THE INFLUENCE OF HEPATITIS C NS5A PROTEIN IN THE STABILISHMENT OF VIRUS-HOST INTERACTIONS.

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INTRODUCTION AND OBJECTIVE Hepatitis C virus causes the hepatitis C disease which affects over 170 million people worldwide. This disease is known to chronificate in up to 80 percent of cases and relies on the viral manipulation of the cellular machinery to persist and evolve, causing fibrosis, cirrhosis and cellular hepatocarcinoma. Identifying host factors that associate with viral proteins is crucial to a better comprehension of disease progression and possible therapeutical targets. The viral non structural protein 5a (NS5A) has been described to interact with several host factors and to manipulate different cellular pathways, such as the cellular cycle. However its complete role in the persistence of the viral infection is yet to be elucidated. Therefore this work aims to determine novel partners of this protein inside the host cell and how it affects the disease progression. Also, as this protein is known to manipulate the progression of cellular cycle, we sought to determine if this protein interacts with the cyclin dependent kinase 9 (CdK9) and the possible effects of this interaction.

MATERIALS AND METHODS In order to determinate the possible interactors of NS5A, this region was amplified from the HCV 1b subgenomic replicon and cloned into the tandem affinity purification vector (pNTAP). To verify the interaction between NS5A and CdK9, the NS5A region was amplified from the HCV 1b subgenomic replicon and cloned into the pCMV2-FLAG and pmCherry-N1 vectors in order to perform pull down, immunoprecipitation and confocal microscopy assays.

RESULTS AND DISCUSSION All of the constructs described above were confirmed by PCR, digestion and sequencing. The cellular expression of the construct NS5A-Flag and NS5A-TAP were tested and confirmed by Western Blotting Assay. The expression of the NS5A-Cherry construct was tested by fluorescence microscopy assay. CONCLUSIONS: The interactions assays are yet to be performed therefore we can only conclude that our molecular tools are functional.

ACKNOWLEDGEMENTS: IFRJ, FAPERJ, CNPq.

KEY WORDS: Hepatitis C, NS5A, Interactions.