Hepatitis C virus core protein interacts with cellular putative RNA helicase.

葉添順

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Abstract

The nucleocapsid core protein of hepatitis C virus (HCV) has been shown to trans-act on several viral or cellular promoters. To get insight into the trans-action mechanism of HCV core protein, a yeast two-hybrid cloning system was used for identification of core protein-interacting cellular protein. One such cDNA clone encoding the DEAD box family of putative RNA helicase was obtained. This cellular putative RNA helicase, designated CAP-Rf, exhibits more than 95% amino acid sequence identity to other known RNA helicases including human DBX and DBY, mouse mDEAD3, and PL10, a family of proteins generally involved in translation, splicing, development, or cell growth. In vitro binding or in vivo coimmunoprecipitation studies demonstrated the direct interaction of the full-length/matured form and C-terminally truncated variants of HCV core protein with this targeted protein. Additionally, the protein's interaction domains were delineated at the N-terminal 40-amino-acid segment of the HCV core protein and the C-terminal tail of CAP-Rf, which encompassed its RNA-binding and ATP hydrolysis domains. Immunoblotting or indirect immunofluorescence analysis revealed that the endogenous CAP-Rf was mainly localized in the nucleus and to a lesser extent in the cytoplasm, and when fused with FLAG tag, it colocalized with the HCV core protein either in the cytoplasm or in the nucleus. Similar to other RNA helicases, this cellular RNA helicase has nucleoside triphosphatase-deoxynucleoside triphosphatase activity, but this activity is inhibited by various forms of homopolynucleotides and enhanced by the HCV core protein. Moreover, transient expression of HCV core protein in human hepatoma HuH-7 cells significantly potentiated the trans-activation effect of FLAG-tagged CAP-Rf or untagged CAP-Rf on the luciferase reporter plasmid activity. All together, our results indicate that CAP-Rf is involved in regulation of gene expression and that HCV core protein promotes the trans-activation ability of CAP-Rf, likely via the complex formation and the modulation of the ATPase-dATPase activity of CAP-Rf. These findings provide evidence that HCV may have evolved a distinct mechanism in alteration of host cellular gene expression regulation via the interaction of its nucleocapsid core protein and cellular putative RNA helicase known to participate in all aspects of cellular processes involving RNA metabolism. This feature of core

protein may impart pleiotropic effects on host cells, which may partially account for its role in HCV pathogenesis.