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Short communication

Identification and analysis of host proteins that interact with the 3′-untranslated region of tick-borne encephalitis virus genomic RNA

Memi Muto[a], Wataru Kamitani[b], Mizuki Sakai[a], Minato Hirano[a], Shintaro Kobayashi[a], Hiroaki Kariwa[a], Kentaro Yoshii[a,*]

[a] Laboratory of Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido 060-0818, Japan
[b] International Research Center for Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Japan

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ABSTRACT

Tick-borne encephalitis virus (TBEV) causes severe neurological disease, but the pathogenetic mechanism is unclear. The conformational structure of the 3′-untranslated region (UTR) of TBEV is associated with its virulence. We tried to identify host proteins interacting with the 3′-UTR of TBEV. Cellular proteins of HEK293T cells were co-precipitated with biotinylated RNAs of the 3′-UTR of low- and high-virulence TBEV strains and subjected to mass spectrometry analysis. Fifteen host proteins were found to bind to the 3′-UTR of TBEV, four of which—cold shock domain containing-E1 (CSDE1), spermatid perinuclear RNA binding protein (STRBP), fragile X mental retardation protein (FMRP), and interleukin enhancer binding factor 3 (ILF3)—bound specifically to that of the low-virulence strain. An RNA immunoprecipitation and pull-down assay confirmed the interactions of the complete 3′-UTRs of TBEV genomic RNA with CSDE1, FMRP, and ILF3. Partial deletion of the stem loop (SL) 3 to SL 5 structure of the variable region of the 3′-UTR did not affect interactions with the host proteins, but the interactions were markedly suppressed by deletion of the complete SL 3, 4, and 5 structures, as in the high-virulence TBEV strain. Further analysis of the roles of host proteins in the neurologic pathogenicity of TBEV is warranted.

Tick-borne encephalitis (TBE) virus (TBEV), a member of the genus Flavivirus in the family *Flaviviridae*, is a major arbovirus that causes thousands of cases of severe neurological illness annually (WHO Publication, 2011). Humans are accidental hosts, and become infected via a tick bite, and unpasteurized goat-milk also can be a source of infection. TBE is a huge public health problem in endemic areas in European and Asian countries (Carletti et al., 2017; Lindquist and Vapalahti, 2008; Mansfield et al., 2009; Yoshii et al., 2017). Mortality rates vary from about 0.5–30%, and neurological sequelae occur in 30–60% of survivors (Grard et al., 2007; Gritsun et al., 2003; Heinz and Kunz, 2004).

TBEV has a single-stranded RNA genome of positive polarity that encodes a long polyprotein in a single open reading frame, flanked by 5′- and 3′-untranslated regions (UTRs). The 5′- and 3′-UTRs are believed to be associated with viral genome replication (Khromykh et al., 2001; Kofler et al., 2006). The 3′-UTR of TBEV is divided into two domains: the 5′-terminal variable region and the 3′-terminal core element. The core element is highly conserved among TBEV strains and contains a sequence that is essential for viral genome replication (Kofler et al., 2006). In recent studies, it was shown that subgenomic flavivirus RNA (sfRNA) is produced by the 3′-UTR of viral genomic RNA, and it was suggested that sfRNA is involved in innate immunity response in mosquito-borne flavivirus infection (Chang et al., 2013; Pijlman et al., 2008; Rouha et al., 2010; Sakai et al., 2015; Schnettler et al., 2014).

The variable region of the 3′-UTR is considered to be essential for the natural transmission cycle of TBEV, but was previously considered not to be involved in viral replication and virulence in mammals (Mandl et al., 1998). The sequence and length of the variable region vary among TBEV strains (Wallner et al., 1995). Notably, few strains contain polyA sequences in the variable region isolated from ticks (Mandl et al., 1998; Růzek et al., 2008), and deletions of sequences in the variable region were identified in strains passaged in mammalian cell culture and in clinical isolates (Formanova et al., 2015; Leonova et al., 2013; Mandl et al., 1998). Those reports suggested that the viral quasispecies with deletions or polyA insertions in the variable region are selected during adaptation from the tick vector to mammalian host environment (Asghar et al., 2014). Our previous studies reported that the deletion in the variable region of the 3′-UTR was involved in the pathogenicity of the strains from the Far-Eastern subtype of TBEV. In a mouse model, Sofjin-HO (accession no. AB062064) showed higher

* Corresponding author at: Laboratory of Public Health, Graduate School of Veterinary Medicine, Hokkaido University, kita-18 nishi-9, kita-ku, Sapporo, Hokkaido, 060-0818, Japan.
E-mail address: kyoshii@vetmed.hokudai.ac.jp (K. Yoshii).

