Molecular cloning of a defective hepatitis C virus genome from the ascitic fluid of a patient with hepatocellular carcinoma

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A defective hepatitis C virus (HCV) genome in the ascitic fluid of a patient with hepatocellular carcinoma was cloned and sequenced up to the 3′ poly(U) stretch. When compared with the published Taiwanese HCV sequence, this defective genome contained deletions of single nucleotides at eight sites, double nucleotides at two sites, triple nucleotides at four sites, quadruple nucleotides at one site and replacement of a short stretch of sequence at one site. For comparison, the corresponding regions containing these mutations were also cloned from a serum sample from this patient. Except for deletions of two triple nucleotides in the hypervariable region, the reading frames of all serum-derived clones were intact. The defective HCV genome encoded a truncated core protein with 90 amino acid residues (the last 20 amino acid residues came from a different reading frame), whereas the serum-derived genome encoded a full-length core protein. When expressed in Huh-7 cells, these two proteins were localized to the nucleus and cytoplasm, respectively. Using specific primer-sets, ascites- and serum-derived genomes were each detected alone in ascitic fluid and serum samples, respectively, whereas both sequences were present in ascitic mononuclear cells. The defective sequence thus constituted the major virus population in the ascitic fluid whereas a putative helper genome coexisted with it inside the ascitic mononuclear cells. This sequence is possibly a defective and interfering genome.

Introduction

Hepatitis C virus (HCV) is a major cause of non-A, non-B hepatitis worldwide. In a majority of patients, the virus causes chronic hepatitis which may lead to complications such as cirrhosis and hepatocellular carcinoma (Houghton, 1996). Since molecular cloning of HCV was achieved in 1989, several complete or nearly complete genomic sequences have been published (Chen et al., 1992; Choo et al., 1989, 1991; Hayashi et al., 1993; Inchauspe et al., 1991; Kato et al., 1990; Ogata et al., 1991; Okamoto et al., 1991, 1992a; Takamizawa et al., 1991). Comparison of the nucleotide and polyprotein amino acid sequences of these isolates revealed considerable heterogeneity, leading to the classification of multiple genotypes (Bukh et al., 1995; Okamoto et al., 1992b; Simmonds et al., 1993). The genomic RNA of HCV is about 9-4 kb in length. With the help of an internal ribosome entry site, a polyprotein of about 3000 amino acid residues is expressed, which is proteolytically processed to produce several structural and nonstructural proteins (Ali & Siddiqui, 1995; Shimotohno et al., 1995; Wang & Siddiqui, 1995). The long open reading frame is followed by a poly(U) homopolymeric tract, except for HCV-1, which contains a poly(A) tract (Han et al., 1991). Recently, a highly conserved sequence-element following the 3′-terminal poly(U) tract was identified (Kolykhalov et al., 1996; Tanaka et al., 1995). This sequence is predicted to form a stable stem–loop structure and is believed to be required for authentic HCV replication.

When HCV sequences were cloned from serum, defective sequences were occasionally identified but in a very small proportion of the virus population (Martell et al., 1992). Interestingly, the nucleotide sequence of the core region from the tissue of a hepatocellular carcinoma revealed deletions and mutations resulting in truncated proteins (Rüster et al., 1996). When the hypervariable regions of HCV sequences from
ascites and serum samples were compared, we also discovered a defective sequence which constituted the major virus population in the ascitic fluid of a patient with hepatocellular carcinoma (Yeh et al., 1996). In this case, all 14 clones obtained from the ascitic fluid contained a frameshift mutation, whereas all 10 clones from a serum sample of the same patient did not. This finding prompted us to clone the complete defective sequence to understand its genetic structure. Selective regions of HCV RNA from the serum sample were also cloned and sequenced in parallel for comparison.

Methods

■ Patient. A male patient, 58 years of age, was diagnosed as having hepatocellular carcinoma by liver biopsy and received surgical resection in December 1993. Unfortunately, recurrence of liver tumours with massive ascites was found in October 1994. Paraenesis was performed for diagnostic purposes and also to relieve symptoms. A serum sample was collected simultaneously. All samples were kept at —70 °C until use. The serum was negative for hepatitis B virus surface antigen (HBsAg; Ausria-II, Abbott) but positive for anti-hepatitis C virus antibody (anti-HCV; UBI HCV EIA, United Biomedica).

