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Short Communication

**TRANS-SUPPRESSION OF GENE EXPRESSION BY HEPATITIS C
VIRAL CORE PROTEIN**

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SUMMARY: We have demonstrated that the truncated hepatitis C (HCV) core protein with its C-terminal hydrophobic domains deleted is translocated to the nucleus of transfected cells (22). In this study, intact and truncated core proteins of HCV were transiently expressed in a human hepatoblastoma cell line, HepG2, and their effects on the expression of the chloramphenicol acetyl transferase (CAT) gene driven by viral and cellular promoters were examined. The intact core protein of 22 kDa which is localized in the cytoplasm of the transfected cells suppressed the expression in all of the promoters tested. They were promoters of the SV40 early region, the *c-fos* oncogene, the retinoblastoma susceptibility gene, the β -interferon gene and the β -actin gene. In contrast, the truncated HCV core protein located in the nucleus did not show such a suppressive activity. The HCV core protein appears to function not only as a viral structural protein but as a regulator of gene expression and it might act as a suppressive factor for the cellular gene expression.

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Hepatitis C virus (HCV) is the major causative agent of post transfusion and sporadic non-A, non-B hepatitis (1-3). It is clinically well known that HCV infection persists for a long time and eventually leads to hepatic cirrhosis and hepatocellular carcinoma (HCC) (4,5). Although the mechanism is unknown, the HCV genome seems to persist in hepatic cells for decades and its gene products may play roles in the hepatocellular carcinogenesis. In Japan, deaths from HCC have been ominously increasing in number in the past two decades (6). At present, over 80% of HCC cases are considered to be related to HCV infection (5). The HCV genome is detectable in liver tissues of HCC (7,8). However, the integration of the viral genome is not found in HCV-infected HCC cells by Southern blotting analysis (7). The mechanism of the close relationship of HCV infection to the development of HCC is not known at all.

HCV contains a positive-stranded RNA genome of about 9.5 kilobases that encodes a large polyprotein precursor of about 3,000 amino acids (9-11). Analysis for its genomic structure revealed that HCV is distantly related to flaviviruses or pestiviruses (12-14). The structural proteins of HCV are considered to be encoded in the 5'-terminal region of the genome; nucleotide about 330 to 1,500 (13,15). Like flaviviruses and pestiviruses, these proteins are cleaved from a large precursor polyprotein by cellular signalase (16). By analogy to these viruses, a non-glycosylated protein of 22 kDa encoded by the N-terminal portion of the open reading frame is considered to be the core protein of HCV (17,18).

HCV is an RNA virus and does not contain the reverse transcriptase. Its life cycle is consequently confined to the cytoplasm (19). However, the core protein of dengue virus, one of flaviviruses, is known to be partially transported into the nucleus (20). We previously found that the truncated HCV core protein was translocated to the nucleus when its cDNA was expressed in insect cells by a recombinant baculovirus or in monkey kidney cells by recombinant plasmids (21,22). Such a truncated core protein of HCV with a smaller size is actually produced at later stage of transfection (Makimura et al., personal communication) or exclusively by a particular HCV isolate, HCV-1 (Makimura et al., personal communication). These lines of research prompted us to investigate to find whether the HCV core protein translocated to the nucleus influences the regulation of the host gene expression.

We first examined to find whether the translocation of the truncated HCV core protein to the nucleus takes place in human liver cells as in COS cells. The HCV core protein was expressed by using an exogenous promoter and HCV cDNA isolated from a healthy HCV carrier (24). The full coding sequence of the

Fig. 1. Construction of the plasmids expressing intact or truncated core protein of HCV. Regions cloned under the $SR\alpha$ promoter of pCDLSR α 296 expression vector are shown. The nucleotide numbers of the cDNA of HCV are from Kato et al (9). C and E indicate coding regions of HCV structural proteins, core and envelope, respectively. The hatched box indicates the highly hydrophobic domain of the core protein (27). Triangles indicate the site of ATG.

core protein [nt318 to 912, (9)] was inserted downstream of the $SR\alpha$ promoter in the pCDLSR α 296 mammalian expression vector (25). This construction was designated as pSR39 in this study (Fig. 1). Similarly we constructed also two other recombinant plasmids with different HCV cDNA inserts. Plasmid pSR37 contains cDNA for the truncated core protein defective in two hydrophobic regions at its C-terminus [nt318 to 699]. Plasmid pSR316 contains the full coding region for core and envelope proteins under the control of $SR\alpha$ promoter [nt318 to 1672]. HepG2 cells were transfected with each plasmid. The cells (2×10^4) cultured on 15-mm coverslips were transfected with 1.5 μ g of each plasmid DNA by the calcium-phosphate coprecipitation method (26). After 48 hr, the cells were fixed with acetone-methanol (1:1) at -20°C and examined for the expression of HCV core protein by indirect immunofluorescence assay (27). The serum of a patient with chronic hepatitis C containing antibody against the core protein was used as the first antibody. The second antibody was a rabbit anti-human immunoglobulin G. As shown in Fig. 2, the core protein expressed by the plasmids pSR39 and

