**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. 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. 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 and Ebola virus VP35 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**

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