■ RT nested PCR (RT–PCR), cloning and sequencing. The ascites and serum samples were first centrifuged to remove all cellular components before analysis. RT–PCR was performed according to the procedure described previously (Yeh et al., 1994b). Briefly, serum or ascites samples (100 µl) were mixed with 600 µl extraction buffer (4 M guanidinium thiocyanate, 2% Sarkosyl, 0.5 M mercaptoethanol, 5 µg tRNA, 5 mM sodium acetate, pH 6). After vortexing, the nucleic acid was extracted with phenol–chloroform and then precipitated with an equal volume of isopropyl alcohol. The pellet was dried under vacuum. For RT, 10 U RNasin (HT Biotechnology) and 30 pmol RT primer were added to the pellet together with 10-5 µl water. The mixture was warmed to 70 °C for 5 min. One µl 5 mM deoxyribonucleoside triphosphate (dNTP), 10 U RNasin, 20 µg BSA and 1 µl Moloney murine leukaemia virus reverse transcriptase (20 U/µl) (Gibco BRL) were then added together with the provided reaction buffer to a final volume of 20 µl. The mixture was brought to room temperature for 15 min, and then the reaction was carried out at 37 °C for 90 min. After RT, PCR was done in two steps with two sets of primers. The reaction was carried out with 10 µl cDNA mixture, 200 µM dNTP, 50 pmol of each primer, 2 U Super Tag (HT Biotechnology) and the provided PCR reaction buffer in a final volume of 100 µl. A DNA thermal cycler (Perkin-Elmer Cetus) was used with a step cycle programme of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 2 min for 25 cycles. For secondary PCR, 5 µl from the first PCR product was used as the source of DNA template. The primers used for RT and nested PCR were deduced from the published Taiwanese HCV sequence and are available from the authors on request. All samples were kept at —70 °C until use.

■ Construction of DNA plasmids encoding truncated and full-length core proteins. To connect the two clones CG1 and C-fs (Fig. 1), 20 ng gel-purified DNA fragments from the two corresponding RT–PCR reactions were mixed together in 200 µM dNTP, 2 U Super Tag and the provided PCR reaction buffer in a final volume of 100 µl. After the reaction had been performed in a DNA thermal cycler with a step cycle programme of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 60 s for 10 cycles, 50 pmol of primer 642 (5’ TGATCATGTCGGCTGCTCA 3’) and primer B (5’ CACCATGACACAAATCTCAACC 3’) were added and the reaction was performed for a further 25 cycles. The resulting product was cloned into pT7Blue T-Vector. After verification by dideoxy sequencing, the fragment containing the core reading frame was isolated by restriction enzyme digestion (HindIII–Smal, from the polynucleotide sites of pT7Blue T-Vector) and inserted into HindIII–Xhol (blunted) polynucleotide sites of the vector pRC/CMV (Invitrogen). Clones derived from both the serum and ascites samples were connected and inserted into pRC/CMV to generate pCMV-SC and pCMV-AC, respectively. A plasmid, pCMV-RCEβ (a gift from J. Ou, USC, Los Angeles, CA), containing HCV-RH isolate from nucleotide 1 to 1292 (aa 1–430) (Lo et al., 1995), was also included in this study for comparison.

■ DNA transfection and immunofluorescence analysis. Hu-h cells were maintained in a 1:1 mixture of Dulbecco’s modified essential medium and F12 medium supplemented with 10% FBS. DNA plasmids were transfected by using the standard CaPO4 precipitation method (Graham & van der Ed, 1973). Cells were glycerol-shocked for 2 min at 4–6 h after transfection. For immunofluorescence analysis, cells were grown on cover slips (Yeh et al., 1990). At 48 h post-transfection, the cells were fixed in acetone at —20 °C for 2 min. Mouse monoclonal antibody to HCV core fragment (aa 1–120; Biogenex) (1:500 dilution) and FITC-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories) (1:20 dilution) were used as the primary and secondary antibodies, respectively.

