

# Detection of hepatitis D virus by cDNA microarray method

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**BACKGROUND:** Viral hepatitis is considered a major public health problem in most areas of the world. In acute and chronic infections, hepatitis D virus (HDV) infection often leads to a more severe disease. This study was designed to prepare microarrays for HDV detection.

**METHODS:** The specific primers of PCR were designed according to the conserved region of HDV. The cDNA microarrays were prepared by spotting PCR products onto the surface of glass slides by robotics. Restriction display PCR (RD-PCR) was used to label the samples.

**RESULTS:** Sequences were aligned, and the results showed that the products of PCR amplification were the specific gene fragments of HDV. Hybridizing signals on gene chip showed the specificity and sensitivity in detecting HDV were satisfactory.

**CONCLUSION:** Using PCR amplified products to construct gene chips for clinical diagnosis of HDV is a quick, simple and effective method.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 423-427)

**KEY WORDS:** hepatitis D virus; PCR; microarray; hybridization

## Introduction

Viral hepatitis is the most common cause of acute and chronic hepatitis.<sup>[1,2]</sup> Chronic infection of hepatitis virus affects over 500 million people worldwide.<sup>[3-5]</sup> Viral hepatitis is associated with signifi-

cant morbidity and mortality and is considered a major public health problem in most areas of the world. The development of sensitive assays may contribute greatly to the diagnosis, treatment, and prevention of the disease.<sup>[6-8]</sup> In the past decade, improved molecular biology-based techniques have yielded highly valuable tools for use in this setting.<sup>[9]</sup> DNA chip is a new category of developing technology, characterized by expedient, large scale, highly automatic and sensitive detection of biological information such as DNA and RNA.<sup>[10,11]</sup>

In this study, we developed a microarray technique for the detection of hepatitis D virus (HDV). The specific primers of PCR were designed with the Primer Premier 5.00 program according to the conserved region of HDV. The PCR fragments were purified and cloned into the pMD18-T vectors. The recombinant plasmids were extracted from positive clones and the target gene fragments were sequenced. The DNA microarrays were prepared by spotting PCR products onto the surface of glass slides by robotics.

## Methods

### Probe template

The full-length plasmid of HDV pDL553 was presented by Dr S. Gudima at the Fox Chase Cancer Center, USA.

### Chemicals and reagents

Premix Taq, dNTP, EcoRI, Sau3AI, pMD18-T vector, T4 DNA ligase were obtained from Takara Corp, Japan. Plasmid Miniprep kits were purchased from Shen Neng Bo Cai Corp, China, PCR primers of HDV and the primers in pMD18-T vector were synthesized by BIOASIA Corp, China. The universal primers cy5-GTTTGGCTGGTGTTGGATC were purchased from Gibco Corp, USA, and CMT-GAPS coated glass slides from Corning Corp, USA.

### Bacterial strains

The *E. coli* strain XL-1 used in the experiments was kept at our laboratory.

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This study was supported by grants from the National Natural Science Foundation of China (No. 39880032) and the Key Sci-Tech Research Project of Guangzhou Municipality (No. 99-Z-022-01), China.

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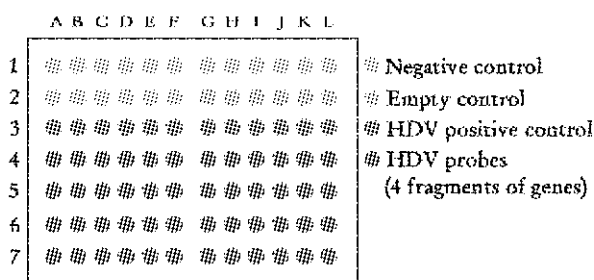


Fig. 1. Arrangement of all spots on HDV microarray.

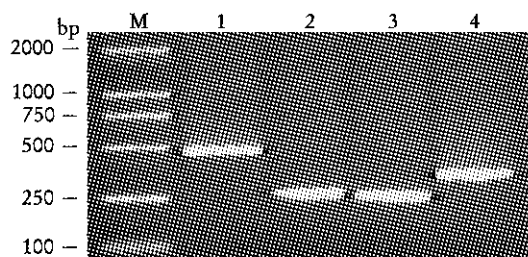


Fig. 2. Agarose gel electrophoresis of PCR products of four fragments by different primer combination for preparation of HDV probes of genechip.