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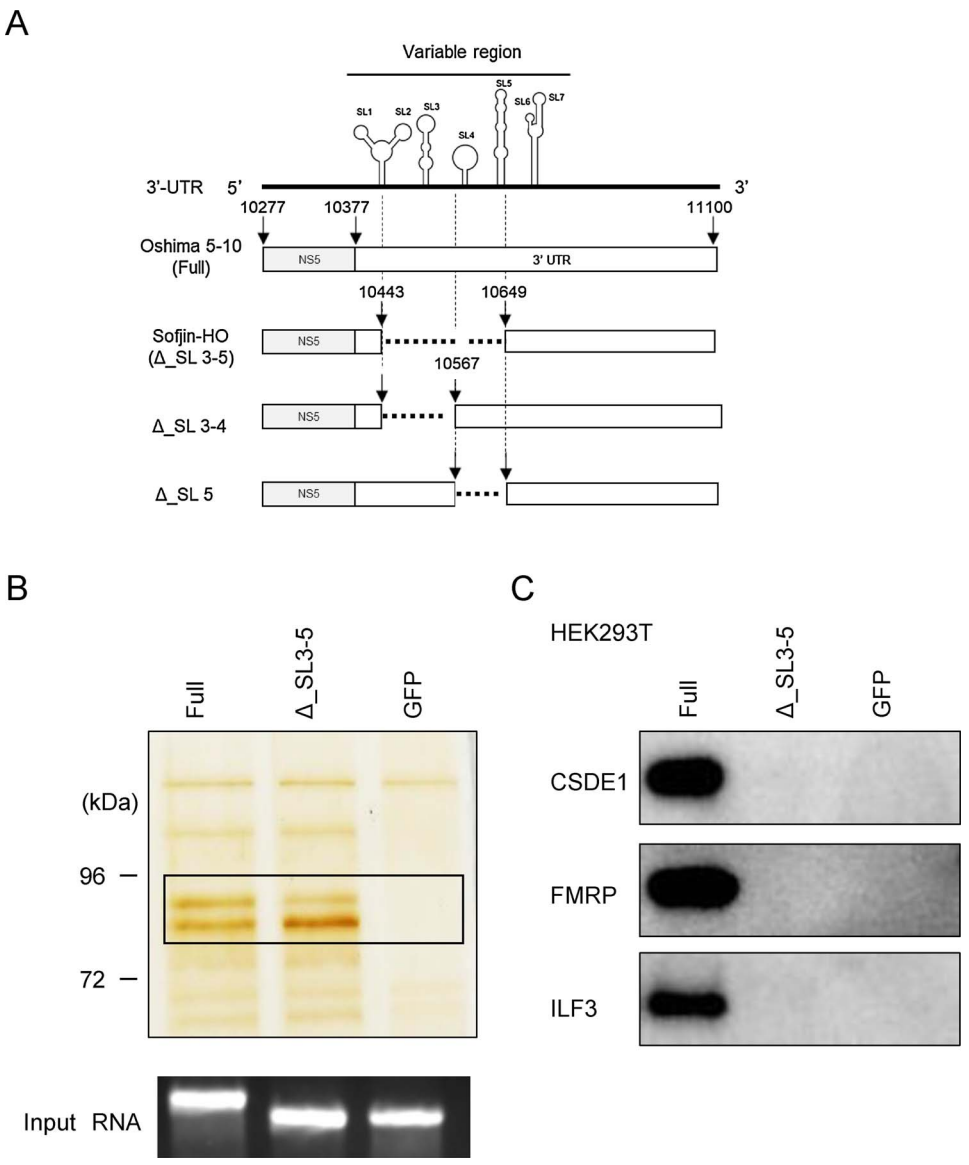

