Research of the month (April 2015)
Preclinical Research

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Role of human heterogeneous nuclear ribonucleoprotein C1/C2 in dengue virus replication

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Abstract

Background: Host and viral proteins are involved in dengue virus (DENV) replication. Heterogeneous ribonucleoprotein (hnRNP) C1/C2 are abundant host cellular proteins that exhibit RNA binding activity and play important roles in the replication of positive-strand RNA viruses such as poliovirus and hepatitis C virus. hnRNP C1/C2 have previously been shown to interact with vimentin and viral NS1 in DENV-infected cells; however, their functional role in DENV replication is not clearly understood. In the present study, we investigated the role of hnRNP C1/C2 in DENV replication by using an *in vitro* model of DENV infection in a hepatocyte cell line (Huh7) and siRNA-mediated knockdown of hnRNP C1/C2.

Methods: Huh7 cells were transfected with hnRNP C1/C2-specific siRNA or irrelevant siRNA (control) followed by infection with DENV. Mock and DENV-infected knockdown cells were processed for immunoprecipitation using hnRNP C1/C2-specific antibody or their isotype-matched control antibody. The immunoprecipitated samples were subjected to RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) for detection of DENV RNA. In addition, the knockdown cells harvested at varying time points after the infection were assessed for cell viability, cell proliferation, percentage of DENV infection, amount of viral RNA, and viral E and NS1 expression. Culture supernatants were subjected to focus forming unit assays to determine titers of infectious DENV. DENV luciferase reporter assay was also set up to determine viral translation.

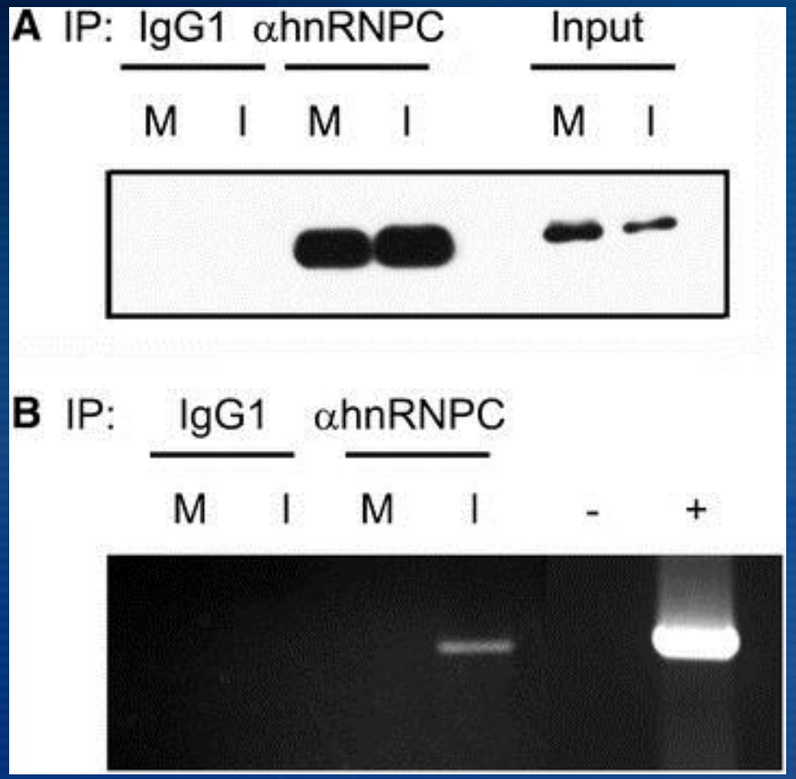
Results: Immunoprecipitation with the anti-hnRNP C1/C2 antibody and subsequent RT-PCR revealed the presence of DENV RNA in the immunoprecipitated complex containing hnRNP C1/C2 proteins. Transfection with hnRNP C1/C2-specific siRNA resulted in a significant reduction of hnRNP C1/C2 mRNA and protein levels but did not induce cell death during DENV infection. The reduced hnRNP C1/C2 expression decreased the percentage of DENV antigen-positive cells as well as the amount of DENV RNA and the relative levels of DENV E and NS1 proteins; however, it had no direct effect on DENV translation. In addition, a significant reduction of DENV titers was observed in the supernatant from DENV-infected cells following the knockdown of hnRNP C1/C2.

Conclusions: Our findings suggest that hnRNP C1/C2 is involved in DENV replication at the stage of viral RNA synthesis.

