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Herpes Simplex Virus 1 Counteracts Viperin via Its Virion Host Shutoff Protein UL41

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The interferon (IFN)-inducible viperin protein restricts a broad range of viruses. However, whether viperin plays a role during herpes simplex virus 1 (HSV-1) infection is poorly understood. In the present study, it was shown for the first time that wild-type (WT) HSV-1 infection couldn't induce viperin production, and ectopically expressed viperin inhibited the replication of UL41-null HSV-1 but not WT viruses. The underlying molecular mechanism is that UL41 counteracts viperin's antiviral activity by reducing its mRNA accumulation.

Viperin is a highly conserved, 361-amino-acid protein. It was first identified as a gamma interferon (IFN-γ)-inducible protein which is directly induced by human cytomegalovirus (HCMV), and its constitutive expression is low (1). The viperin gene (also known as *cig5* or RASD2) can also be categorized as an antiviral interferon-stimulated gene (ISG) which limits the replication of many DNA and RNA viruses (1–14). However, whether viperin plays a role during herpes simplex virus 1 (HSV-1) infection is unknown.

To investigate whether HSV-1 could induce the expression of viperin, HEK293T cells were infected with wild-type (WT) HSV-1 at different multiplicities of infection (MOI) or with Sendai virus (SeV) (15). Infection with SeV induced a significant amount of viperin; however, infection with a low MOI (0.2) of HSV-1 induced only a trace amount of viperin, and infection with a moderate MOI (2) abrogated the expression of viperin (Fig. 1A).

To further explore whether viperin could inhibit the replication of WT HSV-1, HEK293T cells with ectopic expression of viperin-Flag were infected with HSV-1 at an MOI of 0.2. Then cells were harvested at the time points indicated in the figures, and viral plaque assay was performed to determine viral replication (16). As a result, ectopically expressed viperin did not affect the replication of WT HSV-1 (Fig. 1B). The data from Western blot (WB) analysis also showed that viperin did not affect viral protein expression (Fig. 1C). These results demonstrated that ectopic expression of viperin failed to inhibit the replication of WT HSV-1.

The aforementioned data led us to hypothesize that at least one of the HSV-1 proteins could counteract the expression of viperin. As a member of the ISGs, viperin was effectively induced by SeV (Fig. 1) (15). With a high-throughput screen assay of all 84 proteins carried by HSV-1, dual-luciferase reporter gene assays were performed in HEK293T cells cotransfected with viperin-luciferase reporter plasmid and individual HSV-1 protein expression plasmid for 20 h and infected with SeV (17). As a result, ectopically expressed UL41 abrogated the expression of viperin; however, other HSV-1 proteins did not (data not shown). UL41 has been reported to degrade both viral and cellular mRNAs (18–26). Recently, mRNA of tetherin has been reported to be degraded by UL41 (27). Meanwhile, ICP0, an E3 ubiquitin ligase, promotes degradation of many cellular antiviral proteins, such as IRF3, IRF7, IFI16, and ATRX (28–32). To confirm whether ICP0 was involved in degradation of viperin at the protein level, HEK293T cells were cotransfected with UL41 or ICP0 and viperin-Flag plasmids, and the cells were harvested and subjected to WB analysis. UL41 abolished viperin-Flag expression in a dose-dependent manner, but ICP0 did not (Fig. 2).

It was reported that viperin was a target of human RNase endoribonuclease (33) and that UL41 was an endoribonuclease with a substrate specificity similar to that of RNase A (26). Therefore, it is very likely that UL41 abolishes viperin expression via its RNase activity to degrade viperin mRNA. To confirm this hypothesis, HEK293T cells were infected with WT HSV-1, R2621 (UL41-null) HSV-1, or SeV. Then cells were harvested at 8 h postinfection and subjected to reverse transcription (RT)-PCR to analyze the viperin mRNA (Fig. 3A). For normalization, 18S rRNA, which could not be degraded by UL41, was used as an internal control (27). WT HSV-1, but not R2621 HSV-1, significantly reduced the accumulation of viperin mRNA. Similarly, HEK293T cells were infected with WT or R2621 HSV-1 or SeV; 20 h after infection, the cells were harvested and subjected to WB analysis (Fig. 3B). The data showed that, compared with R2621 HSV-1, WT HSV-1 markedly abrogated viperin expression. Collectively, the data demonstrated that UL41 dampens the antiviral activity of viperin by reducing its mRNA accumulation.