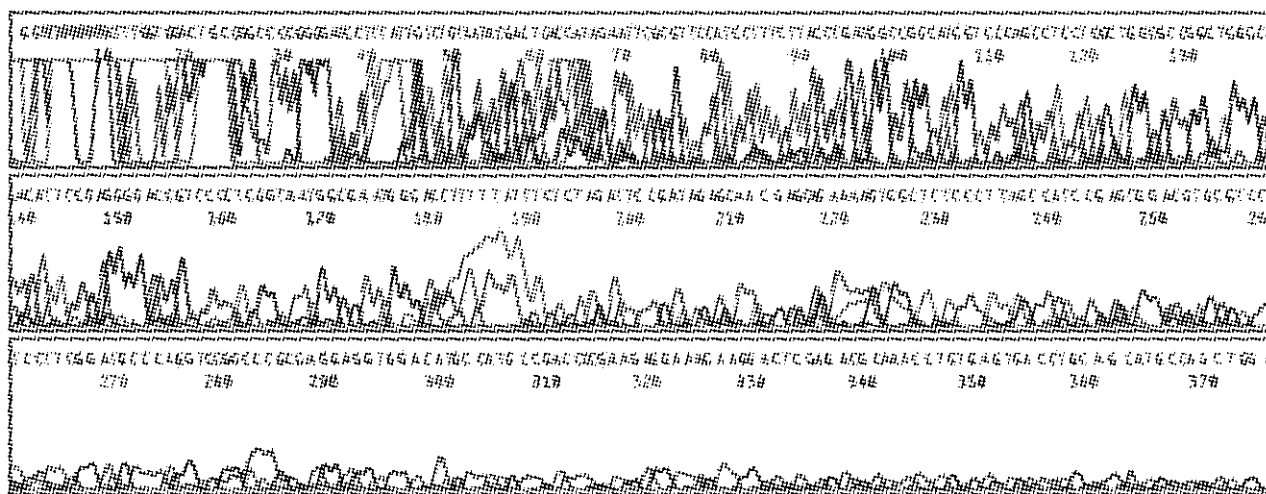


Fig. 3. Sequence analysis of one of DNA fragments from HDV.

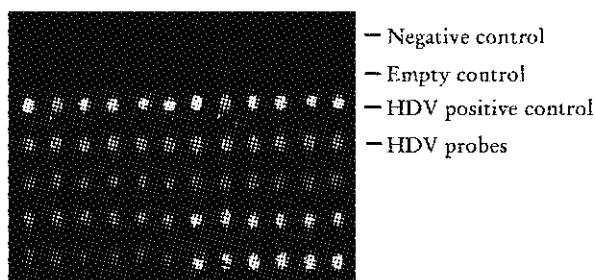


Fig. 4. Scanning plots of hybridizing signals on HDV microarray.

temperature for 10 minutes; 3) 0.1×SSC for 1 minute; 4) Milli Q water; 5) ethanol; respectively. Then the chip was desiccated at room temperature. Scanning was performed using a ScanArray Lite Microarray Analysis System (GSI Lumonics CO, USA).

## Results

### Preparation of HDV probes for microarray

The results of 1.5% agarose gel electrophoresis showed that all of the 4 primer pairs could amplify the HDV cD-

NA and produce the products of expected size (Fig. 2).

### Confirmation of the DNA obtained from HDV by sequence analysis

Blastn with Genebank database, each sequenced PCR product was confirmed to be an HDV genome fragment. Fig. 3 shows one cDNA fragment from HDV located at complete genome region.

### Slide scanning and analysis

Hybridizing signals under a condition of 80% laser energy and 70% GMT on the gene chip showed that the specificity and sensitivity in detecting the HDV were satisfied (Fig. 4). No signal was found on the empty control spots (printing 50% DMSO) and negative control spots (printing gene fragments of eukaryotic cell such as K562 cell, prokaryotic cell such as HIV, and plant gene such as rice. Our institute preserved all the gene fragments). The signal of spots that were hybridized to HDV, HDV positive controls was strong and obvious.