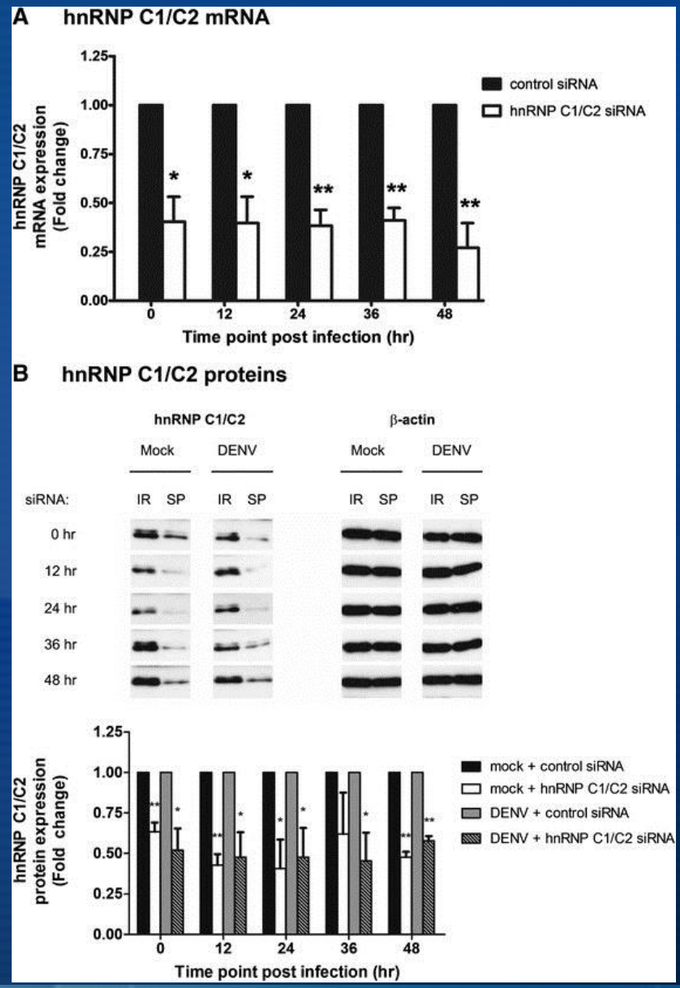
Keywords: hnRNP C1/C2, Dengue virus, siRNA transfection, Virus replication

Role of human heterogeneous nuclear ribonucleoprotein C1/C2 in dengue virus replication

