Clone Q379 Immunoscreened from a Chinese HCV cDNA λgt11 Library

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Abstract. A Chinese HCV cDNA clone, designated as Q379, 379 nucleotides in length, was selected by recombinant immunoscreening from a random-primed Chinese HCV λgt11 library. DNA sequencing demonstrated that Q379 situated from positions 7314 to 7695 at NS5 region corresponded with the American prototype HCV nucleotide sequence. The homologies of Q379 with the equivalent sequences of the prototype were found to be 72.8% in nucleotides and 73.8% in amino acids, respectively. Hydrophobicity profile shows that Q379-encoded polypeptide (126 amino acid residues) contains two hydrophilic areas. This Chinese HCV clone would be valuable in basic and clinical studies of Chinese HCV infection.

HCV genomic RNA was extracted from plasma specimens of Chinese hepatitis C patients, randomly reverse-transcribed to HCV cDNA, and an HCV cDNA λgt11 library of Chinese type was constructed successfully. A positive clone 379 bp in length was selected from the library by immunoscreening and designated as Q379. The sequence analyses demonstrated that the sequence was an HCV cDNA fragment located in a nonstructural (NS5) region of the prototype, and there was no homology with known viral genomes and human DNA sequences.

Materials and Methods

Viral specimens. Plasmas came from hepatitis C patients with no HAV and HBV markers. ALT elevated and anti-HCV positive were employed for the extraction of HCV genomic RNA. Anti-HCV EIA test kits from Ortho Diagnostic System (USA), Kuraray Co. (Japan), and the Long March Co. (China) were used in this study as reagents for anti-HCV detection.

HCV RNA preparation and cDNA synthesis. HCV RNA was extracted with PEG4000-guanidine thiocyanate from viral specimens as described [4, 5]. The first strand of cDNA was synthesized with random primers in the presence of purified HCV RNA template and MMLV reverse transcriptase, followed by adding RNase H and Escherichia coli DNA polymerase to form double-stranded cDNA. T4 DNA polymerase was used to repair the termini of the synthetic cDNA fragments after the treatment with EcoRI methylase.

Construction of HCV cDNA λgt11 gene library [1, 5]. EcoRI adaptors were added to both ends of the termini-repaired HCV cDNA, which were then digested with EcoRI and purified by size fractionation. The purified adapted cDNA was ligated with λgt11 vector arms (in the downstream of Lac Z promoter), and the in vitro packaging was performed to create infectious phage particles (λgt11). Escherichia coli Y1090 cells were infected with the packaged λgt11 and plated out in agarose plates at 43°C for 4 h to produce bacterial plaques. In order to induce the expression of the inserted HCV cDNA, the agarose plates were transferred from 43°C to 37°C and incubated for an additional 3 h in the presence of isopropyl-β-d-thiogalactopyranoside (IPTG).

Immunoscreening and specificity test for HCV cDNA library [1, 7]. The products expressed by λgt11 plaques were transferred from the plates onto nitrocellulose membranes, which were then reacted with hepatitis C sera and goat anti-human IgG conjugate. The positive plaques were picked after color development of the membranes. Rescreening was carried out to obtain the pure positive plaques. The specificity test for the positive plaques-expressed polypeptides was conducted by the reaction of the polypeptides with normal or hepatitis C sera on nitrocellulose membranes.

Amplification and sequencing of positive cDNA clone. Phage DNA was isolated from the lysates, and the insertions were amplified by PCR with the λgt11 oligonucleotide primers, which were located in both sides of the EcoRI site of λgt11 Lac Z gene. The amplified cDNA fragments were isolated from 3% GTG agarose, blunted with Klenow fragment, and then inserted into HindIII site of vector pUC18 DNA. The dideoxynucleotide chain termination method with fluorescent automatic DNA sequencer (Genesis Analysis System 2000, Du Pont, USA) was used for the determination of the nucleotide sequences of the recombinant plasmid DNA.

Results and Discussions

A positive plaque was picked out from about 1.05 x 10^6 phage plaques by immunoscreening. The specific-
ity test demonstrated that the expressed product of the plaque was able to bind specifically with the hepatitis C sera only and had no cross-reaction with the serum samples from normal individuals. Moreover, the product showed a higher positive reaction rate (91.7%) with Chinese hepatitis C patients' sera than with equivalent samples collected from Japan. The findings strongly suggest that this positive plaque not only has a significant correlation to HCV infection, but is more specific for Chinese patients.

The insertions in kg11 DNA were amplified by PCR and subcloned into plasmid vector pUC18, followed by endonuclease digestions and DNA sequencing. The results confirmed that the cloned positive sequence was an HCV cDNA clone from Chinese HCV genome 379 nucleotides in length (Q379), and positions at 7314-7695 NS5 region corresponded with the American prototype HCV nucleotide sequence [2]. The homologies of Q379 with Japanese isolates HCV-J [3] and BK [8] were 89.8% and 91.6% at the nucleotide level, 96.6% and 96.0% at the amino acid level, respectively. In a comparison of Q379 with the equivalent sequences of the HCV prototype, they shared 72.8% homologies of nucleotide sequences, and 73.8% homologies of amino acid sequences (Fig. 1). Based on the typing standard of Houghton et al., Q379 along with HCV-J and HCV-BK should belong to HCV-II group [6].

In addition, the comparison of sequence mutant frequency of Q379 demonstrated a much higher frequency of mutation at the third position of each codon with Japanese isolates HCV-J (81.6%) and HCV-BK (80.6%) than that with American isolate HCV-1 (58.6%). The nucleotide mutation at codon

![Fig. 1. Comparison of nucleotide and amino acid sequences of Q379 with the equivalent sequences of American and Japanese HCV isolates.](image-url)