IPD Project Details

Project ID: IPD9067

Project Title: Identification of the host proteins binding to the CHIKV genomic RNA

Description: RNA-binding proteins (RBPs) play a crucial role in RNA regulation. The RBPs are involved in a variety of functions, including viral RNA stabilisation, translation regulation, and controlling the synthesis of the complementary replication intermediate RNA. In several alphaviruses, Sindbis virus, Ross River virus, and Chikungunya virus (CHIKV), host RBPs are the key replication regulators, thereby controlling the viral life cycle. Though the coding region of alphaviruses exhibits sequence diversity, their non-coding regions are highly conserved at both sequence and structural levels. This conservation suggests that these sequences are crucial for the virus replication. Also, numerous reports have demonstrated that various RBPs bind to the conserved non-coding regions (NCRs) of the viral genome, thereby modulating its replication. We, therefore, hypothesised that host RBPs binding to the conserved sequences within the NCRs of CHIKV may have a role in the viral replication. To validate the hypothesis, we used an RNA pull-down-based approach to identify the host proteins binding to the conserved RNAs specifically. To this end, biotinylated RNA, representing the conserved NCR sequence, was used as a bait to pull down the RBPs from the host cell lysates. The bound proteins were then eluted and analysed by mass spectrometry. The identified RBPs were potential regulators of viral genome replication, providing insights into the host-virus interactions. Overall, this study aims to identify the host RBPs interacting with the CHIKV genome and understand their role in CHIKV replication.

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Sample Preparation: Cell lysate (protein pool) was prepared from three different cell lines: HEK293T, Vero, and ERMS. RNA pull-down was performed using conserved CHIKV RNAs, 3NCR40, 3NCR48, 5NCR51 and 5NCR-RC50. These RNAs were biotin labelled. We have also included one random RNA (Random48), which serves as a nonspecific control. For each RNA, three experimental conditions were included: 1. Streptavidin beads control (beads+protein) to assess the proteins binding non-specifically to the beads. 2. RNA incubated with protein and then pulled down from streptavidin beads (RNA+protein) to capture proteins binding to RNA. 3. RNA incubated with protein in the presence of polyIC (dsRNA mimic) to reduce non-specific RNA-

protein interactions. After washing thoroughly, bound proteins were eluted from the beads by incubating them with 95% formamide at 65°C for 5 minutes. The eluates were transferred to fresh tubes and then buffer-exchanged to 50 mM ammonium bicarbonate. Proteins were then digested with trypsin at 37°C for 18 h. The peptide samples were then subjected to ESI-MS for protein identification.

Peptide Separation: To enrich RBPs specifically interacting with CHIKV RNAs, an affinity-based RNA pull-down was performed. Biotinylated RNA was incubated with proteins from different cell lines and was immobilised to streptavidin beads to pull down the proteins binding to CHIKV RNA. Multiple controls were used to determine specific RNA-protein interactions. Streptavidin beads without RNA were used to identify proteins binding non-specifically to the beads. Random48 was used to identify proteins binding to RNA in a sequence-independent manner. polyIC was used to reduce non-specific RNA interactions. These provide enrichment for proteins specifically interacting with conserved RNA sequences. For proteomic analysis, enriched protein samples were subjected to in-solution trypsin digestion, generating peptide mixtures. Peptides were separated by reverse phase MS and analysed by ESI-MS using an LC-MS platform. Data-dependent acquisition was used for peptide sequencing and protein identification. The resulting MS spectra were analysed using Protein Pilot software. We were interested in the proteins that were present in both RNA+protein and RNA+protein+polyIC but not in the protein-only condition.

Protein Characterization: All raw files were searched using Protein Pilot software version 5.0.2 against the SwissProt database R 6.12.2017. Default search parameters were applied. Protein identification was filtered using an unused score ?0.47. A false discovery rate (FDR) of 1% was used. Proteins present in RNA+protein and RNA+protein+polyIC but not in the protein control were selected. Any of those proteins that are interacting with Random48 were excluded. Proteins detected in at least four out of six total analyses (three biological and two technical replicates) were considered potential interactors. This strategy gives the protein specifically interacting with the conserved CHIKV RNAs.

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Data in disease_details No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications:

PubMed ID: