Title: ***"Deciphering the unconventional role of capsid protein in Chikungunya Virus infection"***

ABSTRACT

Capsid proteins have an essential role in virus genome protection and maintaining virus integrity. Recently, the capsid proteins of several RNA and DNA viruses have been reported to exhibit non-structural functions through subcellular trafficking. Chikungunya virus (CHIKV) capsid protein also localizes in the nucleus, but the purpose is not yet known. In the present study, we attempted to understand the unconventional roles of the nuclear-localized CHIKV capsid protein. The nucleic acid binding is the inherent feature of capsid protein and is crucial for both encapsidation and non-encapsidation functions of the protein so, it is important to understand the binding nature of the protein. To address this, we identified the nucleic acid binding activity of the protein through gel shift assay. The experiment was performed using different non-specific and CHIKV genome sequence-specific RNA and DNA molecules. The results showed non-specific nucleic acid binding activity of the capsid protein, suggesting it as one of the possible ways through which capsid interacts with host factors.

The capsid proteins belong to the group of supercharged proteins that are known to possess cell internalization ability. So, we assessed the DNA delivery activity of CHIKV capsid by transfecting GFP-tagged plasmid DNA using the capsid protein. The outcomes of the experiments demonstrated the DNA delivering ability of the protein. Detailed analysis of the protein sequence unveiled the involvement of two NLS sequences namely, NLS1 and NLS2 sequence in the above-specified functions of CHIKV capsid protein. Both NLS1 and NLS2 sequence peptides showed DNA-binding activity. Moreover, the NLS1 peptide was found capable of delivering DNA also. The additional function of DNA delivery by the NLS1 peptide was explained through structural characterizations using circular dichroism (CD) and Nuclear Magnetic Resonance (NMR) spectroscopy. The above results suggest that the NLS domains facilitate the non-structural functions of CHIKV capsid protein. The capsid protein may utilize these functions to interfere with host cellular trafficking or to translocate the viral genome across the membrane in the absence of infectious virions. Lastly, we checked the influence of CHIKV capsid protein on host gene expression through transcriptome analysis. We compared the transcriptome profiles of capsid transfected, and CHIKV infected Huh7 cells to identify differentially expressed genes (DEGs) in common between the two. The functional and pathway analysis of these shared DEGs (CXCL11, ALDH3B2, G6PC2, FSHR, etc.) showed their association with host cellular and metabolic processes. It also suggested the correlation of the identified DEGs and pathways with CHIKV-associated comorbid situations such as diabetes, hypertension, ocular manifestations, etc. Overall, the findings of the study highlight some crucial non-structural functions of CHIKV capsid protein. The capsid protein might carry out these functions to deal with the antiviral response and promote viral infection concurrently. Therefore, the study sheds light on the multi-functionalities of capsid protein. It further suggests reconsidering the role of capsid protein in virus infection.