Virus Research 169 (2012) 54–62

Contents lists available at SciVerse ScienceDirect

Virus Research

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

Two PDZ binding motifs within NS5 have roles in Tick-borne encephalitis virus replication

Wessam Melik[a], Karin Ellencrona[a], Michael Wigerius[a,b], Christer Hedström[a], Annelie Elväng[a], Magnus Johansson[a,c,*]

[a] School of Life Sciences, Södertörn University, S-141 89 Huddinge, Sweden
[b] Department of Pharmacology, Faculty of Medicine, Dalhousie University, 1459 Oxford Street, Halifax, NS, Canada B3H 4R2
[c] Örebro Life Science Center (ÖLSC), Örebro University, S-701 82 Örebro, Sweden

ARTICLE INFO

Article history:
Received 2 March 2012
Received in revised form 2 July 2012
Accepted 3 July 2012
Available online 10 July 2012

Keywords:
Flaviviruses
Tick-borne encephalitis virus replication
PDZ domains
NS5
Replicon

ABSTRACT

The flavivirus genus includes important human neurotropic pathogens like Tick-borne encephalitis virus (TBEV) and West-Nile virus (WNV). Flavivirus replication occurs at replication complexes, where the NS5 protein provides both RNA cap methyltransferase and RNA-dependent RNA polymerase activities. TBEVNS5 contains two PDZ binding motifs (PBMs) important for specific targeting of human PDZ proteins including Scribble, an association important for viral down regulation of cellular defense systems and neurite outgrowth.

To determine whether the PBMs of TBEVNS5 affects virus replication we constructed a DNA based sub-genomic TBEV replicon expressing firefly luciferase. The PBMs within NS5 were mutated individually and in concert and the replicons were assayed in cell culture. Our results show that the replication rate was impaired in all mutants, which indicates that PDZ dependent host interactions influence TBEV replication. We also find that the C-terminal PBMs present in TBEVNS5 and WNVNS5 are targeting various human PDZ domain proteins. TBEVNS5 has affinity to Zonula occludens-2 (ZO-2), GIAP C-terminus interacting protein (GIPC), calcium/calmodulin-dependent serine protein kinase (CASK), glutamate receptor interacting protein 2, (GRIP2) and Interleukin 16 (IL-16). A different pattern was observed for WNVNS5 as it associate with a broader repertoire of putative host PDZ proteins.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Tick-borne encephalitis virus (TBEV) and West-Nile virus (WNV) are both arthropod-borne flaviviruses that have a major impact on global health. TBEV causes a febrile illness that commonly progress into encephalitis with mortality rates as high as 20–30% (Gritsun et al., 2003; Mandl, 2005; Pletnev and Men, 1998). The WNV is a milder neurotropic flavivirus transmitted by *Culex* mosquitoes, which was the subject of much attention in 1999 when the virus switched continent and a more virulent virus appeared in the US (Hayes, 2001).

Flaviviruses possess a positive sense RNA genome that encodes a single polyprotein, which is co- and posttranslationally processed into three structural and seven non-structural (NS) proteins in the order C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5 (Chambers et al., 1990; Gritsun et al., 2003; Mandl, 2005). Flavivirus replication occurs at replication complexes (RCs) present within induced invaginations at the ER membrane (Miller et al., 2007; Overby et al., 2010). The RCs includes most of the NS proteins (Kapoor et al., 1995; Li et al., 1999; Mackenzie et al., 1999), where the NS5 protein provides the RNA cap methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp) activities coupled to the N-terminal and C-terminal domains, respectively (Ackermann and Padmanabhan, 2001; Egloff et al., 2002; Koonin, 1993; Malet et al., 2007; Yap et al., 2007). Each of these enzymatic properties is essential for viral replication. Even though flavivirus replication occurs in the cytoplasm (Li et al., 1999; Mackenzie et al., 1999), the dengue virus and the yellow fever virus NS5 proteins have been found to localize predominantly within the host-cell nucleus by utilizing the nuclear import machinery (Brooks et al., 2002; Buckley et al., 1992; Johansson et al., 2001; Kapoor et al., 1995; Pryor et al., 2007).

PDZ (PSD-95/Dlg/ZO-1) domains are protein-interaction modules involved in maintaining cell polarity and regulation of synaptic dynamics (Hung and Sheng, 2002; Roche et al., 2002). Class 1 PDZ domains typically recognize proteins that carry the PDZ binding motif (PBM) X-(S/T)-X-ϕ (where X is any amino acid and ϕ is a hydrophobic residue, usually V, I or L) in their C-terminus (Harris and Lim, 2001), but internal PBMs have also been identified (Hillier et al., 1999; Penkert et al., 2004). Interestingly, small differences

* Corresponding author at: School of Life Sciences, Södertörn University, SE-141 89 Huddinge, Sweden. Tel.: +46 8 608 4853; fax: +46 8 608 4510.
E-mail address: magnus.johansson@sh.se (M. Johansson).

0168-1702/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2012.07.001

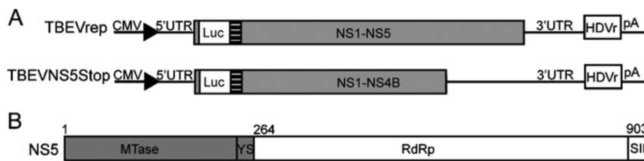

Fig. 1. Construction of DNA based subgenomic replicon models with mutations in the PBMs of NS5. (A) Schematic representation of the TBEVrep based on a cloned W-TBEV as described in material and methods. In the replicons, most of the sequence encoding the structural genes were removed and replaced with sequences encoding the GFP reporter gene and the autoprotease of FMDV (striped box). The constitutive promoter/enhancer of CMV, the HDVr ribozyme and the SV40 polyadenylation signal (pA) are indicated. TBEVNS5Stop contains two stop codons terminating translation after NS4B. (B) Schematic representation of TBEV NS5 with indicated domains methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp). The C-terminal and internal PBMs are indicated as SII and YS, respectively.

within the PBM of the influenza A virus (IAV) NS1 protein has been highlighted as a virulence determinant (Obenauer et al., 2006) and for the flaviviruses we recently demonstrated diverging C-terminal PBMs within the TBEVNS5 and WNVNS5 proteins, -SII and -TVL respectively (Werme et al., 2008). In addition, TBEVNS5 contain a novel internal PBM within the MTase domain that recognizes human Scribble (hScrib), regulating synaptic membrane exocytosis-2 (RIM2) and Zonula occludens-1 (ZO-1) (Ellencrona et al., 2009; Werme et al., 2008). The interaction between TBEVNS5 and hScrib was further found to be important for blockage of the innate interferon response in mammalian cells (Werme et al., 2008), and in blockage of NGF induced neurite outgrowth in PC12 cells (Wigerius et al., 2010).

Immense variations have been observed in the PBMs of different flavivirus NS5 proteins. However, the biological relevance of PDZ binding especially regarding replication, which is one of the best-characterized functions of the NS5 protein, remained largely elusive. Virus replicons provide a useful tool for studies of fundamental viral processes, such as replication and cellular tropism (Khromykh et al., 2001; Varnavski et al., 2000; Yamshchikov et al., 2001). Here we have used the genome of a Swedish TBEV (strain Torö-2003) as template to develop a DNA based sub-genomic replicon expressing the firefly luciferase gene (*luc*). By introducing suitable mutations into the replicon we show that the PBMs in TBEVNS5 influences virus replication. Furthermore, investigation of the C-terminal PBMs of TBEVNS5 and WNVNS5, revealed new host-binding partners. Taken together, our results highlight PDZ domains as an important target during flavivirus host invasion that could potentially serve as a target for novel antiviral therapies.