■ Specific detection of serum- or ascites-derived clones. To specifically detect either the serum- or ascites-derived clones in samples, two sets of primers were designed according to the hypervariable regions: specific primers for serum-derived clones, 5’ ATGTAGTGCC- CGTGGCCTACGTGTTT 3’ (sense; nt 1171 to 1198 (positions corresponding to the published Taiwanese strain); see also Fig. 3) and 5’ AAGACAGCAGCAATACATGA 3’ (anti-sense; nt 1438 to 1418); specific primers for ascites-derived clones, 5’ GCAGACGACATAC- CACCGCCGGCTCA 3’ (sense; nt 1171 to 1198) and 5’ GGGGCAT- CGTGCAGCTAGT 3’ (anti-sense; nt 1438 to 1418). These two sets of primers had the same sequences as those of the serum and ascites-derived clones but had 20 and 10 nucleotide mismatches between each other for the sense and anti-sense primers, respectively. These two sets of primers were used in the second step PCR for specific detection, while the primers for RT and first step PCR were the same as those for cloning HVR-fs (available from the authors on request). The step cycle programme of the first step PCR was modified to 94 °C for 30 s, 58 °C for 30 s and 72 °C for 22 cycles, and the second step PCR to 94 °C for 30 s, 62 °C for 30 s and 72 °C for 30 s for 20 cycles.
To the determination of this PCR experiment, HVR-fs clones from both ascitic fluid and serum samples were used as templates for in vitro transcription using MEGAscript T7 Kit (Ambion) to produce fragments of RNA. These RNA fragments were diluted serially to obtain concentrations of $10^6$–$10^7$ copies/ml.

To confirm its specificity, the PCR product was further analysed by direct sequencing. The gel-purified DNA fragment was analysed using SequiTherm Cycle Sequencing kits (Epicentre Technologies). The procedure was performed according to the manual provided by the manufacturer and [$\alpha$-32P]dATP was used for radiolabelling. The primer used for direct sequencing was from either one of the second step PCR primers.

**Minus-strand RT–PCR.** The experiment was performed according to a previously described procedure with minor modifications (Yeh et al., 1996). Briefly, ascitic fluid was centrifuged at 3000 × g for 10 min. The pellet (containing mononuclear cells) was washed once with TBS (10 mM Tris–HCl pH 7.2, 150 mM NaCl) and dispersed in 250 µl water before being submitted to RT–PCR. The outer primers used in minus-strand RT–PCR were 5’ CCGACAGCAGAAGCTACTGTC 3’ (sense; nt 92 to 279) and 5’ CGGGATCGAATTCACCCCGG 3’ (anti-sense; nt 279 to 529) and 5’ TTTCACGCAGAAAGCGTCTAG 3’ (sense; nt 299 to 529) and 5’ CGGCAACAGGTAAACTCCAC 3’ (anti-sense; nt 114 to 95). The inner primers were 5’ CCCGTGAGGAAGTACTGTGTC 3’ (sense; nt 299 to 279) and 5’ CGGCAACAGGTAAACTCCAC 3’ (anti-sense; nt 114 to 95). The primer used for direct sequencing was from either one of the second step PCR primers.

Results

Cloning of a defective ascitic HCV genome up to the poly(U) stretch by RT–PCR

We cloned the HCV genome from the ascitic fluid of a patient with hepatocellular carcinoma (patient-HCC) by the procedure of RT followed by nested PCR (Fig. 1). RT–PCR using the primers deduced from the published Taiwanese clone was quite efficient. Fragments as long as 1.2 kb (CG10) were amplified but attempts to produce longer fragments failed. Ten to sixteen clones were sequenced for each fragment. For each set of clones derived from fragments CG4, NS2-fs, CG7, CG10, CG11 and CG12, one to two clones were different from the others in the same set and contained substitutions of up to four nucleotides. For HVR-fs, the region with the most diverse sequences, sequence analysis revealed five different kinds of clones with eight, three, one, and one clones (of the 16 analysed) of each kind. However, there were only zero to six nucleotide differences among them. For the remaining fragments, all clones derived from the same fragment were identical and no sequence variation was observed.

The HCV genome contained 9400 nucleotides plus a poly(U) stretch (Fig. 2). When compared with the previously published Taiwanese strain (Chen et al., 1992), several nucleotide deletions were identified. The 5’ noncoding region was very conserved with only two nucleotide substitutions (nt −222 and −1) and one single nucleotide deletion (between nt −171 and −170). Strikingly, in the coding region, there were five sites of deletions which were capable of disrupting the reading frame (marked in Fig. 2; see also Fig. 3): a single nucleotide deletion in C-fs, HVR-fs and NS3-fs, deletion of a nucleotide duplet in NS2-fs and deletion of a nucleotide quadruplet in NS3-fs. These frameshift mutations were observed in all of the 10 C-fs, 16 HVR-fs, 12 NS2-fs and 11 NS3-fs sequenced clones. Unusual mutations were also found in clone NS3-fs, in which five nucleotides (nt 3401 to 3405 in the published Taiwanese strain) were replaced by a short stretch of sequence containing 13 nucleotides (Fig. 3).

