کارگاه‌های آموزشی مرکز اطلاعات علمی

- مقاله نویسی علوم انسانی
- اصول تنظیم قراردادها
- آموزش مهارت های کاربردی در تدوین و چاپ مقاله
HEV-ORF3 Encoding Phosphoprotein Interacts With Hepsin

Chunyan Wang 1; Liang Guo 1; Dayi Yu 2; Xiuguo Hua 1; Zhibiao Yang 1; Congli Yuan 1; Li Cui 1*

1Shanghai Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China
2Animal Disease Control Center of Min Hang District, Shanghai, China
*Corresponding Author: Li Cui, Shanghai Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai Jiao Tong University, 800 Dong Chuan Road, Shanghai, China. Tel: +86-2134206367, Fax: +86-2164785582, E-mail: lcui@sjtu.edu.cn

Received: July 31, 2013; Revised: October 12, 2013; Accepted: November 1, 2013

1. Background

Hepatitis E virus (HEV), a member of the family Hepadnaviridae, is believed to be a major pathogen responsible for endemic infections as well as large epidemics of acute clinical hepatitis (1). Infection caused by Hepatitis E virus (HEV) has been a significant global health concern and has been identified and described as a zoonotic infection by earlier researchers (2). Epidemics of HEV infection has been reported in many developing countries of Asia and Africa, however specific sporadic cases of HEV infection have also been reported in industrialized countries (3). Among the known subtypes, genotype 4 hepatitis E virus has been a predominant pathogen infringing the Chinese, especially since the last decade or so (4, 5). The genome of HEV is a single-strand RNA of 7.2 kbs that is positive-sense with 5'-methylguanine cap and 3' poly(A) stretch and contains three partially overlapping open reading frames (ORFs), called ORF1, ORF2 and ORF3 (6, 7). ORF3 encodes around 120 amino acids of phosphoprotein that can associate with the cytoskeleton using one of its hydrophobic domains and can homodimerize through a 43-amino-acid interaction domain (6). Previous researches have suggested that the pORF3 can promote cell survival and proliferation and dampen innate host response through an attenuated acute phase response (8). Meanwhile, the pORF3 is important to virion egress from infected cells and the PSAP motif has a justified role as a functional domain for HEV budding (9, 10).

2. Objectives

The major objectives of the current study were to screen HEV-pORF3 interacting proteins from hepatocytes cDNA library using yeast two-hybrid systems, and to further confirm the interactions by Co-IP and western-blotting assays. These novel research findings will hopefully bring new descriptions to better understand the specific function of pORF3.

3. Materials and Methods

3.1. Materials

Preservation of genotype 4 hepatitis E virus was carried out at our laboratory. The Cyto-Trap two-hybrid system used in the current study including pMyr and pSos vectors, CDC25H cells and corresponding control plasmids were purchased from Stratagene. The pcDNA3.1-His-Flag-tag (pcDNA3.1 HF), a mammalian expression vector...
rived from pcDNA3.1 was purchased from Life Technologies and modifications for purification were carried out through His-tag and Co-IP with Flag-tag. The vector M51 (Genecopoeia, America) was used to explore the coordinated co-expression of two genes with the same vector and the Green fluorescent protein (GFP) sequence, which allowed for direct observation of expression results.

3.2. Plasmid Construction

To construct the pSos-bait ORF3 for Cyto-Trap two-hybrid system assay, the ORF3 cDNA of HEV was amplified using the primers listed in Table 1 and cloned into pSos vector by BamHI and SalI restriction sites. To generate an N-terminal with HF-tagged ORF3 expression construct for Co-IP, the cDNA of ORF3 was amplified using the primers listed in Table 1 and the PCR product was cloned into the pcDNA3.1-His-Flag vector via the HindIII and EcoRI restriction sites to create pcDNA3.1-HF-ORF3. The gene of hepsin (HPN) of a transmembrane serine protease corresponding to bases from 132 bp to 1250 bp was amplified through primers presented in Table 1 and the resulting amplification product was then sub-cloned into the M51 vector for Co-IP.

