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Modulation of MAPK-JNK1/2 by hepatitis E virus ORF3 protein in hepatoma cells

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Background & Aim: Hepatitis E virus (HEV) has recently emerged to cause chronic infection, including neuropathy and arthralgia in some patients. Though *in vitro* modulation of cellular MAPK-ERK cascade by HEV-ORF3 protein is suggested to have a role in host pathobiology, activation of JNK1/2 has not been studied so far. To address this issue, we used the HEV genomic replicon system as well as expression vectors.

Material & Methods: *In vitro* transcribed pSKE2-WT (HEV-SAR55) replicon as well as expression vectors pTriEX-ORF2 and pTriEx-ORF3 were transfected into human hepatoma S10-3 cells (0.5×106 cells/well; 12-well plate; in triplicate). Following 24 hr incubation, RNA/DNA receiving cells were re-seeded in 8-chamber slides as well as in two sets (RNA-I & RNA-II and DNA-I & DNA-II) in 96-well culture plates. On day 4 post-transfection, HEV replication was determined by double immune-staining for ORF2 (chimp-sera/Alexa488-conjugated goat anti-human IgG) and ORF3 (Rabbit-anti-ORF3/Alexa 568-conjugated goat anti-rabbit) expressions in RNA-transfected cells or single staining for ORF2 and ORF3 in DNA-transfected cells. For a time-course (day 2, 4, 6 and 8) JNK1/2 profiling, ELISA-based detection of JNK1/2 protein phosphorylation was performed, using BIOMOL Super Array Case-Kit.

Results: While positive staining for ORF2/ORF3 in HEV RNA-transfected cells confirmed an active viral replication, the ELISA-based relative quantitation of JNK1/2 phosphoprotein in the same culture showed an optimal phosphorylation on day4. The JNK1/2 phosphorylation in RNA receiving cells was elevated by ~54% as compared to negative-control. Further, ~66% activation of phospho-JNK1/2 was observed in ORF3 over-expressing cells, compared to mock-control. Notably, these experiments when repeated in serum-free media under the same conditions did not show significant differences in the results.

Conclusion(s): The data therefore, shows regulation of MAPK-JNK1/2 by HEV-ORF3 in hepatocytes, mimicking HEV infection. This may have a possible pro-cell survival role in persistent infection in immunosuppressed individuals or extra hepatic manifestations of HEV. Molecular studies are underway to further validate these results.

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