Functional Role of Conserved RNA Sequences in the DENV2 Genome

Harmanpreet Kaur, Nana Halane and Anna Groat Carmona

University of Washington Tacoma

Dengue virus (DENV) is a positive-sense, single-stranded RNA virus that remains a major global public health concern. While the DENV genome encodes viral proteins, it also contains conserved RNA sequence elements that regulate viral replication, including the cyclization sequence as well as the upstream and downstream AUG regions. In this study, two conserved coding-region RNA sequence elements, Envelope-Coding Region 1 (EnvCR-1) and NS1-Coding Region 1 (NS1CR-1), were identified using bioinformatics. Sequence alignments (Clustal Omega) were used to compare sequence homology within these regions to background genomic sequences across the four DENV serotypes. EnvCR-1 and NS1CR-1 sequence conservation was also evaluated in closely related flavivirus serogroups, including Japanese encephalitis virus and tick-borne encephalitis virus. These analyses indicated that both sequence elements are conserved among DENV serotypes, suggesting potential biological importance. To investigate whether these sequences function independently of their protein-coding roles, silent mutations preserving the encoded amino acid sequence were incorporated through overlap-extension PCR. The resulting mutant DNA amplicons were cloned into intermediate pGEM-T vectors and propagated in *Escherichia coli*. Diagnostic restriction digests, agarose gel electrophoresis, and Sanger sequencing were used to evaluate amplicon orientation and mutation incorporation within the intermediate vector; however, verifying amplicon orientation and successful mutation incorporation required additional screening and troubleshooting. Candidate constructs will be used in future studies to determine whether EnvCR-1 and NS1CR-1 regulate DENV replication independently of their protein-coding functions. This work provides a foundation for investigating conserved coding-region RNA elements and improving our understanding of DENV replication strategies.