利用噬菌體呈現法來尋找與 D 型肝炎病毒 RNA 交互作用的宿主因子

Search for cellular factors interacting with hepatitis delta virus RNA using phage-display cDNA library

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一、中文摘要

D 型肝炎病毒有一些獨特的生物機能例如自我催化之切割及連結的活性、RNA 編輯活性、與 D 型肝炎抗原相關之病毒複製。這些活性應有其它細胞因子與 D 型肝炎病毒 RNA 或抗原交互作用而產生。噬菌體呈現法利用噬菌體將外來蛋白表現在其表面蛋白上。它是研究蛋白間交互作用或蛋白及核酸交互作用很有用的工具之一。我們用 T7 噬菌體製造了一個人肝對補去氧核甘酸的基因庫。利用 biotin 標記的 D 型肝炎病毒 RNA 探針來篩檢基因庫，我們得到了一些可能作用的基因。這包括人類白蛋白，一 leucine zipper 蛋白，apolipoprotein E, phosphodiesterase 3B，及一些粒腺體中的蛋白。其中只有白蛋白通過 in vitro 結合試驗。我們正在研究白蛋白與 D 型肝炎病毒 RNA 交互作用是否有生物學上的意義。

關鍵詞：D 型肝炎病毒、噬菌體呈現法

Abstract

Hepatitis delta virus (HDV) possesses several distinct biological activities such as autocatalytic cleavage and ligation activity, RNA-editing activity, hepatitis delta antigen (HDAg) dependent replication transactivation and inhibition. There should be some cellular factors interacting with HDV RNA or HDAg to accomplish these activities. The phage display technique utilizes phages to express foreign proteins or peptides in fusion with one of their coat protein. It is an ideal tool to study protein-protein interaction and protein-nucleic acid interaction. We constructed a T7 phage expression CDNA library from a human liver and selected with biotin-labeled genomic HDV RNA probe. We obtained several candidate genes including human albumin, a leucine zipper protein, apolipoprotein E, phosphodiesterase 3B and some mitochondria-related proteins. Only human albumin stood the in vitro binding test including northwestern and gel mobility shift assay. We are currently investigating the biological meaning of this HDV RNA

and human albumin interaction.

Keywords: Hepatitis delta virus, phage display

二、緣由與目的

Hepatitis delta virus (HDV) is a satellite virus of hepatitis B virus. Superinfection with HDV in chronic hepatitis B carriers may induce fulminant hepatic failure or aggravate the underlying liver disease. HDV contains a single-stranded circular RNA genome of 1.7 kilobases in length, which has extensive intramolecular complementary sequences. Its RNA structure resembles that of viroids, virusoids, and plant satellite virus RNAs. HDV RNA has an autocatalytic cleavage and ligation activity, and appears to replicate by a rolling-circle mechanism. It has been shown that HDV RNA replication requires the presence of a virus-encoded protein, hepatitis delta antigen (HDAg). Posttranscriptional RNA editing specifically modifies some of the HDV RNA, leading to the production of the large form of the HDAg, which is essential for virus assembly. Because HDAg has no known RNA polymerase acitivity or RNA-editning activity, it is likely that HDAg or HDV RNA might interact with one or more cellular factors to promote HDV replication or accomplish RNA editing. Using yeast two hybrid system, a cellular factor (delta-interacting protein A) has been identified to interact with HDAg. Yet no cellular factor has been reported to interact with HDV RNA.

Phage-display library utilized filamentous phage, M13, to express foreign peptides or proteins in fusion with a coat protein, called gene III protein. The gene III protein helps the expressed target proteins to be displayed on the surface of phage particles. These particles are screened with a bait protein or any ligands coated on a plate or column. This phage-display technique has been used for the construction of single-chain antibody libraries and combinatorial antibody fragment libraries. Many antibodies have been isolated from these libraries. Most of the bait ligands are proteins, but single-stranded DNA and RNA have also been successfully used to obtain anti-ssDNA and anti-RNA antibodies. In this project we we will screen phage display cDNA libraries from human liver with HDV RNA. The cellular factors obtained will further be confirmed by in vitro and in vivo binding assay and tested for their possible functions. Hopefully

this result will aid to our understanding of the mechanism of several biological activities of HDV.

三、結果與討論

Initially we used a commercial phagemid cDNA library which was later found to be faulty. We therefore constructed a cDNA library using T7Select Phage Display System (Novagen, Inc.). Most of the inserts were between 0.3 kb to 1.3 kb.
The representative of the library was checked by PCR with ras and actin primer. Both reactions resulted in expected size fragment. We changed our strategy of panning using biotinylated HDV RNA binding to Streptavidin-Paramagnetic beads. Using this method we can do 2 to 3 rounds of panning in one day. According to literatures, the frequency of target phage was about one in one hundred after third round of panning. After 5 rounds of panning we did plague lift and probed with digioxigen-labeled HDV RNA probe. The background was too high despite we varied concentrations of probe and competing tRNA and cellular RNA. We picked and amplified around one hundred of plagues and blotted onto nylon membrane and doing northwestern binding assay. The positive rates seemed too high (>90%). We sequenced around 20 plaques using PCR.
No sequences were alike. We thought that whole phage probably possessed too many proteins that caused the background.
To express the protein coded by the insert only, we designed an upstream primers which had T7 promoter sequence, Kozak sequence and in frame ATG in 5' end. After PCR amplification we do in vitro translation using single-tube T7 quick translation system (Promega).
No translated protein was obtained. We designed a second PCR to incorporate ribosome entry site from pCITE (Novagen). However in vitro translation of this PCR product still resulted in nothing.
We looked back to find what the problems were. On initial panning we used 1μg of biotinylated HDV RNA, which amounted to about 10^12 molecules, to bind against 10^10 recombinant phages. This would cause too much non-specific binding. We decided to decrease the panning HDV RNA to 10 pg. The ratio to non-specific competing tRNA and cellular RNA (10 μg each) became 1:10^6. By this new panning condition we sequenced about 20 plaques each around after 4th round of panning. There are few clones repeatedly pres

ent in each round. These clones represent candidate cellular proteins. They included human albumin, a leucine zipper protein, apolipoprotein E, phosphodiesterase 3B and some mitochondria-related proteins. These genes were cloned into expression vectors and northwes tern blot and gel mobility shift assay were done for retest of in vitro binding. Human albumin showed positive result for both assays. We are currently investigati ng the in vivo binding and biological significance of this interaction. It is intersting to note that although HBV and HDV shares the same coat proteins, they may use different mechanisms to enter cells and have different cell tropism. Whether human albumin takes some role in HDV cell tropism remains to be studied.

四、計畫成果自評

Our initial goal was to find cellular factors that contribute to the replication of HDV RNA. Although a leucine zipper transactivator was found, but it did niot stand the in vitro binding test. More rounds of panning may be needed to get meaningful transcription factors. Un expectedly we found that human albumin may interact with HDV RNA. Further studies will be done on this subject . From this project we learned a lot of tips in constructing phage cDNA library and panning againt baits. This will aid our studies for other protein-protein or protein-nucleic acid interactuions.

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