- **Association of hnRNP C1/C2 proteins with DENV RNA**



- **Knockdown of hnRNP C1/C2 by specific siRNA transfection**



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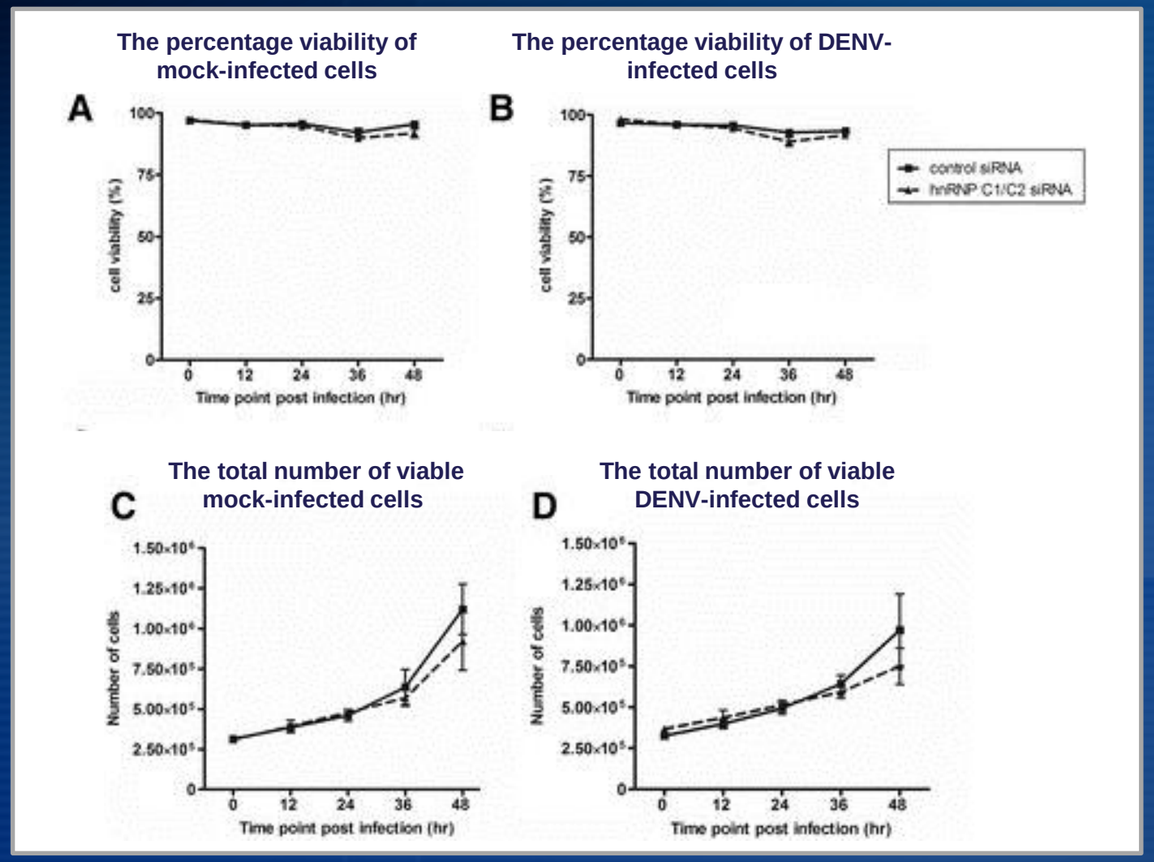
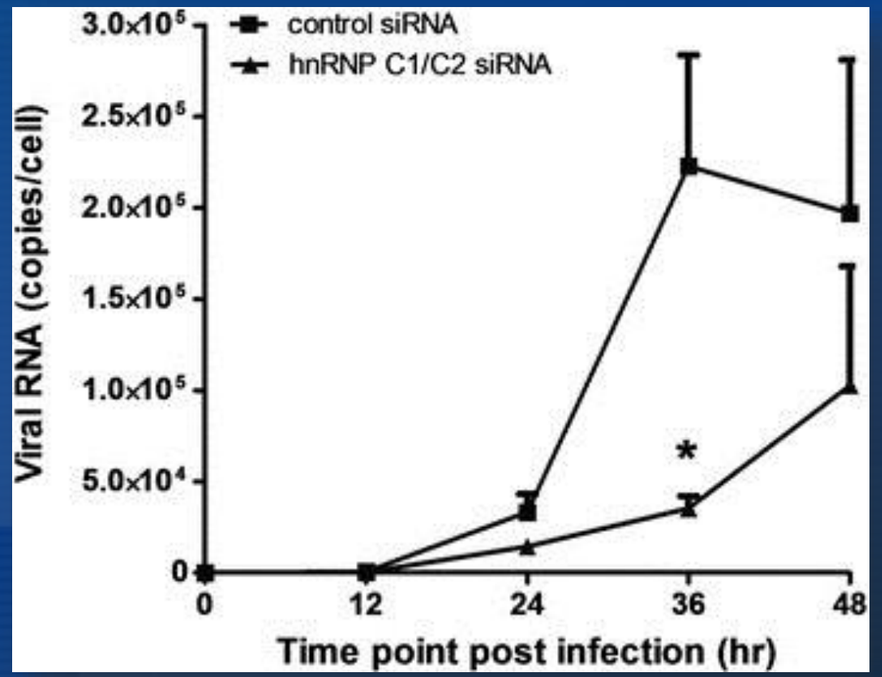
Effects of hnRNP C1/C2 knockdown on cell viability and proliferation



Effects of hnRNP C1/C2 knockdown on DENV RNA and protein syntheses



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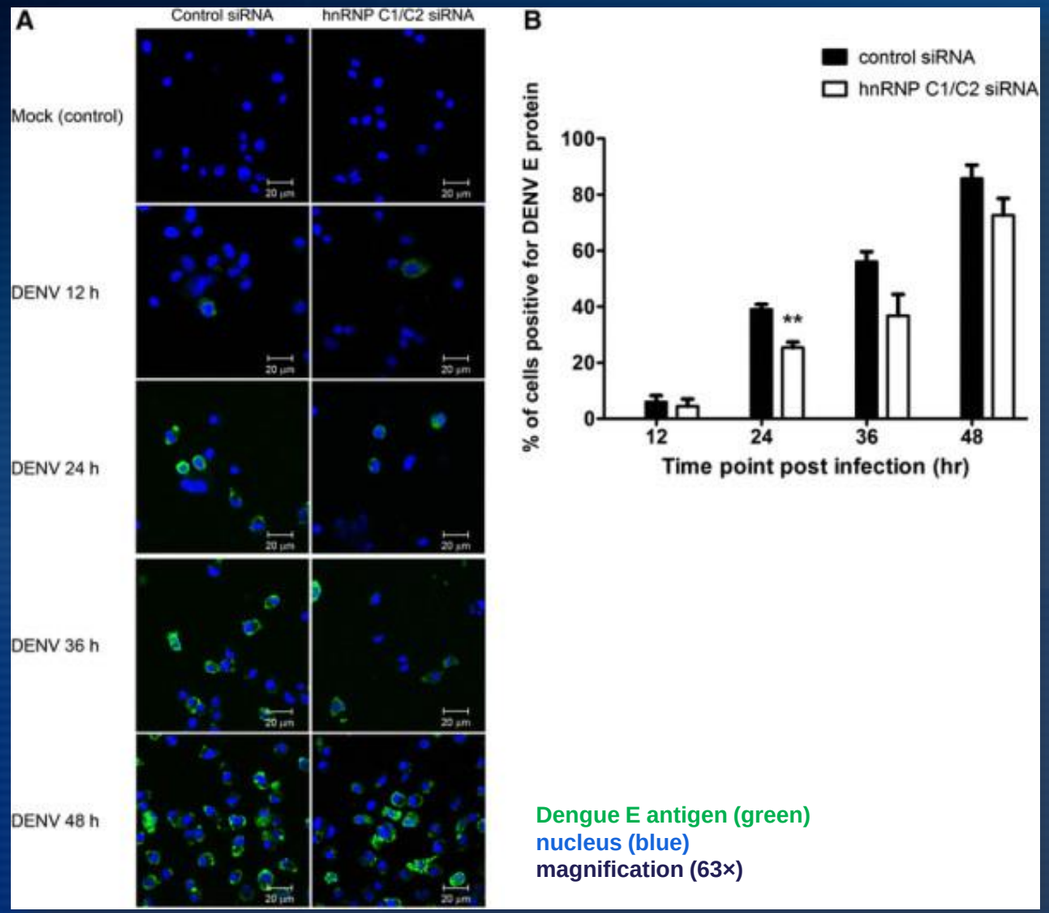
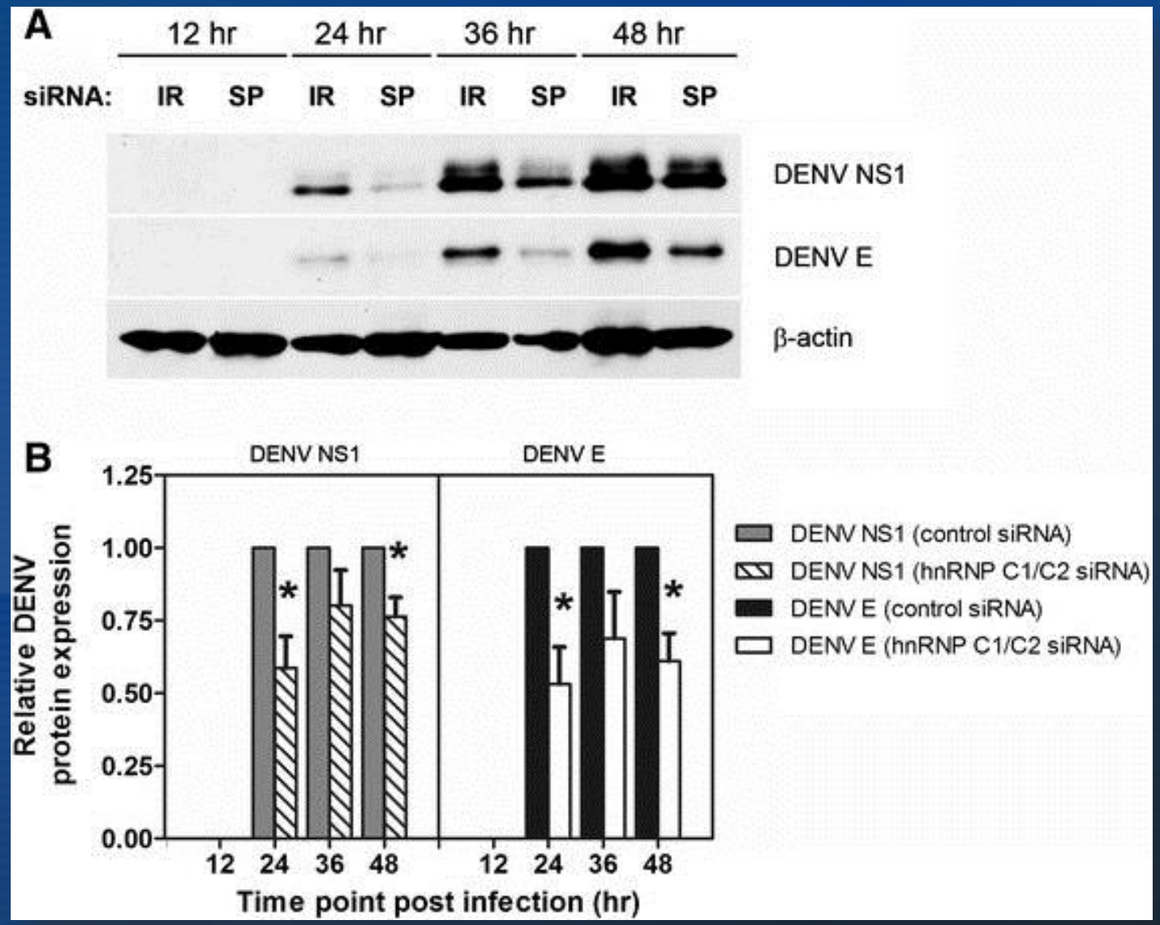
- **Effect of hnRNP C1/C2 knockdown on the extent of DENV infection**



