Devitalizing Dengue Virus:

Identification and Mutagenesis of Conserved Genomic RNA Sequence and Structural Elements.

Colleen Selness, David Slattery, Rachel Ramirez and Anna M. Groat-Carmona

Conserved RNA sequence and structure elements can have important regulatory functions in RNA viruses, mediating various aspects of their lifecycle. Dengue virus (DENV) is an ssRNA vector-borne flavivirus, spreading between human hosts via Aedes mosquito vectors. Conserved RNA elements in the untranslated regions (UTR’s) of the DENV genome have been extensively studied and found to have functional roles in viral replication. Our research sought to identify conserved RNA elements within the coding region of the DENV genome that may be beneficial to the viral lifecycle. Screening for conserved RNA elements across the DENV1-4 serogroup and closely related serogroups (Japanese Encephalitis [JEV] and Tick-Borne Encephalitis [TBEV]) required the use of multiple bioinformatics platforms. Our efforts led to the identification of two conserved RNA sequence elements, Tapas Ranjan 1 and 2 (TJ 1/2), as well as a conserved RNA structural element, Spire of Dengue (SoD). Mutations were engineered to disrupt our candidate RNA elements in the DENV2 genome using the pD2/IC plasmid which would allow us to produce viral RNAs and study their effects in different cell lines. Mutagenesis was conducted in a manner that conserved the wild-type (WT) amino acid sequence, reading frame, peripheral secondary structure, and codon usage in two cell types (Aedes aegypti, and Mesocricetus auratus) to maintain efficacy. Our candidate RNA structural element (SoD) was mutated in a way that altered the primary nucleotide sequence while maintaining the WT structure, as well as in a way where both the structure and nucleotide sequence were disrupted. If our candidate RNA elements are important for the viral life cycle, these mutations would disrupt DENV2 replication, which could be determined from the execution and analysis of plaque assays. These findings hold potential to expand our understanding of viral replication, and lead to the identification of possible new antiviral targets.