Fig. 1. (A) Schematic of the 3′-untranslated region (UTR) of tick-borne encephalitis virus (TBEV) genomic RNA. The SL 3–5 (nt 10,443–10,649) were deleted in the Sofjin-HO strain and Δ_SL3-5. The SL 3 and 4 (nt 10,443–10,567) were deleted in Δ_SL3-4. The SL 5 (nt 10,568–10,649) was deleted in Δ_SL5. (B) Cellular proteins that interact with the 3′-UTR of TBEV. HEK293T cell lysates were mixed with the biotinylated 3′-UTR of TBEV, and precipitated using streptavidin beads. The proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and silver stained. Black square indicates the range of proteins subjected to mass spectrometry analysis. The bottom panel shows input biotinylated RNA for the pull down assay. (C) Proteins that co-precipitated with the 3′-UTR of TBEV were analyzed by western blotting.

Table 1
Primers for the construction of plasmid expressing each host protein.

Primer name	Primer sequence (5′ to 3′)
CSDE1_Fw	TGACGATAAACTCGAATGGAGAACGTTTTTACTGTG
CSDE1_Rv	TGGATCCCCGCGGCCTTAGTCAATGACACCAGCTT
FMRP_Fw	TGACGATAAACTCGAATGGAGGAGCTGGTGGTG
FMRP_Rv	TGGATCCCCGCGGCCTTAGGGTACTCCATTCACG
ILF3_Fw	TGACGATAAACTCGAATGCGTCCAATGCGAATTTT
ILF3_Rv	TGGATCCCCGCGGCCTTATCTGTACTGGTAGTTCAT

virulence than Oshima 5–10 strain (accession no. AB062064) (Chiba et al., 1999). The Oshima strain putatively forms seven Stem Loop (SL) structures in the variable region (Fig. 1A), while the SL 3–5 structures were deleted in the Sofjin strain. By reverse genetics analysis, introduction of the deletion into the Oshima strain drastically increased the virulence (Sakai et al., 2015). However, the role of this region in virus pathogenicity remains unclear.

Host factors that bind to the viral 3′-UTR RNA including sfRNA play important roles in infections by mosquito-borne flaviviruses, such as Dengue virus (DENV), Japanese encephalitis virus (JEV), and West Nile virus (WNV) (Bidet et al., 2014; Manokaran et al., 2015). However, few study has focused on TBEV, and prion-like T-cell-restricted intracellular antigen 1 (TIA-1) and TIA-1-related protein (TIAR) were the only identified proteins interacting the TBEV RNA (Albornoz et al., 2014). In this study, we hypothesized that differences in the variable region of the 3′-UTR of TBEV affect the interaction of this virus with host factors. We thus identified and characterized host proteins that bind to the 3′-UTR of TBEV.

To identify host proteins that bind to the variable region of the 3′-UTR of TBEV, biotinylated RNAs for 100 nt of the 3′ end of the coding sequence and the 3′-UTR of the Oshima (Full) or Sofjin (Δ_SL3-5) strain, and the coding sequence for EGFP as a control, were synthesized *in vitro* using a Biotin RNA Labeling Kit (Roche, Basel, Switzerland). One μg of the RNAs were mixed with 200 μg of proteins in lysate of HEK293T cells and precipitated using streptavidin magnetic beads (GE Healthcare, Buckinghamshire, UK) (Fig. 1A). The co-precipitated proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver stained using a SilverQuest Staining Kit (Invitrogen, Carlsbad, CA, USA) (Fig. 1B). Proteins bound to the viral RNA were detected at 72–90 kDa. The bands were excised from the gels, and each was trypsinized and subjected to liquid chromatography-electrospray tandem mass spectrometry (LC-ESI–MS/MS) (LTQ Orbitrap Velos + ETD, Thermo Fisher Scientific) to identify co-precipitated proteins. All of the proteins were analyzed with Mascot Server2.5 (MATRIX SCIENCE, Tokyo, Japan), and those from cells containing the