Dengue E antigen (green)
nucleus (blue)
magnification (63×)

- **Effect of hnRNP C1/C2 knockdown on DENV protein expression**



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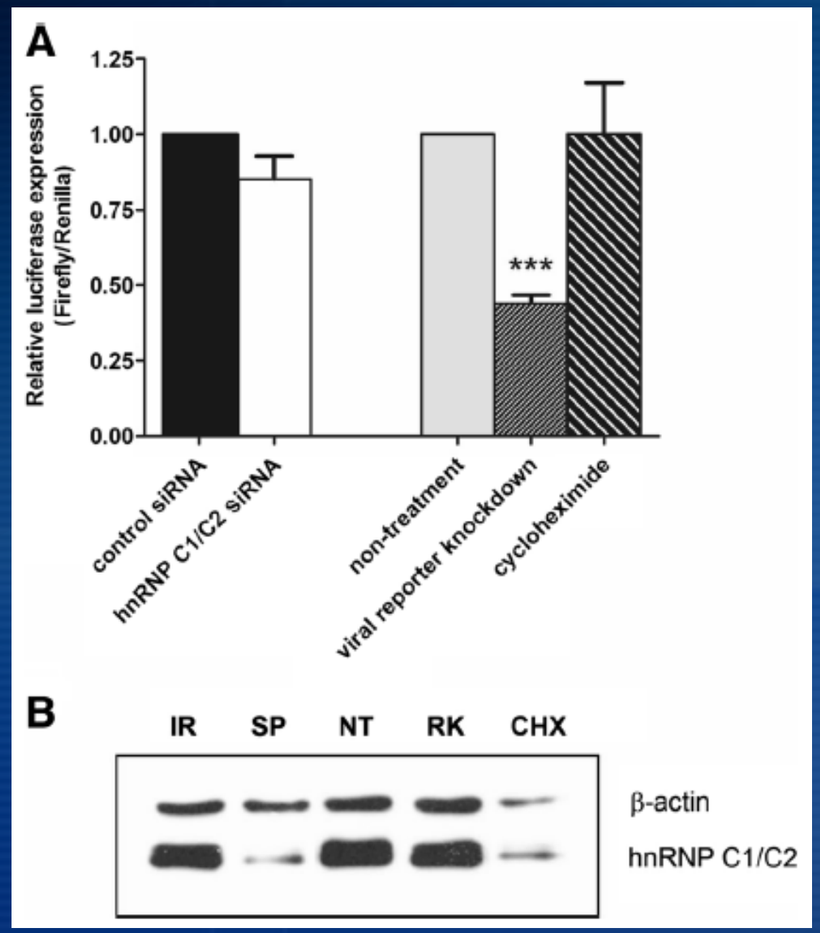
- **Effect of hnRNP C1/C2 knockdown on DENV protein translation**



- **Effect of hnRNP C1/C2 knockdown on release of infectious DENV**