2. Materials and methods

2.1. Construction of DNA based TBEV sub-genomic replicons

A cloned TBEV strain (Torö-2003) (AH013799) (Melik et al., 2007), was used as the template to construct TBEV replicons expressing luciferase as a reporter. This replicon was identical to the replicon described in (Wigerius et al., 2010) (Fig. 1A), except that the EGFP gene was replaced with *luc*. Briefly, the replicon is driven by the CMV promoter expressing the 5′-untranslated region (UTR) and the 3′-UTR flanking an open reading frame including 60 nucleotides of the C gene fused in frame with the luciferase gene, the autoprotease gene of Foot and mouth disease virus 2a (FMDV 2A), 84 nucleotides of the E gene and all the NS proteins. The hepatitis delta virus antigenomic ribozyme (HDVr) sequence was inserted immediately downstream of the TBEV 3′-end followed by the Simian virus 40 (SV40) polyadenylation signal (pA) (Fig. 1A). To generate the control replicon, TBEVNS5Stop, two stop codons were introduced within the NS5 protein replacing residues G^9 and W^{12}, respectively (Fig. 1A). In addition, replicons expressing NS5 with simultaneous mutations in either one or both PBMs (*$YS^{223} \rightarrow AA$ and $SII^{903} \rightarrow AIA$*) (Fig. 1B) were generated by directed mutagenesis.

2.2. Plasmids

All recombinant DNA techniques and cloning procedures were carried out by standard procedures (Sambrook et al., 1989). The TBEVNS5 and TBEVNS3 (Torö-2003, AH013799) and WNVNS5 (M12294) genes were amplified by PCR introducing suitable endonuclease restriction sites. Full-length NS5, NS5 mutants and NS3 were cloned into the pPiczB plasmid for yeast expression and pEYFP-C1 (Clontech) or pKH3 (kindly provided by Dr Ian Macara and Ben Margolis) for mammalian cell expression. The QuikChange XL-Site-Directed mutagenesis kit (Stratagene) was used to introduce amino acid changes following the manufacturer's instructions. To verify introduced mutations the constructs were sequenced at Eurofins MWG Operon, Ebersbeg, Germany.

2.3. Yeast protein expression and crude extract preparation

The pPiczB-NS5 plasmids were electroporated into *Pichia pastoris* and introduced into the genome according to the manufacturer's instructions (Invitrogen). *P. pastoris* was grown at 30 °C for 72 h supplemented with 0.5% methanol every 24 h to induce high NS5 expression. The Cells were lyzed with acid-washed glass beads (Sigma) according to the manufacturer's instructions and sonicated in 50 mM sodium phosphate buffer, pH 7.4, 5% glycerol and 1 mM phenylmethylsulfonyl fluoride (PMSF), and the supernatant was recovered after 10 min centrifugation at 12,000 × g, 4 °C.

2.4. Luciferase assay

COS-7 cells (1×10^5) were seeded into 12 well plates and were transfected with the different TBEV replicons (TBEVrep, TBEVrepYS → AA, TBEVrepSII → AIA, TBEVrepYS/SII → AA/AIA and TBEVNS5Stop) after 12 h, respectively. 350 ng of replicon plasmids was mixed with 100 ng pGL4.74[hRluc/TK]–a reporter constitutively expressing Renilla luciferase (Promega) and were transfected with lipofectamin 2000 according to manufacturer's instructions (Invitrogen). Cells were lyzed every 12 h post-transfection for 72 h using the Dual-Luciferase^{TM} Reporter Assay according to manufacturer's instructions (Promega). Measurements for lucifearase were assayed in Lumi-star luminometer in triplicate in three independent experiments and the ratios of firefly luciferase to renilla luciferase were calculated and presented as relative luciferase units (RLU). The rmcdr package in the software application R was employed to analyze data. Statistical differences between means were determined using general linearized model followed by Tukey's post hoc test. Values are presented as mean ± SD. Experimental reproduction of results and the amount of data in account of statistical evaluations is indicated in figure legends.

2.5. siRNA treatment

Targeting and non-targeting siRNAs were designed by ambion. The hScrib-targeting double stranded siRNAs was previously reported (Takizawa et al., 2006), and were #1: 5′-CAGGATGAAGTCATTGGAACA-3′ and #2: 5′-CCGCAGGAGGAGGATGGAGAA-3′. For the transfection of siRNAs, each siRNA (Final concentration 10 nM) was mixed with 350 ng of replicon plasmids and 100 ng pGL4.74[hRluc/TK], and transfected and assayed as above.

Download English Version:

https://daneshyari.com/en/article/6143100

Download Persian Version:

https://daneshyari.com/article/6143100

Daneshyari.com