عنوان مقاله:

Construction and Eukaryotic Expression of Recombinant Large Hepatitis Delta Antigen

محل انتشار:

مجله گزارش های بیوشیمی و زیست شناسی مولکولی, دوره 2, شماره 1 (سال: 1392)

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خلاصه مقاله:

Background: Hepatitis delta virus (HDV) is a subviral human pathogen that exploits host RNA editing activity to produce two essential forms of the sole viral protein, hepatitis delta antigen (HDAg). Editing at the amber/W site of HDV antigenomic RNA leads to the production of the large form (L-HDAg), which is required for RNA packaging. Methods: In this study, PCR-based site-directed mutagenesis by the overlap extension method was used to create the point mutation converting the small-HDAg (S-HDAg) stop codon to a tryptophan codon through three stages. Results: Sequencing confirmed the desirable mutation and integrity of the L-HDAg open reading frame. The amplicon was ligated into pcDNA۳.۱ and transfected to Huh۷ and HEK ۲۹۳ cell lines. Western blot analysis using enhanced chemiluminescence confirmed L-HDAg expression. The recombinant L-HDAg localized within the nuclei of cells as determined by immunofluorescence and confocal microscopy. Conclusion: Because L-HDAg requires extensive post-translational modifications, the recombinant protein expressed in a mammalian system might be fully functional and applicable as a tool in HDV molecular studies, as well as in future vaccine research.

کلمات کلیدی:

Hepatitis Delta Virus, L-HDAg, SOEing-PCR