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Interaction of hepatitis C virus F protein with prefoldin 2 perturbs tubulin cytoskeleton organization

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Abstract

By use of the yeast two-hybrid system, hepatitis C virus (HCV) F protein was found to interact with a cellular protein named prefoldin 2. The interaction was confirmed by confocal immunofluorescence microscopy as well as coimmunoprecipitation experiments. Prefoldin 2 is a subunit of a hexameric molecular chaperone complex, named prefoldin, which delivers nascent actin and tubulin proteins to the eukaryotic cytosolic chaperonin for facilitated folding. Functional prefoldin spontaneously assembles from its six subunits (prefoldin 1–6). In the yeast three-hybrid system, it was found that expression of HCV F protein impeded the interaction between prefoldin 1 and 2. By performing immunofluorescence experiment and non-denaturing gel electrophoresis, it was shown that expression of HCV F protein resulted in aberrant organization of tubulin cytoskeleton. Since HCV replication requires intact microtubule and actin polymerization, HCV F protein may serve as a modulator to prevent high level of HCV replication and thus contributes to viral persistence in chronic HCV infection.

Keywords: Hepatitis C virus; F protein; Tubulin; Cytoskeleton; Prefoldin 2; Yeast two-hybrid system; Aberrant organization; Interaction; Replication; Persistence

Hepatitis C virus (HCV) is the major etiologic agent of blood-transmitted non-A, non-B hepatitis worldwide [1]. Presently, approximately 170 million people are infected. HCV infection results in chronic hepatitis in about 70–80% of infected individuals, a condition that may lead to severe sequels, such as cirrhosis, hepatic failure, and hepatocellular carcinoma [2,3]. HCV belongs to the *Flaviviridae* family. It contains a positive-stranded RNA genome of about 9.6 kb, which encodes a polyprotein of 3011 amino acid residues [4]. Translation of this polyprotein is initiated by an internal ribosome entry site located at the 5′ noncoding region. This polyprotein can be further proteolytically cleaved into several distinct protein fragments named C, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The C protein fragment, also named the core protein, is the major constituent of the viral capsid. This protein contains 191 amino acid residues and has a molecular mass of approximately 21 kDa (P21). Owing to the existence of an alternative cleavage site, an additionally core protein product of 173 amino acid residues (P19) has also been reported [5–7]. Of particular interest is that an additional protein of about 16–17 kDa (P16) was expressed in some isolates [6]. By performing Western blot analysis, differential antibody titers were observed for P16 and P21 in chronic HCV infected patients, leading to the speculation that P16 harbored new antigenicity [8]. Subsequently, the P16 protein (or F protein) was shown to be generated by a +1 translational frameshift by ribosomes, occurring between codons 9 and 11 of the polyprotein [9,10]. This +1 frameshift was presumably attributed to the presence of a stretch of adenines in the core coding region, which was known to promote frameshifting in vitro. However,

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recent evidence indicated that the translation of F protein could also be initiated at a non-AUG codon in a +1 reading frame, whereby the mechanism of ribosomal frameshift needed not be applied [11–13]. Despite the dispute, the existence of specific antibodies against HCV F protein in patients' serum was confirmed by independent studies, suggesting that the protein is indeed being made during chronic HCV infection [8,14].

The F protein was found to associate with the endoplasmic reticulum and was very unstable [15,16]. Its function in HCV life cycle is unclear to date. In this study, we provided evidence showing that F protein interacted with prefoldin 2 (PFD2), a subunit of a hexameric molecular chaperone complex, named prefoldin (PFD). This chaperon complex was built from two related classes of subunits and present in all eukaryotes and archaea [17–19]. PFD interacts with nascent polypeptide chains to stabilize non-native proteins (such as actins and tubulins) for subsequent folding in the central cavity of a chaperonin [20–23]. To do so, PFD binds specifically to cytosolic chaperonin (c-cpn) and transfers target proteins to it [21]. In this report, we discovered that over-expression of HCV F protein interfered with normal function of PFD.