The above-described data led us to hypothesize that viperin could inhibit replication of UL41-null virus. To test this assumption, HEK293T cells with ectopic expression of viperin-Flag were infected with WT or R2621 HSV-1 and harvested at the indicated time points for WB analysis and viral plaque assay (16). As a result, ectopically expressed viperin significantly reduced the expression of UL46 and UL42 from R2621 but not WT HSV-1 (Fig. 4A). The

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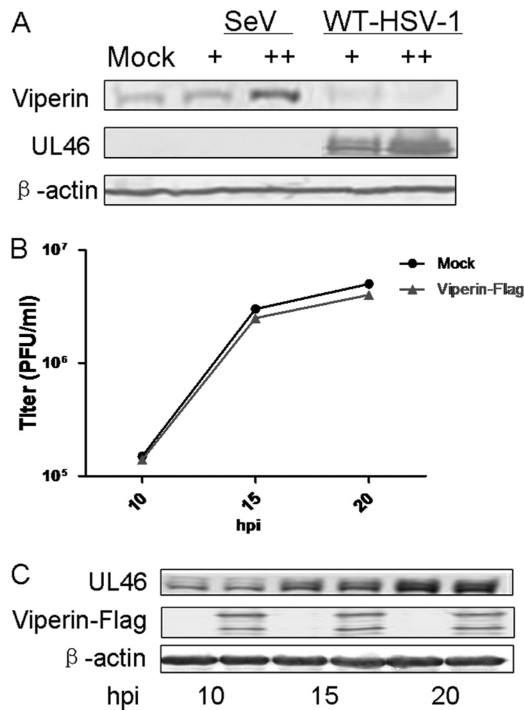

FIG 1 Ectopically expressed viperin did not inhibit the replication of WT HSV-1. HEK293T cells were infected with WT HSV-1 at an MOI of 0.2 or 2.0 or with SeV. (A) Twenty hours after infection, cells were harvested and subjected to WB analysis with antibodies against UL46, β-actin, or viperin. HEK293T cells were transfected with vector or with viperin-Flag plasmid. Twenty-four hours after transfection, the cells were infected with HSV-1 at an MOI of 0.2, and then cells were harvested at the indicated time points after infection and subjected to viral plaque assay (B) or WB analysis with antibodies against UL46, Flag, or β-actin (C). The results are from triplicate samples with standard deviations.

viral plaque assay showed that ectopic expression of viperin significantly inhibited the replication of R2621 but not WT HSV-1 (Fig. 4B).

The fact that ectopically expressed viperin inhibits the replication of the R2621 mutant does not mean that the lower constitutive expression of viperin would play an important role in inhibition of viral replication. To address this issue, HEK293T cells were transfected with a viperin-specific small interfering RNA (siRNA) prior to infection, and then the replications of WT and R2621 HSV-1 were compared with that in cells that had been transfected with a nontargeting siRNA (9). As presented, knockdown of viperin did

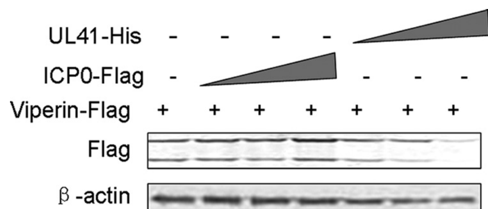

FIG 2 UL41, but not ICP0, decreased the expression of viperin. HEK293T cells were cotransfected with viperin-Flag and UL41-His or ICP0-Flag plasmids. Twenty-four hours after transfection, cells were harvested and subjected to WB analysis with antibodies against Flag or β-actin. The data represent results from one of the triplicate experiments.

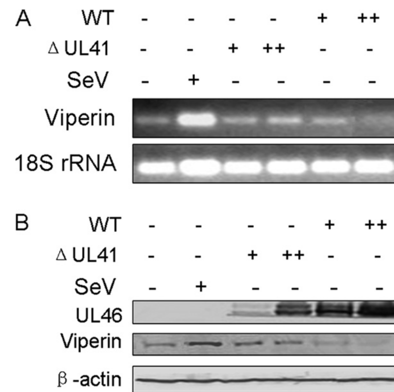

FIG 3 HSV-1 UL41 protein reduced the accumulation of viperin mRNA. (A) HEK293T cells were infected with WT or R2621 HSV-1 at an MOI of 0.5 (+) or 5.0 (++) or with SeV. Eight hours postinfection, cells were harvested and then subjected to RT-PCR (Roche). (B) HEK293T cells were infected as described for panel A with an MOI of 0.2 (+) or 2.0 (++). Twenty hours postinfection, cells were harvested and subjected to WB analysis with antibodies against viperin and β-actin.

not affect the replication of WT HSV-1 but did promote the replication of the R2621 HSV-1 (Fig. 4C).

To rule out the involvement of other late proteins in viperin regulation other than UL41, Us11 was chosen, as ΔUs11 HSV-1 had been constructed in our lab (34). Us11 is an RNA binding tegument protein that prevents the activation of protein kinase R (PKR) and oligoadenylate synthetases (OAS) and impairs type I IFN responses by antagonizing retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5) (34–36). HEK293T cells were transfected with viperin-Flag or vector plasmids prior to being infected with ΔUs11 HSV-1 at an MOI of 0.2, and the titers were tested. As shown, ectopic expression of viperin did not affect the replication of ΔUs11 HSV-1 (Fig. 4D). Taken together, these results indicated that UL41, but not other viral proteins, promotes HSV-1 replication by ablation of the antiviral activity of viperin.

Viperin restricts the replication of many RNA viruses, including HIV, hepatitis C virus (HCV), SeV, and influenza virus, and also DNA virus HCMV (1, 2, 9, 11, 14, 15, 37). Viperin effectively affects the replication of influenza virus by inhibiting its release from the plasma membrane of infected cells (10) and inhibits HCV replication by localizing and interacting with HCV nonstructural protein 5A at the lipid-droplet interface (6). Surprisingly, we found that HSV-1 infection abolished viperin expression and ectopic expression of viperin could not restrict the replication of HSV-1, and HSV-1 UL41 protein was demonstrated for the first time to dampen the antiviral activity of viperin.

To establish effective infection, HSV-1 has evolved multiple mechanisms to evade host innate immunity (34, 38–44). UL41 is an mRNA-specific RNase that triggers rapid degradation of host mRNAs to facilitate the sequential expression of viral proteins (19, 20, 22, 26, 45–48). Our data demonstrated that ectopic expression of UL41 or WT HSV-1 infection reduced the accumulation of viperin mRNA, suggesting that UL41 degraded viperin mRNA to promote the replication of HSV-1.

In brief, we have demonstrated for the first time that HSV-1 UL41 dampens expression of viperin to abrogate the antiviral activity of viperin by reducing its mRNA accumulation. These find-

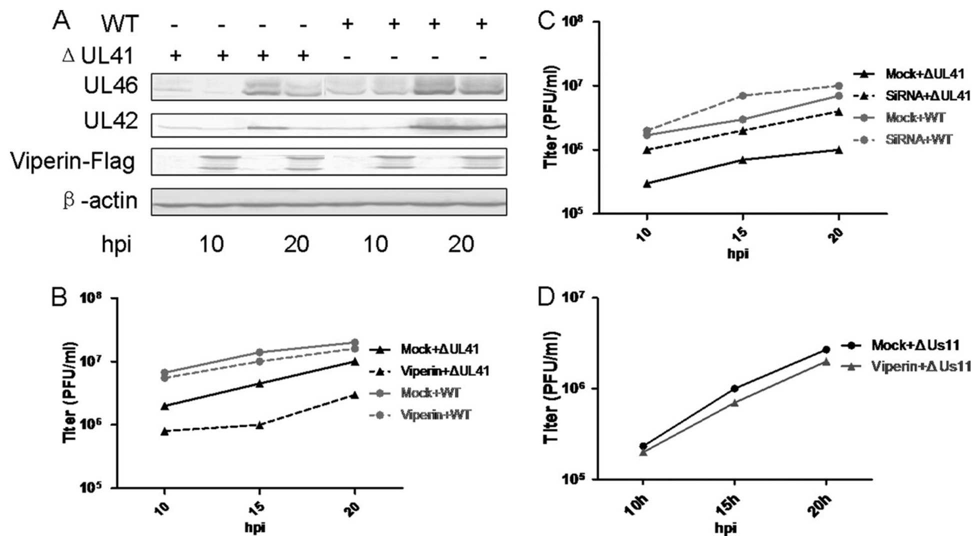

FIG 4 Viperin counteracted the replication of UL41-null HSV-1. HEK293T cells were transfected with vector or with viperin-Flag plasmid. Twenty-four hours after transfection, the cells were infected with the indicated viruses at an MOI of 0.2, and then cells were harvested at the indicated time points postinfection and subjected to WB analysis with antibodies against UL46, UL42, Flag, and β-actin (A) or viral plaque assay on Vero cells (B). (C) HEK293T cells were transfected with control or with siRNA specific to viperin. Twenty-four hours after transfection, the cells were infected and subjected to viral plaque assay as described for panel A. (D) HEK293T cells were transfected and infected with ΔUs11 HSV-1 at an MOI of 0.2 and subjected to viral plaque assay as described for panel A. The data represent results from one of the triplicate experiments.

ings will lead us to better understand the mechanisms employed by HSV-1 to evade host antiviral activity and develop novel effective therapeutics to modulate HSV-1 pathogenesis.

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