If these frameshift mutations were ignored, there were still six sites at which mutations led to amino acid deletions (Table 1). At four of them (NS3-fs, CG1, CG11 and CG13), nucleotide triplets were deleted, whereas at the other two (CG10 and CG12), separate but nearby nucleotide deletions resulted in both amino acid deletion and missense mutations.

Cloning of HCV RNA fragments from a serum sample of the same patient

Using the same cloning and sequencing strategy, we selectively cloned the corresponding fragments containing important deletions from the serum sample of patient-HCC. In total, seven, seven, eleven, six, eight, seven, seven, eight and six clones were analysed for C-fs, CG1, HVR-fs, NS2-fs, NS3-fs, CG10, CG11, CG12 and CG13, respectively. Except for HVR-fs, only one to five nucleotide substitutions were found among clones derived from the same fragment. Surprisingly, none of the frameshift mutations detected in the ascitic clones were found in the serum-derived clones (Fig. 3). In the 5’ noncoding region, the sequence was identical to that of the ascitic clone except for the absence of the deletion between nt −171 and −170 of the ascitic clone. In C-fs, all deletions, including the frameshift mutation, were absent in serum-derived clones. In HVR-fs, which contained the hypervariable region, 10
Fig. 2. For legend see facing page.
different sequences were observed in the serum-derived clones with up to 16 nucleotide substitutions among clones. However, only two kinds of predicted amino acid sequences were present and most of the nucleotide substitutions did not result in heterogeneity at the amino acid level. Two single amino acid deletions (nt 1183 to 1185 and 1220 to 1222) were observed for all clones but no frameshift mutation was observed. Only the most prevalent clone for HVR-fs is shown in Fig. 3. For NS2-fs and NS3-fs, both deletions leading to frameshift mutations and the unusual mutations at nt 3401 to 3405 (replacement of CHGF...
oligonucleotides) were absent. No mutation leading to amino acid deletions was found. For NS5 regions (CG10, CG11, CG12 and CG13), none of the four amino acid deletions (Table 1) was present in the serum-derived clones. Instead, the predicted amino acid residues in these four sites were the same as those in the Taiwanese strain.

The defective HCV genome encodes a truncated nuclear core protein

The frameshift mutation in C-fs resulted in early termination of translation of the genome such that the only protein product would be a truncated core protein of 90 amino acid residues (Fig. 2). The last 20 amino acid residues came from a different reading frame and were not related to the original HCV core protein. This protein contained all three putative nuclear localization signals (Ravaggi et al., 1994). When we expressed it in Huh-7 cells, the protein was indeed localized to the nucleus (Fig. 4). In contrast, the serum-derived intact core protein was localized to the cytoplasm.

Specific detection of ascites- or serum-derived clones in ascitic fluid, serum and ascitic mononuclear cells

To understand whether HCV replicated in the ascitic mononuclear cells of this patient, minus-strand-specific RT–PCR was performed. Minus strand HCV RNA was only detected when ascitic mononuclear cells were collected from
Table 1. Summary of amino acid 'deletions' in the ascitic HCV clone

Since there were frameshift mutations, the reading frame was in fact interrupted before these amino acid residues could be translated. Therefore, these 'deletions' exist only when the frameshift mutations are ignored.

<table>
<thead>
<tr>
<th>Region (clone)</th>
<th>Nucleotide no.*</th>
<th>Amino acid no.*</th>
<th>Sequence change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core (CG1)</td>
<td>433–435</td>
<td>145</td>
<td>GGG (G) → del.</td>
</tr>
<tr>
<td>NS3 (NS3-fs)</td>
<td>3721–3723</td>
<td>1241</td>
<td>CCG (P) → del.</td>
</tr>
<tr>
<td>NS5 (CG10)</td>
<td>6802–6825</td>
<td>2268–2275</td>
<td>GTATCCGTTCGGCCGGAGATCTGTGTCGTCCGGCGGAGATCTG (VSVPAEIL) → (VSSGGDL)</td>
</tr>
<tr>
<td>NS5 (CG11)</td>
<td>7903–7908</td>
<td>2635–2636</td>
<td>GGTTC (GF) → GTC (V)</td>
</tr>
<tr>
<td>NS5 (CG12)</td>
<td>8170–8184</td>
<td>2724–2728</td>
<td>GCTGGAAGCTCCAG (AAKLQ) → GCTGGAAGCCAG (AGSQ)</td>
</tr>
<tr>
<td>NS5 (CG13)</td>
<td>8821–8823</td>
<td>2942</td>
<td>GGC (G) → del.</td>
</tr>
</tbody>
</table>

