00134 A Single Nucleotide Change in Dengue NS2B Alters Virus Replication

Ng Wy Ching, Sun Bo, Kwek Swee Sen, Tan Hwee Cheng, Ooi Eng Eong
Duke-NUS Medical School

Aims: The two successful flavivirus live attenuated vaccines (LAVs) against yellow fever (YFV) and dengue viruses (DENV), namely YF17D and DENV2 PDK53 strains were developed through serial passaging of the respective clinical isolates to obtain an attenuated phenotype characterised by small plaque size. We have recently shown that the small plaque phenotype was due, at least in part, to a robust interferon (IFN) response from infection with either virus that prevented the spread of infection to neighbouring cells. In this study, we aim to determine if mutations elsewhere in the genome would derive a virus with the same phenotype, defined by small plaque size, increase viral replication and elevated interferon response, as PDK53.

Methodology: We generated random mutations within the genome of a clinical DENV2 isolate by using 5-fluorouracil (5-FU) and sorted for mutants that induce robust type-I IFN response using Huh7 cells expressing IFNβ-EGFP promoter. Using full genome sequencing and reverse genetics, we generated these mutant viruses by Gibson assembly for further characterisation.

Result: We identified a single mutation within the NS2B of DENV2 virus (T4472C; amino acid I114T) that gave rise to small plaque size, increased viral replication and elevated IFN response in vitro, similar to PDK53 and significantly different to the parental clinical isolate as well as DENV2 16681. Similar observations were also made on monocyte derived dendritic cells (MDDCs), suggesting that NS2B could be targeted for DENV LAV development. The identification of this single mutation provide insights into the function of NS2B in suppresing type-I IFN induction, possibly in compromising its recently discovered ability to inhibit the cGAS/STING pathway.

Conclusion: Our findings suggest that alternative LAV candidates could be generated through chemical-induced mutagenesis, fluorescence sorting and reverse genetics.