

Varicella-Zoster Virus Induces PKR Activation and its Localization to the Nuclei of Infected Cells

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Background

PKR is a serine-threonine kinase encoded by the human *EIF2AK2* gene and belongs to the eIF2 α kinase family, which comprises four enzymes activated in response to various stresses. These stresses can be extrinsic, such as a viral infection or heat shock, or intracellular, such as endoplasmic reticulum stress. Upon activation, these kinases phosphorylate eIF2 α on serine 51, leading to the inhibition of cap-dependent translation and a global reduction of protein synthesis. This blockage leads to preferential translation of genes involved in the integrated stress response. During viral infection, PKR senses double-stranded RNA and triggers an innate antiviral response by suppressing viral protein synthesis, thereby limiting viral replication. Beyond its roles in antiviral defences and stress responses, PKR contributes to the control of cell fate by modulating apoptosis and cell cycle progression, regulates inflammatory signalling and is implicated in a wide range of human diseases.

Varicella-Zoster Virus (VZV) is an enveloped double-stranded DNA virus of the *Herpesviridae* family (*Alphaherpesvirinae*) distinguished from other alphaherpesviruses by its strict tropism for human cells. The long-standing co-evolution between VZV and its human host has led to the parallel development of cellular immune defences and viral immune evasion strategies. VZV has evolved multiple mechanisms to evade and manipulate host immune responses to ensure viral replication, notably by modulating both interferon production and interferon signalling.

Methods

Experiments were performed in spontaneously immortalized human retinal pigment epithelial cells (ARPE-19), using the wild-type parent Oka (pOka) strain of VZV. A “co-culture” infection system was employed, as VZV produces virtually no cell-free viral particles *in vitro*. Protein localization was assessed by immunofluorescence using a Zeiss LSM 980 confocal microscope, while protein expression levels were analysed by Western blotting.

Results

Our work shows that VZV infection activates PKR and induces its localization in nuclear foci in infected cells. Western Blot analyses confirmed that PKR and Phospho-PKR are not restricted to the cytoplasm but also localize in the nucleus of infected cells.

Interestingly, PKR activation patterns differ between physiological conditions and poly(I:C)-induced artificial stimulation, as shown by Western Blot and Immunofluorescence. This demonstrates differential activation of PKR between infection by a wild-type virus and induction by synthetic double-stranded RNA transfection.

Several DNA viruses have been reported to display specific double-stranded RNA labelling in infected cells, notably HSV-1, a member of the same viral family as VZV. In contrast, immunofluorescence experiments reveal that, during VZV infection, specific double-stranded RNA labelling is restricted to a minor subset of infected nuclei. This phenomenon cannot account for the widespread nuclear PKR labelling observed across VZV-infected cells.

Deletion mutant studies suggest that the VZV kinases ORF47 and ORF66 do not directly mediate PKR phosphorylation. This suggests a more complex indirect phosphorylation mechanism. Immunofluorescence experiments reveal that the nuclear foci, containing PKR and its phosphorylated form, colocalize with nuclear speckles. This colocalization could reflect either a nuclear role for PKR in the regulation of mRNA splicing or a viral strategy aimed at sequestering PKR in nuclear structures to promote viral replication.

Implications

This study provides insights into the ability of a DNA virus to selectively activate a double-stranded RNA-dependent kinase involved in innate immune signaling, while maintaining efficient replication of the wild-type virus. These findings contribute to a deeper understanding of viral immune evasion strategies.

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