3.3. Screening of Interacting Proteins by Yeast Two-Hybrid System

During this study, cytoplasm-based yeast two-hybrid systems were adopted, to dissect the proteins interacting with pORF3 from human hepatocytes cDNA library. All experiments were carried out strictly according to the manufacturer’s instructions. Briefly, the plasmids of pSos-ORF3 as baits and that of human hepatocytes cDNA library were co-transformed to the temperature-sensitive yeast strain cdc25H cells. Transformed yeast cdc25H cells with specific galactose-induced growth at the per-
Table 2. The Blast Results of Positive Clone Within in GenBank

<table>
<thead>
<tr>
<th>Clone</th>
<th>Name and Location</th>
<th>Homology, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>821</td>
<td>Homo sapiens albumin (ALB), bases: 72 to 1151</td>
<td>96</td>
</tr>
<tr>
<td>475</td>
<td>Homo sapiens mitochondrion genome, bases: 15065 to 15889</td>
<td>99</td>
</tr>
<tr>
<td>763</td>
<td>Homo sapiens chromosome 1 genomic contig, bases: 36379 to 36479</td>
<td>96</td>
</tr>
<tr>
<td>194</td>
<td>Homo sapiens histidine-rich glycol-protein (HRG), bases: 823 to 1679</td>
<td>99</td>
</tr>
<tr>
<td>235</td>
<td>Transmembrane protease, serinet (HPN), bases: 132 to 1250</td>
<td>94</td>
</tr>
<tr>
<td>376</td>
<td>Homo sapiens phosphorinositide-3-kinase (pi110δ), bases: 3589 to 4661</td>
<td>95</td>
</tr>
<tr>
<td>788</td>
<td>Complement component 3, bases: 2988 to 3998</td>
<td>97</td>
</tr>
<tr>
<td>72</td>
<td>Homo sapiens ribosomal protein S11 (RPS11), bases: 16 to 613</td>
<td>98</td>
</tr>
</tbody>
</table>

missive temperature (37°C) were selected. 15 putatively positive clones were screened and co-transformed to the same cells once again with pSos for further verification. After re-verification, eight positive clones were confirmed and their cDNAs were further analyzed by means of DNA-sequencing and identified using the GenBank Blastp program (Table 2). The hepsin, a transmembrane protease, was selected to verify the interaction with the pORF3 through Co-IP.

4.2. Co-Immunoprecipitation (Co-IP)

For verification of the interaction established by the yeast two-hybrid screening, we used a Co-IP assay. With the Green fluorescent protein (GFP) sequence in M51 vector, the fluorescence microscopy detection results of M51-HPN (pCMV-HPN-IRES-GFP) presented green fluorescence, which reflects the success of transformation and construction of recombinant plasmid (Figure 1). Our Co-IP and western blotting results indicated that HPN interacts with pORF3, specifically pORF3 in human cells (Figure 2).

5. Discussion

The current study was carried out to investigate the interactions between HEV-pORF3 and hepsin. Hepatitis E virus (HEV) is the causative agent of hepatitis E, a form of acute viral hepatitis that is endemic in developing countries. It is estimated that about 2 billion, a third of the world’s population, live in areas where they are at risk of HEV infection (3, 4). The pORF3 is definitely an essential element for the establishment of HEV infections in animals but its exact functions are still unclear and obscure. Previous studies have suggested that pORF3 prolongs endomembrane growth factor signaling and also promotes cell survival to contribute positively in viral replication and pathogenesis, and PASP motif in pORF3 is responsible for viron egress from infected cells (8-11).

In the present study, yeast two-hybrid system was employed to select the pORF3 interacting proteins from human hepatocytes cDNA library. Eight selected positive clones and their DNA sequences were analyzed through DNA sequencing and the GenBank Blastp program (Table 2).
2) was used for identification. Hepsin, a type II transmembrane serine protease, was selected for further studies and confirmation of outcomes. Results of Co-IP and western blotting techniques indicated that the hepsin has specific interactions with pORF3.

It has been reported that hepsin, a cell surface protease is associated with growth and progression of cancers, particularly prostate cancer and its over-expression is found in more than 90% of human prostate cancer cases (12-14). Hepsin is also thought to be involved in diverse cellular functions, including blood coagulation and the maintenance of cell morphology (15, 16). Furthermore, the ORF3 protein is likely to cause an imbalance in physiological processes like coagulation and fibrinolysis by interacting with certain host proteins and eventually triggering the corresponding pathological processes (17). Therefore, from novel findings of the current study, it was concluded that pORF3-Hepsin interactions might lead to certain disturbances and imbalances in coagulation; we suggest further detailed and specific studies to unfold the domain of interaction and the real biological significance of pORF3-Hepsin interactions.

Acknowledgements
Current research studies were supported by the National Natural Science Foundation of China (31070132).

Authors’ Contribution
Chunyan Wang and Liang Guo developed the novel idea, designed and carried out this study. Li Cui and other authors gave many constructive opinions and offered essential assistance.

Financial Disclosure
Each author certifies that he or she has no commercial associations (i.e. consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

Funding/Support
This work was supported by the National Natural Science Foundation of China no. 31070132.

References
کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله