# Inhibition of RIG-I-dependent innate immunity by herpes simplex virus type I Us11 protein

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KEY MESSAGES

1. Double-stranded RNA binding protein PACT activates RIG-I and thus optimally induces the production of type I interferon.
2. PACT associates with RIG-I in virus-infected cells. Activation of RIG-I by PACT triggers host anti-viral responses.
3. Herpes simplex virus 1 (HSV-1)-encoded Us11 protein inhibits the production of type I interferon in virus-infected cells. Mutant HSV-1 virus incapable of expressing Us11 protein induces higher amounts of type I interferon.
4. Us11 associates with PACT, and inhibits PACT-dependent type I interferon production.
5. Other viruses may apply the same strategy to shut down host antiviral responses by inhibiting PACT-dependent type I interferon production.

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## Introduction

Infection with herpes simplex virus type 1 (HSV-1) is very common and causes oral and/or genital herpes. In addition, HSV-1 can infect the nervous system, resulting in encephalitis.[1] HSV-1 Us11 is a multifunctional protein required for full resistance to interferons, and inhibits protein kinase R (PKR) through an interaction with cellular dsRNA-binding protein PACT.[2] Nonetheless, exactly how Us11 antagonises innate immunity is not fully understood. In this project, we aimed to fully characterise this new mechanism by which Us11 counteracts PACT to inhibit RIG-I-dependent interferon production.

## Methods

In vitro affinity binding and complex formation assays were performed with recombinant Us11, PACT and RIG-I expressed and purified from *Escherichia coli*. Co-fractionation and co-localisation experiments were carried out to verify the interaction between Us11 and PACT in transfected and HSV-1-infected cells. The roles of PACT and RIG-I in interferon-induced anti-HSV cellular response as well as the mechanisms by which Us11 inhibits PACT and RIG-I were investigated. In particular, the influence of Us11 on PACT-RIG-I complex formation was determined.

## Results

PACT was a potent activator of RIG-I and resulted in optimal interferon production. PACT and RIG-I mediated interferon production was critical to combat viral infection. Experimentally, RNAi depletion of PACT led to inhibition of virus-induced and RIG-I-dependent activation of IFN production in three different cell lines, lung epithelial carcinoma A549, normal diploid fibroblast IMR-90, and primary mouse embryonic fibroblast MRFs. In addition, the stimulation of RIG-I by PACT did not require PKR or Dicer, but was mediated through a direct interaction with the C-terminal domain of RIG-I leading to activation of ATPase activity and plausibly a conformational change associated with the recruitment of downstream effectors.

We revealed a novel inhibitory role of HSV-1 Us11 protein in PACT-RIG-I mediated interferon production. Us11 protein associated with PACT, and thus inhibited the activation of RIG-I. This interaction required both dsRNA binding domains of Us11 and PACT, and this interaction was dsRNA-insensitive. Using HSV-1 wild type and mutant viruses, viruses that express Us11 demonstrated a stronger inhibition on interferon production. This inhibition was caused by the sequestration of PACT by Us11 protein.

## Discussion

Our findings revealed PACT as a new target of a viral interferon-antagonising protein. We demonstrated the importance of the C-terminal dsRNA-binding domain of Us11 in its interaction with PACT. The same domain was also used in the interaction with RIG-I, melanoma differentiation-associated protein 5 (MDA5), Dicer, PKR and 2'-5'-oligoadenylate synthase. It would be of great interest to determine

whether Us11 also affects the normal function of these cellular proteins. Particularly, the interplay of Us11, PACT, RIG-I, and MDA5 in the activation of type I interferon production merits further investigation. Because other viral interferon-antagonising proteins such as influenza A virus NS1[3] and Ebola virus VP35[4] can also interact with PACT and perturb RIG-I function, suppression of PACT-augmented activation of RIG-I might represent a common viral countermeasure to combat the host antiviral response used by other viruses.

## Conclusions

Our study revealed a novel mechanism by which HSV-1 circumvents innate antiviral immunity through Us11 inhibition of PACT and RIG-I. This finding provides a new opportunity in the development of novel antiviral agents against HSV-1 infection.

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**References**

1. Roizman B, Knipe DM, Whitley RJ. Herpes simplex viruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editors. Philadelphia: Lippincott Williams & Wilkins; Fields Virology, 5th ed: 2501-601.
2. Peters GA, Khoo D, Mohr I, Sen GC. Inhibition of PACT-mediated activation of PKR by the herpes simplex virus type 1 Us11 protein. J Virol 2002;76:11054-64.
3. Li S, Min JY, Krug RM, Sen GC. Binding of the influenza A virus NS1 protein to PKR mediates the inhibition of its activation by either PACT or double-stranded RNA. Virology 2006;349:13-21.
4. Cárdenas WB, Loo YM, Gale M Jr, et al. Ebola virus VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon production induced by RIG-I signaling. J Virol 2006;80:5168-78.