* Numbered according to the Taiwanese HCV strain. The nucleotide numbers in the ascitic clone (as in Fig. 2) are also given in parentheses for clones CG10, CG11 and CG12.
† Predicted amino acid residues are shown in parentheses after the nucleotide sequence and are underlined. TW strain, Taiwanese HCV strain; AS clone, defective ascitic genome; del., deleted.

10 ml ascitic fluid but was not detectable when only 1 ml ascitic fluid was used (Fig. 5a, lanes A1 and A2). Ascites from patients with hepatitis B-related liver cirrhosis with similar amounts of mononuclear cells, and serum samples with high HCV RNA levels were included as controls (Fig. 5a, lanes C1, C2 and S1–4). The HCV RNA concentrations for S1, S2, S3 and S4, determined by QUANTIPLEX HCV RNA 2.0 assay, were 13±5, 120, 200 and 30 MEq/ml, respectively. The HCV RNA concentration in cell-free ascitic fluid was 2±6 MEq/ml, whereas for 10^6 mononuclear cells (collected from 10 ml ascitic fluid) dissolved in 250 µl water it was < 0.2 MEq/ml. This was the mixture submitted to minus-strand-specific RT–PCR for sample A2.

Specific primer-sets were designed for second step PCR to specifically detect ascites- or serum-derived clones in the samples using more stringent conditions (see Methods). Using
in vitro-transcribed HCV RNA with known concentrations as standards, the sensitivity of this PCR reaction was found to be $10^4$ copies/ml and this method could specifically detect one species of HCV RNA in the presence of up to $10^6$ copies/ml of the other species. Using this method, only the ascites-derived clone was detected in the ascitic fluid (cell-free fraction) and only the serum-derived clone was found in the serum sample (Fig. 5b). In contrast, both clones were detected in the ascitic mononuclear cells. Our PCR conditions, however, cannot detect minimal amounts of HCV RNA ($<10^4$ copies/ml). Each band was also isolated from the gel and analysed by direct sequencing to confirm the specificity of the product.

**Discussion**

Hepatitis C virus is capable of replication in extrahepatic sites (Lerat et al., 1996; Muller et al., 1993; Ounanian et al., 1995; Wang et al., 1992; Willems et al., 1994). Although minus-strand-specific RT–PCR has frequently been used to demonstrate HCV replication, false positivity of this assay has always been a concern. Another way to demonstrate independent HCV replication in different sites is to compare the composition of quasispecies between these sites. If the compositions of quasispecies were markedly different, it would be highly likely that HCV replicated independently in these locations. In our previous study, we found that different HCV quasispecies were present in ascites and serum samples in two cases (Yeh et al., 1996). Further study revealed that such quasispecies existed in four of eight cases with late stage chronic hepatitis C (data not shown). In a recent report, it was found that the composition of HCV quasispecies was different between tumour and non-tumour tissues from liver and serum, but was similar between serum and peripheral blood mononuclear cells (Saito et al., 1996). Presumably, HCV is capable of replication in a relatively isolated environment (in our case, the peritoneal cavity) and thus quasispecies distinguishable from those in the serum sample developed. The cells permissive for HCV replication in the peritoneal cavity are presumably the ascitic mononuclear cells, as has been shown in one of the two reported cases (Yeh et al., 1996). For the case in this study, minus strand HCV RNA could only be detected in mononuclear cells collected from 10 ml and not 1 ml ascitic fluid, indicating a very low level of minus strand HCV RNA (Fig. 5). The amounts of plus strand HCV RNA in the ascitic fluid and serum samples were 2.6 and 13.5 MEq/ml, respectively, when determined by QUANTIPLEX HCV RNA 2.0 assay. The amount of HCV RNA in the ascitic mononuclear cells was difficult to assess. However, when 10^6 mononuclear cells were lysed in 250 μl water, the resulting HCV RNA concentration was <0.2 MEq/ml. Since minus strand HCV RNA was detected only in this sample, it is unlikely to be a false positive result.

