Immunofluorescence based characterization of Dengue Non-Structural Protein 1 (NS1) intracellular localization in hepatic and non-hepatic cells

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Dengue virus (DENV) non-structural protein 1 (NS1) is a secreted glycoprotein essential for viral replication and known to play a key role in disease pathogenesis. It is known to interact with multiple host factors and understanding its intracellular behavior is important for characterizing its host protein interactions. In this study, we investigated the subcellular localization of NS1 in hepatic and non-hepatic cell lines overexpressing the DENV NS1 protein using immunofluorescence coupled with fluorescence confocal microscopy. Four cell lines HepG2, HC-04 (hepatic), and HEK293T, MDA-MB-231 (non-hepatic) were reverse transfected with mammalian expression plasmid, which express the DENV NS1 gene in abundance mimicking the protein expression during viral infection. After 72 hours, cells were fixed, permeabilized, and probed with Anti-DENV NS1 antibody. Probed samples were further treated with fluorescent conjugated secondary antibody to facilitate detection of NS1 protein via fluorescence microscopy. NS1 expression was successfully detected in all four cell lines. In HEK293T, MDA-MB-231, and HepG2 cells, NS1 showed a punctate cytoplasmic distribution consistent with previous reports of NS1 localization during active viral infection. This pattern suggests retention in the endoplasmic reticulum or association with secretory vesicles. In contrast, HC-04 cells showed a more diffuse cytoplasmic signal, which may reflect differences in protein processing or trafficking in this hepatic-derived line. Overall, our results demonstrate that transfected NS1 exhibits localization patterns similar to those observed during natural DENV infection, reinforcing the relevance of this model system for studying DENV NS1 intracellular behavior. In future studies, co-localization of DENV NS1 protein with organelle-specific markers will be conducted to identify the subcellular compartments involved in DENV NS1 trafficking and its intracellular functions during a viral infection.

Keywords: *Dengue virus NS1, Immunofluorescence, Fluorescence microscopy, Intracellular localization*

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