Materials and methods

The yeast two-hybrid system. To identify the interacting protein of HCV F protein, the yeast two-hybrid system was applied (Matchmaker LexA two-hybrid system; Clontech Laboratories, Palo Alto, CA). Primers containing an engineered *Eco*RI or *Xho*I site were first synthesized: P1, 5′-GAATTCAACGTAACACCAACCGCCGCC-3′ (sense) and P2, CTCGAGCTACGCCCCCCAAGGGGGCGC-3′ (antisense). The engineered sites were underlined. P1 and P2 were designed according to the 5′ and 3′ portion of HCV F protein. The coding region of HCV F protein was amplified by RT-PCR from pCMV-RCEβ, which contains the HCV-RH isolate from nucleotides 1–1292 (aa 1–430). The amplified sequence was digested with restriction enzymes and inserted (in-frame) into *Eco*RI–*Xho*I sites of pLexA, a plasmid encoding the 202-residue LexA protein and *HIS3* marker. The plasmid is named pLexA-HCV-F. A cDNA library (Clontech) was used for screening, of which the cDNA inserts was built into pB42AD, a plasmid encoding the 88-residue B42 acidic activator and *TRP1* marker. The plasmids were cotransfected into EGY48[p8op-lacZ] yeast cells, a yeast strain (EGY48) transformed with the autonomously replicating plasmid p8op-lacZ (containing *URA3* marker). This system allows double selection with both leucine prototrophy and lacZ reporter. Cotransformants were selected in SD/-His/-Trp/-Ura medium. The presence of protein–protein interaction was demonstrated by growth of the cotransformants as blue colonies on an SD/Ga./Raf/-His/-Trp/-Ura/-Leu plate containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

Cell line and DNA transfection. The human embryonic kidney 293 cells (HEK293 cells) and Vero cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. DNA transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

Coimmunoprecipitation. The coding region of HCV F protein in pLexA-HCV-F was isolated by restriction enzyme digestion, blunt-ended and inserted into the blunted *Xma*I site of pEGFP-C1 (Clontech) to obtain pEGFP-HCV-F, wherein the coding sequences of HCV F protein and EGFP were in-frame. The coding region of PFD2 was amplified by RT-PCR using normal liver mRNA (purchased from Invitrogen). The primers used for amplification were PF2L, 5′-GAATTCATGGCGGAGAACAG CGGTCG-3′ (sense) and PF2R, 5′-CTCGAGCTAGGAGACCA ACACTCCAGCTGAG-3′ (antisense). The engineered restriction enzyme sites were underlined. The amplified product was digested with restriction enzymes and inserted (in-frame with the V5 epitope) into the *Eco*RI–*Xho*I sites of pcDNA3.1/V5-His (Invitrogen) to obtain pcDNA-PFDN2. The two plasmids, pEGFP-HCV-F and pcDNA-PFDN2, were cotransfected into HEK293 cells for coimmunoprecipitation experiment. Cells were lysed in 1 ml of RIPA (10 mM Tris [pH 7.0], 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) for immunoprecipitation using mouse monoclonal anti-V5 antibody (Invitrogen). EGFP was detected by rabbit polyclonal anti-EGFP antibody (Clontech).

The yeast three-hybrid experiment. Three plasmids were constructed for the yeast three-hybrid experiment. The *Eco*RI–*Xho*I fragment of PFD2 coding sequence was isolated from pcDNA-PFDN2 and inserted (in-frame with LexA) into pLexA to obtain pLexA-PFDN2. The coding sequence of PFD1 was amplified by RT-PCR using normal liver mRNA (Invitrogen). The primers used for amplification were PF1L, 5′-GAATTCGCACGACCCAAGATGGCC-3′ (sense) and PF1R, 5′-CTCGAGCTACTGGGCCCTTCGTGCCATC-3′ (antisense). The engineered restriction enzyme sites were underlined. The amplified product was digested with restriction enzymes and inserted (in-frame with B42) into the *Eco*RI–*Xho*I sites of pB42AD to obtain pB42AD-PFDN1. To construct pYES-NLS-HCV-F, the sequence of the nuclear localization signal of B42 was amplified from pB42AD using primers PNLS-L, 5′-AAGCTTATGGGTGCTCCTCCAAAAAAGAA G-3′ (sense) and PNLS-R, 5′-GAATTCCCCTTGAATCGGCATTGAC TC-3′ (antisense). The product (90 bp) was digested with restriction enzyme before it was used to replace the *Hin*dIII–*Eco*RI fragment in pYES2/NT (Invitrogen), a plasmid contained *URA3* marker. The resulting plasmid, pYES-NLS, encoded the B42 nuclear localization signal, MGAPPKKKRKVAGINKDIEESMPIQG. The coding sequence of HCV F was isolated from pLexA-HCV-F by restriction enzyme digestion and inserted into *Eco*RI–*Xho*I sites of pYES-NLS to obtain pYES-NLS-HCV-F, wherein the B24 nuclear localization signal was in-frame with the HCV F protein. The three plasmids were cotransfected into EGY48 yeast cells and the cotransformants were selected in SD/-His/-Trp/-Ura medium. The presence of interaction between PFD1 and PFD2 was demonstrated by growth of the cotransformants on an SD/Ga./Raf/-His/-Trp/-Ura/-Leu plate.

