Virology 516 (2018) 147–157

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Expression of flavivirus capsids enhance the cellular environment for viral replication by activating Akt-signalling pathways

Adriana M. Airo[a,1], Matt D. Urbanowski[b,1], Joaquin Lopez-Orozco[b], Jae Hwan You[b], Tamara D. Skene-Arnold[c], Charles Holmes[c], Vladimir Yamshchikov[d], Natasha Malik-Soni[e], Lori Frappier[e], Tom C. Hobman[a,b,f,g,*]

[a] Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada
[b] Department of Cell Biology, University of Alberta, Edmonton, Canada
[c] Department of Biochemistry, University of Alberta, Edmonton, Canada
[d] Oregon Public Health Laboratory, Hillsboro, OR, United States
[e] Department of Molecular Genetics, University of Toronto, Toronto, Canada
[f] Li Ka Shing Institute of Virology, University of Alberta, Canada
[g] Women & Children's Health Research Institute, University of Alberta, Canada

ARTICLE INFO

Keywords:
Flavivirus
Capsid
Akt-signalling
Apoptosis
Protein phosphatase-1
Dengue virus

ABSTRACT

Flaviviruses depend on multiple host pathways during their life cycles and have evolved strategies to avoid the innate immune response. Previously, we showed that the West Nile virus capsid protein plays a role in this process by blocking apoptosis. In this study, we examined how expression of capsid proteins from several flaviviruses affects apoptosis and other host processes that impact virus replication. All of the tested capsid proteins protected cells from Fas-dependent apoptosis through a mechanism that requires activated Akt. Capsid expression upregulated other Akt-dependent cellular processes including expression of glucose transporter 1 and mitochondrial metabolism. Protein phosphatase 1, which is known to inactivate Akt, was identified as a DENV capsid interacting protein. This suggests that DENV capsid expression activates Akt by sequestering phosphatases that downregulate phospho-Akt. Capsid-dependent upregulation of Akt would enhance downstream signalling pathways that affect cell survival and metabolism, thus providing a favourable environment for virus replication.

1. Introduction

Insect-borne viral infections, the majority of which are caused by RNA viruses, are a significant global health concern. Some of the more well known members of the genus *Flavivirus* include Zika virus (ZIKV), Yellow fever virus (YFV), Dengue virus (DENV), Japanese encephalitis virus (JEV), and West Nile virus (WNV). Despite their medical importance, with the exception of YFV and more recently, DENV, no specific treatments, prophylactic or therapeutics are available for these pathogens. Accordingly, there is a significant need for anti-viral therapies and understanding how flaviviruses interact with host cells is sure to facilitate development of novel drug targets.

All viruses depend on host cell factors for replication and virion assembly, a situation that allows the host to establish barriers to infection by restricting the availability of key biomolecules or committing to apoptosis to eliminate infected cells. Subversion of the cell death machinery has been extensively described for many DNA viruses but is understudied in RNA viruses. Flavivirus-encoded proteins interact with a plethora of host factors and can alter anti-viral signalling through a variety of mechanisms (Marceau et al., 2016; Reid et al., 2015). DENV, a particularly well-studied pathogen, is known to employ multiple strategies to block the interferon response. For example, the NS2B/3 complex cleaves the anti-viral protein Stimulator of Interferon Genes (STING), which prevents induction of interferon α/β (Aguirre et al., 2012) and the viral NS5 protein induces degradation of STAT2, a transcription factor required for transcription of interferon α/β (Ashour et al., 2009). Recently it was reported that Zika virus (ZIKV) NS5 also inhibits type-1 interferon through degradation of STAT2 (Grant et al., 2016; Kumar et al., 2016). Through a different mechanism, WNV infection interferes with the type-1 interferon response by preventing phosphorylation and activation of the Janus kinases JAK1 and Tyk2 (Guo et al., 2005) and depletion of the IFN-α receptor subunit 1 (IFNAR1) (Evans et al., 2011).