According to the sequence of the non-coding region, the ascites-, serum-derived and the reported Taiwanese clones were all genotype 1b (Simmonds et al., 1993). The sequence homology within the same genotype is usually above 90% if the hypervariable region is excluded (Okamoto et al., 1992a). When clones C-fs, NS2-fs and NS3-fs were used for comparison, the sequence homology was 98% between the ascites- and serum-derived clones but 88% between the ascites-derived and reported Taiwanese clones, implicating the same origin of the ascitic and serum clones. In this view, the infected ascitic mononuclear cells possibly originated from the peripheral blood mononuclear cells. After migrating into the peritoneal cavity, a relatively isolated environment, HCV continues to replicate and develop a distinct sequence. We did not collect the PBMC at the same time as the ascites and serum cells.
samples were obtained. However, PBMC collected 3 months later were available and only serum-derived clones were detected by the specific primer-sets method. This result was consistent with the previous report (Saito et al., 1996). However, when ascitic mononuclear cells were examined, both serum- and ascites-derived clones were present (>10^4 copies/ml). Judging from the relative densities of PCR products, the ascites-derived clone was the minor species and the serum-derived clone was the predominant one (Fig. 5b). The ascitic clone possibly developed after migration of mononuclear cells to the peritoneal cavity.

A striking finding was that there are deletions leading to frameshifts in the ascitic clone and the open reading frame is terminated shortly after the first frameshift mutation. As a result, a truncated core protein is produced (90 aa). These frameshift mutations appeared in every clone from the same PCR fragments, indicating that they were the major species of the virus population. A major concern was whether these mutations resulted from PCR, cloning or sequencing artifacts. Several factors argued against this. Firstly, all sequence data were obtained from sequencing of both strands. Secondly, many deletions were in triplets and represented single amino acid deletions on the same putative polyprotein reading frame (except for the one in CG11). Finally, using the same cloning and sequencing strategy, these deletions were absent in the serum-derived clones. Therefore, this ascitic HCV genome not only constituted the major virus population in the ascitic fluid but was also defective. Since both ascites- and serum-derived clones were present in the ascitic mononuclear cells but only the defective ascitic clone was present in the ascitic fluid (after removal of the cellular components), the serum-derived clone in the ascitic mononuclear cells probably served as a helper sequence which enabled the encapsidation and secretion of the defective genome. Conversely, the maturation of helper virus itself was interfered with and the helper sequence failed to exit the mononuclear cells. At present, the molecular mechanism for selective secretion of a defective genome is not clear. It is possible that the area responsible for encapsidation in the defective genome (encapsidation signal) is not occupied by the translation machinery and is more readily accessed by HCV core protein. Alternatively, a clonal origin of cells may preferentially allow replication of the defective genome because of the presence of certain cellular factors.

Although we have not analysed the virus sequences in the hepatocellular carcinoma tissue in this study, another group has performed such experiments (Rüster et al., 1996) and discovered a mixed population of truncated core proteins in the tumour tissue. Although nuclear localization of full-length core protein is still unresolved, a truncated or processed core protein which contained the putative nuclear localization signals was localized to the nuclei (Ravaggi et al., 1994; Lo et al., 1995). It is likely that the carboxyl portion of HCV core protein, which contains a hydrophobic domain, is conformationally interfering with the function of nuclear localization signals. On the other hand, a recent study indicated that HCV core protein cooperated with ras to transform primary rat embryo fibroblasts, but the molecular mechanism was not understood (Ray et al., 1996). We speculate that nuclear transport of HCV core protein plays a role in hepatocarcinogenesis and a truncated core protein accumulates more efficiently in the nuclei and facilitates its action.

In summary, we have identified a defective HCV genome which constituted the major virus population in the ascitic fluid from a patient with hepatocellular carcinoma. In contrast, a non-defective HCV genome existed in the patient’s serum and both clones were present in the ascitic mononuclear cells. This finding raised the possibility that the ascitic clone was a defective interfering HCV genome. With the aid of a helper sequence in the ascitic mononuclear cells, it could replicate and was secreted into the ascitic fluid.

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References


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