Interaction between Sin Nombre Hantavirus Nucleocapsid protein (N) and Ribosomal protein S19 (RPS19)

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Results

Hantaviruses, bunyaviridae family members, cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) with mortalities of up to 15% and 50%, respectively (1). Hantaviruses are enveloped, negative strand RNA viruses with their genomes encoding viral RNA dependent RNA polymerase (RdRp), glycoproteins (G1 and G2) and nucleocapsid protein (N), respectively. Each of the three segments contain partially complementary nucleotides at the 5’ and 3’ end at under base pair forming and panhandle-like structures. Nucleocapsid protein (N) plays diverse roles during hantavirus infection. Primarily, N protein is involved in the encapsidation and packaging of viral genome. Hantavirus N protein helps in the preferential translation of viral mRNAs by specifically binding to the 5’UTR of viral mRNAs and recruiting translation initiation machinery (2,3). N protein also binds 5’ Cap and facilitates translation initiation by acting as eIF4F surrogate. We have recently found that N protein interacts with 40S ribosomal subunit via ribosomal protein S19 (RPS19) (4). In this study, we mapped N protein for RPS19 binding domain and asked whether N protein deficient in RPS19 binding augments reporter mRNA translation.

Material & Methods

Fluorescence binding: Fluorescence binding studies were carried out using previously reported methods (5), except the percentage of wild type N or mutant N bound to panhandle-like RNA structure at each input concentration of the RNA was calculated using 175 nucleotides of sequence 5′-CCUUAUCUUGGGUAGGAU-3′. Fluorescence measurement was performed in 50 mM HEPES-KOH pH 7.5, 100 mM KCl, 10 mM MgCl2, 10 mM DTT, 1 mM EDTA, 0.01% CHAPS. 20 nM of synthetic RNA was preincubated with 40 nM Hela cell nuclear extracts (gift from Dr. William Schmaljohn, NIH). To each reaction, 10 nM wild or mutant N protein was added, and binding was determined using a SynergyMx microplate reader (BioTek). The fluorescence intensity was calculated using the equation: ΔF = Fobs - Fblank. Each binding reaction was performed in triplicate.

Conclusions

• Identification of RPS19 binding domain at the N-terminus of N protein between the amino acids 151 to 175.
• Fusion of N151-175 amino acids with GFP was sufficient to pull down RPS19.
• WT-N augmented reporter mRNA translation both in-vivo as well as in-vitro, while as N-mutant deficient in RPS19 binding didn’t.
• Inhibition of cap dependent translation of reporter mRNA (GFP), in particular was rescued only when wt-N, but not the mutant N deficient in RPS19 binding.
• N-mutant deficient in RPS19 binding was able to bind mRNA cap, viral RNA panhandle and formed stable trimers similar to wt-N protein.
• N-mutant deficient in RPS19 binding was structurally similar to wt-N.

Acknowledgements

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References


Figure 2: (A) SDS-PAGE showing the purified wild type N and N151-175 variant (Panel A). Far UV CD spectra of the wild type N (19 m) and N151-175 variant (13 m) in phosphate buffer, 11.40 ± 0.20°C (Panel B). Hela cells were transfected with plasmid expressing either GFP or GFP fused with the N-terminus of RPS19 binding domain of protein (GFP-RPS19). Cells were kept 48 hours post-transfection and visualized (a) as uninfected (b) and (c) anti-GFP antibody used in western blot analysis. (d) shows that only wild type N variant lacking RPS19 binding domain binds mRNA Scap similar to wild type N (Fig 4).

Figure 3: Panel A and B: Hela cells expressing freely luciferase (panel A) or GFP (panel B) and wild type N (N151-175) expressing SNVN151-175 variant of N (panel C) and wild type N (N151-175) expressing SNVN151-175 variant of N and the effects of RPS19 binding domain on N mediated augmentation of luciferase mRNA translation. (a) bars represent the standard deviation; calculated from three independent experiments. Panel B: Translation of luciferase mRNA in rabbit reticulocyte lysates in the presence of increasing concentrations of wild or mutant type N (lanes 2 and 4 in SNVN151-175 variant lanes 1 and 3). Translation products were analyzed with SDS/Methione during denaturing PAGE and detected with 125I labelled anti-Methione antibody (C top). Redissolved luciferase mRNA was heat denatured during transcription with or without 5S Methione and examined by polyacrylamide gel electrophoresis (A lane 2 compared to lane 3). The intensity of bands in the Panel C was measured using ImageJ software and normalized to the band intensity in the Panel C lane 2. (b) Diagrammatic representation of wild type N and mutants used in this study. This line represents the deletion pattern (Panel A-B). Hela cells transfected with either empty vector or plasmid expressing N and N mutants. Cell lysates were used to pull down RPS19 protein using RPS19 antibody and N protein mutants using anti-t7 tag antibody. On western blot analysis, pull-down data was subjected to gel filtration analysis, and bands were subjected to semi-native PAGE. For further information see Ganaie SS et al. Biochim J. 2014 PMC4315661.

Figure 4: Binding profiles for the interaction of wild type N (filled square) and SNVN151-175 variant (filled circle) with a capped (panel A) and uncapped (panel B) RNA. Purification of SNVN151-175 variant was in accordance with the information in Figure 2. For further information see Ganaie SS et al. Biochim J. 2014 PMC4315661.