Fig. 2. Indirect immunofluorescence of HepG2 cells producing intact or truncated core protein. The cells cultured on 15-mm coverslips were transfected with 1.5 μ g of DNA of plasmid pSR39 (39), pSR37 (37), or pSR316 (316) and vector pCDLSR α 296 (SR). Arrow-heads indicate the positive fluorescence in the cytoplasm (39 and 316) and in the nucleus (37).

pSR316 which contain the full coding region of the core protein was detected mainly in the cytoplasm. The truncated core protein expressed by the plasmid pSR37 lacking two C-terminal hydrophobic regions, however, was located exclusively in the nucleus. We could not detect the truncated HCV core protein in the cytoplasm. Translocation of the HCV core protein with the hydrophobic C-terminal domain deleted to the nucleus is consistent with the result previously obtained from the experiments using an other mammalian cell line, monkey COS cells, transfected with these recombinant plasmids (21,22). By Western blotting analysis, as expected, the size of the intact core proteins expressed by pSR39 and pSR316 was 22 kDa and the truncated core protein by pSR37 17 kDa (data not shown). Translocation of the truncated HCV core protein was recently confirmed by Shih et al. (28) in another liver cell line HuH-7 cells.

We then examined the effect of the HCV core protein expression on a different gene. We first co-transfected HepG2 cells with each plasmid which expresses the intact or truncated core protein with CAT reporter plasmids containing the SV40 early region promoter. The CAT assay was performed according to the method of Gorman et al (29). Forty-eight hours after the transfection with CAT plasmid, the cells were rinsed twice with PBS and harvested by scraping with a rubber policeman. The cells suspended in 200 μ l of 0.25 M Tris-HCl (pH 7.8) were disrupted by five cycles of freeze-thawing. The cell debris was removed by centrifugation and the lysate was examined for protein content by the method of Bradford (30). A sample of the cell lysate was incubated with 0.2 μ Ci of [14 C]chloramphenicol (Amersham) and 1 mmol of acetyl CoA (Sigma) in 0.25 M Tris-HCl (pH 7.8) at 37 C. The reaction products were extracted with ethylacetate and separated by ascending thin-layer chromatography with chloroform and methanol (95:5). The radioactive materials were located by autoradiography. As shown in Fig. 3, the intact core protein expressed by plasmids pSR39 and pSR316 suppressed the expression of the chloramphenicol acetyl transferase (CAT) gene driven by the SV40 viral oncogene promoter. However, plasmid pSR37 did not show such a suppression. Such *trans*-suppression of intact core protein was observed in the CAT gene expression driven by cellular gene promoters as well. Functions of promoters of the chicken *c-fos* oncogene (31) and the human β -interferon gene (32) were also suppressed by pSR39 and pSR316, but not by pSR37. It was of interest that the CAT gene expression by the promoter of a tumor suppressor gene, retinoblastoma susceptibility gene (33), was suppressed similarly. Even the expression by the promoter of the housekeeping β -actin gene (34) was also suppressed by the intact core protein.

These results suggest that the intact HCV core protein has a general *trans*-suppressive activity against cellular gene expression. This suppressive activity may be on the translation level, because the intact core protein is located only in the cytoplasm. Replication of hepatitis B virus (HBV) was suppressed in another human liver cell line, HuH-7 cells, when it was cotransfected with HCV cDNA. In this case, however, the suppression was considered to be at a transcription level (28). Whether this suppression reported here occurs on the transcription or translation level has to be examined. Analysis of the transcripts may be necessary.

The core protein in the virion binds to viral RNA in the cytoplasm to form a nucleocapsid. If the HCV core protein binds not only to viral but to various cellular mRNAs under certain circumstances, it may down-regulate the translation of

Fig. 3. Effects of intact or truncated HCV core protein on the expression by various promoters. HepG2 cells cultured on 6-cm plates were cotransfected with 6 μ g of indicated core protein expressing plasmid together with 2 μ g of CAT reporter plasmid by the calcium-phosphate coprecipitation method. After 48 hr, the cells were harvested and lysed by five cycles of freeze-thawing. The CAT activities were estimated by use of [14 C]labeled chloramphenicol. The promoters were SV40 early region promoter (SV2), retinoblastoma susceptibility gene promoter (Rb), chicken *c-fos* oncogene promoter (fos), human β -interferon gene promoter (IFN) and chicken β -actin gene promoter (β -Act).

such mRNAs resulting in general suppression of cellular gene expression. Satolli et al. (35) recently demonstrated the RNA-binding activity of the HCV core protein by showing that the N-terminal portion of the HCV core protein binds to HCV- as well as HBV-RNA. The *trans*-suppressive activity of the HCV core protein does not directly explain the development of HCC. However, it is of interest to note that the HCV core protein possibly down-regulates the expression of cellular tumor suppressor genes. This may be one of the multiple triggers of cascades resulting in hepatocarcinogenesis. Another possible biologically significant features of this finding may be that the slow, persistent infection with HCV is relat-

ed to the down-regulation of the general gene expression of HCV-infected cells and autologous viral replication.

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