilber Lourmat, Collégien, France). The band intensities were quantified by Fusion-Capt analysis software (Vilber Lourmat). A representative image of two to three independent experiments is shown.

**Confocal analysis**

Full details are provided in the Supplementary Materials.

**Luciferase reporter assay**

Full details are provided in the Supplementary Materials.

**RNA-seq analysis**

Full details are provided in the Supplementary Materials.

**Statistical analysis**

Full details are provided in the Supplementary Materials.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**ACKNOWLEDGMENTS**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (NRF-2016R1C1B2006493), and research funding from Green Cross. We thank Takashi Fujita (Kyoto University, Japan) for sharing plasmids.

**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.03.041.

**REFERENCES**


Heineman TC, Cohen JL. The varicella-zoster virus (VZV) open reading frame 47 (ORF47) protein kinase is dispensable for viral replication and is not required for phosphorylation of ORF63 protein, the VZV homolog of herpes simplex virus ICp22. J Virol 1995;69:7367–70.