## Discussion

In China, chronic viral hepatitis is considered a major public health problem.<sup>[12,13]</sup> Hepatitis D virus (HDV), a defective virus, requires the supply of hepatitis B surface antigen (HBsAg) envelope by hepatitis B virus (HBV) for its assembly and transmission.<sup>[14,15]</sup> During both acute and chronic infections, HDV infection often leads to a more severe disease.<sup>[16-18]</sup>

In clinical diagnosis of hepatitis virus, the antibody-based methods such as ELISA<sup>[19-21]</sup> and the technique of molecular biology such as PCR<sup>[22,23]</sup> and molecular hybridization have been considered practical to detect viral infection. But immunoassays present with a problem that antibody assays are insufficient for diagnostic purposes because of the period of diagnostic window and patients with hypimmunity. In viral diagnosis, common nucleic acid hybridization might have a good specificity, but the sensitivity is not so satisfactory. In the protocol of PCR, cross contamination, false negative and false positive, often occur and simply producing DNA is not the sufficient evidence that one has amplified the right product. The DNA microarray offers a potential solution to these problems and provides us with a simple and sensitive method to diagnose the infection of hepatitis virus.<sup>[24-26]</sup> Compared with traditional diagnostic techniques, the method has advantages of integration, micromation, and autoimmunization.<sup>[27]</sup> Generally, one could draw on this resource when needed to clinically diagnose different hepatitis viruses subtypes and the strain of drug resistance simultaneously on a microarray slide and the captured DNA could be assayed in a single reaction. The DNA gene chip could also offer a dependable basis for the clinical laboratory diagnosis, treatment, monitoring response to therapy, as well as the occurrence, development and prognosis of hepatotropic viral infections.<sup>[28-30]</sup>

The clinical diagnostic microarray was prepared by immobilizing the capture target genes of pathogens on a slide,<sup>[31]</sup> which was treated specifically. The DNA or RNA, which was extracted from the patient's serum, was labeled with fluorochrome and was hybridized to the target DNA. In this study, the microarray was prepared by spotting PCR products of the conserved sequences of HBV and HDV onto the surface of glass slides by the Cartesian 5500 MicroArrayer. The controls were immobilized on it at the same time. The control system was composed of three parts: (i) empty controls were DM-SO without any gene fragments; (ii) negative controls were gene fragments of plants (rice), eukaryocyte (K562 cell) and prokaryocyte (*E. coli*) which were not homologous with HBV and HDV; and (iii) positive controls were the reconstructive gene fragments which added to the samples while fluorescent labeling. Ten of 12×12 microarrays could be immobilized on a glass slide simultaneously. The chip we had prepared was cost-effective, and the operation of the experiment was simple and more convenient. When the results were verified by

molecular hybridization, we could overcome the cross contamination of PCR. With negative control and positive control, we could ensure the results in detecting each specimen. The subjective factors in judging the results could be reduced greatly by the analytic computer software.<sup>[32]</sup> Also, the sensitivity of the assay can be enhanced by increasing the amount of capture DNA on the slide or by pretreatment of the sample DNA.

There are two ways for preparing DNA microarray probes. The first one is PCR amplification of the DNA fragments with a molecular clone, and the other is artificial synthesis of oligonucleotide arrays by a DNA synthesizing machine. We prefer to the first method, making use of Primer Premier 5.00 to design special primers of HDV. Because of the convenience of PCR, we could directly prepare DNA microarray probes after purification of the PCR product. As for the small genome of HDV (1.7 kb), the method could be a practical one as it is speedy, simple and valuable in clinical application.

Using their creative invention, the technique of restriction display-PCR (RD-PCR),<sup>[33,34]</sup> Professors Ma and Zheng prepared microarray probes on a massive scale, which were nearly the same length, printed them on the chip, and developed clinical diagnostic gene chips. The application of this method for preparing gene chip probes is used in our laboratory at present.<sup>[35-37]</sup> Further application of the technique in labeling the sample could also bring about good results and speed up the multi-virus detection by microarray technology.<sup>[38-40]</sup> By RD amplification of the enzyme-digested samples, the sample fragments can be significantly labeled with fluorescent signals, and can produce a much stronger hybridization than the conventional labeling methods.

## Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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Received May 12, 2004

Accepted after revision June 26, 2004