In addition to antagonizing the interferon system, evidence suggests

* Corresponding author at: Department of Cell Biology, University of Alberta, Edmonton, Canada.
E-mail address: tom.hobman@ualberta.ca (T.C. Hobman).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.virol.2018.01.009
Received 16 October 2017; Received in revised form 4 January 2018; Accepted 9 January 2018

that flaviviruses activate cell survival signalling shortly after infection (Lee et al., 2005; Scherbik and Brinton, 2010), which in turn may serve to block apoptosis. Previously, we demonstrated that the WNV capsid protein inhibits apoptosis through a mechanism that involves phosphatidylinositol 3 (PI3) kinase activation of Akt kinase (Urbanowski and Hobman, 2013). To determine if this mechanism was conserved among flaviviruses, we examined how expression of capsid proteins from seven different flaviviruses affects apoptosis and other Akt-dependent processes. We observed that all the flavivirus capsid proteins tested induced phosphorylation of Akt albeit to different levels. As well as resistance to apoptosis, capsid-expressing cells had increased mitochondrial metabolism, elevated ATP levels and increased expression of Glucose Transporter 1 (GLUT1). Together, these data are consistent with a scenario in which flavivirus capsid proteins prepare the host cell for virus replication by increasing the activity of survival and energy producing pathways.

2. Materials and methods

2.1. Mammalian cell culture

Mammalian cell lines were maintained at 37 °C in a 5% CO_2 atmosphere. Human alveolar epithelial carcinoma (A549) cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA), and cultured in DMEM with 10% fetal bovine serum (FBS) (GIBCO Invitrogen, Carlsbad CA). Human embryonic lung (HEL-18) fibroblasts were obtained from Eva Gönczöl (Wistar Institute, Philadelphia, PA) (Megyeri et al., 1999) and cultured in RPMI 1640 medium supplemented with 10% FBS and nonessential amino acids (Life technologies Inc., Burlington, ON, Canada).

2.2. Expression plasmids

All plasmids were propagated in the *E.coli* strain DH5α under standard growth conditions in Luria-Bertani medium with the appropriate antibiotics. The NY99 strain of WNV replicon (p8113.1) were constructed as described previously (Borisevich et al., 2006). pDsRed-Monomer-C1 was purchased from Clontech Laboratories. BFP2 cDNA was amplified by PCR from pBFP2-Acta and inserted into pEGP-N1 to create pEBFP-N1. The BFP2 sequence of pBFP2-Acta was originally from pTag-BFP2-C plasmid purchased from Evrogen. The PPP1CA encoding cDNA fragment was released from pEYFP(C1)-PP1alpha (Addgene plasmid#44231) by digestion with EcoR1 and SalI and then ligated into the corresponding sites in PEGFP-C1. Lentiviral expression plasmids containing flavivirus capsid cDNAs were produced using the pTRIP-MCS-IRES-AcGFP vector, which has been described previously (Urbanowski and Hobman, 2013). Oligonucleotide primers were designed to amplify the desired capsid sequence and introduce a myc epitope tag into the 5' end of the cDNA. The resulting PCR products were then cloned into the lentiviral vector pTRIP-MCS-IRES-AcGFP. All oligonucleotide primers used in this study are listed in Table 1.

2.3. Antibodies

Antibodies were from the following sources: rabbit anti-human activated caspase-3 antibody for flow cytometric analyses from BD Biosciences (Franklin Lakes, NJ); rabbit anti-human activated caspase-8 antibody for flow cytometric analyses, Akt and phospho-Akt (S473) antibodies all from Cell Signaling Technologies (Danvers, MA); Rabbit anti-PPP1CC antibody from Bethyl Laboratories Inc. (Montgomery, TX); mouse anti-WNV NS3 from R&D systems (Minneapolis, MN); rabbit anti-GAPDH antibody from Abcam (Cambridge, MA); mouse IgM anti-human Fas from Millipore (Billerica, MA); donkey anti-rabbit HRP and goat anti-mouse HRP antibodies from Jackson Immunoresearch (West Grove, PA); goat anti-mouse Alexa Fluor 680 from Life Technologies (Burlington, ON); donkey anti-rabbit Alexa Fluor 750 from Life Technologies (Burlington, ON); rabbit anti-GFP from Eusera (Edmonton, AB); Goat-anti GFP from Abcam (Cambridge, MA); Rabbit and Guinea Pig anti-WNV capsid antibodies were produced in-house and by Pocono Rabbit Farm & Laboratory (Canadensis, PA) as described previously (Hunt et al., 2007). Mouse anti-myc 9e10 antibody (American Type Culture Collection); mouse anti-actin antibody from Abcam (Cambridge, MA); Rabbit anti-GLUT1 from Sigma-Aldrich (St. Louis, MO).

