Characterization of the Interaction Between Dengue Virus NS5 and the Human Protein HMGB1.

Aguilera, E.A.[1], Allonso, D.[1], Andrade, I. S. [1], Silva, E. M.[1], Fantappié, M. R.[2] and Mohana-Borges, R.[1]

[1] Laboratório de Genômica Estrutural, IBCCF, UFRJ, Rio de Janeiro, Brazil

[2] Instituto de Bioquímica Médica Leopoldo de Meis, Programa de Biologia Molecular e Biotecnologia, UFRJ, Rio de Janeiro, Brazil

INTRODUCTION: Nonstructural 5 protein (NS5) is the most conserved among dengue virus (DENV) serotypes. It is a bifunctional enzyme that contains the S-adenosyl methionine transferase (MTase) and RNA-dependent RNA polymerase (RdRp) catalytic domains. Although NS5 functions occur in the cytoplasm of infected cells, it is also localized in the cell nucleus. There is no described role for nuclear localization of DENV2 NS5, therefore the interaction studies between NS5 and nuclear proteins are important for understanding the DENV infection. Furthermore, an increase in pro-inflammatory cytokines levels has been observed in severe dengue cases such as high mobility group box 1 protein (HMGB1), a DNA-bending protein that participates in the modulation of gene transcription. In inflammatory conditions, it can be released to the extracellular environment and it can act as a pro-inflammatory cytokine. **OBJECTIVES:** The aim of this study is to identify and characterize the interaction between the DENV2 NS5 and HMGB1 proteins. **MATERIAL AND METHODS:** The interaction between DENV2 NS5 and HMGB1 proteins was confirmed by ELISA assays. The NS5 activity in the presence of HMGB1 was measured by a polymerase assay using the γ-[2'(2-benzothiazoyl)-6'-hydroxybenzothiazole)-adenosine-5'-triphosphate (BBT-ATP) as probe. The HMGB1 bending activity in the presence of the NS5 protein and its domains was measured by a fluorescence resonance energy transfer (FRET) assay using oligonucleotides labeled at opposite ends with carboxyfluorescein (FAM) and 6-carboxytetramethylrhodamine (TAMRA) probes. **DISCUSSION AND RESULTS:** We identified the HMGB1 protein as a NS5- interacting partner and also it was able to interact with both RdRp and MTase domains. The functional assays showed that the presence of HMGB1 did not interfere with the NS5 polymerase activity. However, it was observed that NS5 RdRp domain altered the HMGB1 role, reducing the DNA bending. **CONCLUSIONS:** These results suggest that this interaction might affect the HMGB1 nuclear activity and modulate gene transcription.

Key words: DENV2 NS5 protein, HMGB1 and protein-protein interaction.
Patrocínio: FAPERJ, CNPq and CAPES