2.4. SDS-PAGE and immunoblotting

Cells were lysed in SDS-PAGE loading buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA, 0.02% bromophenol blue) which was then heated to 95 °C for 5 min. Subsequently, samples were incubated with 500U of Benzonase nuclease (EMD Biosciences, Darmstadt Germany) for 5–10 min at room temperature before freezing at − 80 °C or immediate loading on to polyacrylamide gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes at 320 mA for 2 h using Towbin transfer buffer (25 mM Tris-HCl pH 8.3, 192 mM glycine, and 20% methanol). Membranes were then blocked for at least one hour in TBST (20 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 0.1% Tween-20) and 5% skim milk or 5% bovine serum albumin (BSA) prior to incubation with primary antibodies overnight. Membranes were briefly washed with TBST and then incubated with secondary antibodies for 30 min and then washed again with TBST. Proteins were visualized using chemiluminescent supersignal ECL (Thermo Scientific, Rockford, IL) or fluorescent LiCor Odyssey system (LiCor Biosciences, Lincoln, NE) detection systems.

2.5. Production of lentiviral particles and transduction of cells

Pseudotyped lentiviruses for expression of flavivirus capsids were recovered from transfected HEK293T cells, and titred as previously described (Urbanowski and Hobman, 2013). Transduction of A549 cells was performed in DMEM containing 3% FBS and 5 μg/mL polybrene and infected with lentivirus encoding pTRIP-AcGFP or pTRIP-AcGFP-Cap lentiviruses in 6-well plates for caspase assays, 12-well plates for ATP measurements and GLUT1 expression, and in 96-well plates for the Alamar Blue assay.

2.6. Detection of caspase-3 and caspase-8 in transduced cells

In all experiments in this study, cells were transduced according to previously described protocols, with the exception that a multiplicity of transduction (MOT) of 3 was used. Briefly, cells were challenged with 250 ng/mL of anti-Fas antibody and 1 μg/mL cycloheximide for 6–8 h following 48 h of incubation and a medium change. Cell culture supernatants were then collected, and adherent cells removed from the plate by trypsinization. After washing in PBS, cells were resuspended in PBS containing 2% paraformaldehyde and placed on ice for minimum of 1 h. Cells were pelleted again, washed once with PBS, and permeabilized with PBS containing 0.2% Triton X-100 and 10% FBS for 10 min. Following a wash with PBS, cells were incubated with 0.25 μg of rabbit anti-active caspase-3 or 0.25 μg of rabbit anti-active caspase-8 in 50 μl PBS containing 1% BSA for 60 min. Cells were then washed in PBS and incubated for 30 min with 0.1 μg of the appropriate fluorescent secondary antibody in PBS containing 1% BSA. Following a single wash in PBS, cells were resuspended in PBS containing 1% BSA, 5 mM EDTA and analyzed via flow cytometry utilizing FACS Canto II flow cytometer (BD Biosciences) or LSRFortessa X20 SORP flow cytometer (BD Biosciences). To detect caspase-3 activation in transduced cells treated with LY294002, A549 cells were transduced as described previously but in addition to treatment with anti-Fas antibody and cycloheximide, cells were treated with either 50 μM LY294002 or DMSO alone and incubated for 6–8 h before fixation and permeabilization. Cells were