Immunofluorescence. The coding sequence of HCV F was isolated from pLexA-HCV-F by restriction enzyme digestion and inserted into *Eco*RI–*Xho*I sites of pYES2/NT to obtain pYES-HCV-F. The *Hin*dIII–*Xho*I fragment (containing the Xpress epitope in-frame with the HCV F coding sequence) was isolated from pYES-HCV-F and inserted into pcDNA3.1/V5-His to obtain pcDNA-Xpress-HCV-F. This plasmid was used for immunofluorescence experiments to examine the effect of HCV F expression on α-tubulin. Cells were fixed in cold acetone at −20 °C for 2 min. The Xpress antigen was detected by mouse monoclonal anti-Xpress antibody (Invitrogen) and α-tubulin was detected by mouse monoclonal TU-16 antibody (Abcam, Cambridge, MA). To visualize the nuclei, cells were simultaneously stained with DAPI (200 ng/mL). Confocal microscopy was performed using Leica TCS SP2 Laser Scanning Spectral Confocal System.

Denaturing and non-denaturing gel electrophoresis. To construct pcDNA-HCV-F, the 5′ portion of the HCV core sequence and part of the non-coding sequence was amplified by PCR from a HCV clone. The primers were PC1, 5′-AAGCTTGTGGTACTGCCTGATAGGG-3′ (sense) and PC2, 5′-GAATTCGTCTTTGAGGTTTAGGATTTG-3′ (antisense). The PCR product was digested with *Hin*dIII and *Xba*I before it was used to replace the *Hin*dIII–*Xba*I fragment of pcDNA-Xpress-HCV-F. The resulting plasmid, pcDNA-HCV-F, contained only the coding sequence of the HCV F protein without any other fusion protein. This plasmid was used for the denaturing and non-denaturing electrophoresis experiments to examine the effect of HCV-F expression on α-tubulin polymerization. Non-denaturing gel electrophoresis was performed in the absence of SDS in the gel or running buffer. Transfection efficiency was monitored by cotransfection of pCMVβ (Clontech) into cells (in a ratio of 1/10 in the transfected DNA mixture). pCMVβ directed the expression of β-galactosidase, which was subsequently measured by

Luminescent β-galactosidase Detection Kit II (Clontech) according to the manufacturer's protocol.

Results

Interaction of HCV F protein with PFD2 in the yeast two-hybrid system

Using HCV F protein as bait, the yeast two-hybrid system was used to screen through a human liver cDNA library. Twenty-five positive cotransformants were selected from 5×10^6 clones in the screening process. The plasmids containing the cDNA inserts (in pB42AD vector) were isolated and individual plasmids were cotransfected with pLex-HCV-F into the EGY48[p8op-lacZ] yeast cells again. The protein–protein interaction was verified in 11 clones. Of these 11 clones, 6 were found to be identical and encoded the whole PFD2 sequence in-frame with the B42 peptide.

Verification of the interaction by coimmunoprecipitation experiments

To verify the interaction between HCV F protein and PFD2, two plasmids, pEGFP-HCV-F and pcDNA-PFDN2, were constructed to express the EGFP-HCV-F and PFD2-V5 fusion proteins (Fig. 1A). HEK293 cells were transfected with different combination of these two plasmids and pEGFP-C1 (encoding only EGFP) (Fig. 1B, bottom). The cells were harvested for coimmunoprecipitation and Western blot analysis. The results showed that similar amounts of EGFP-HCV-F and PFD2-V5 were expressed when cells were transfected with pEGFP-HCV-F and pcDNA-PFDN2 either individually (Fig. 1B, lanes 3 and 4) or in combination (Fig. 1B, lane 5). As a control, the pEGFP-C1 vector was also transfected either alone (Fig. 1B, lane 2) or together with pcDNA-PFDN2 (Fig. 1B, lane 6). Coimmunoprecipitation was performed using anti-V5 antibody to pull down the PFD2-V5 fusion protein. Subsequently, the EGFP-HCV-F fusion protein was detected using anti-EGFP antibody. The results showed that the EGFP-HCV-F protein coimmunoprecipitated with the PFD2-V5 protein (Fig. 1B, lane 5).

Verification of the interaction by immunofluorescence analysis

To further verify the interaction between the two proteins, immunofluorescence experiments were conducted in Vero cells, of which the subcellular localization of the proteins could be clearly demonstrated. It was found that although EGFP alone was distributed in both the cytoplasm and nucleus, it accumulated to a higher concentration in the nucleus (Fig. 2A, lower panel). In contrast, the PFD2-V5 protein mostly resided in the cytoplasm, with the appearance of a reticular pattern, suggesting its association with membranous structures (Fig. 2A, upper panel).

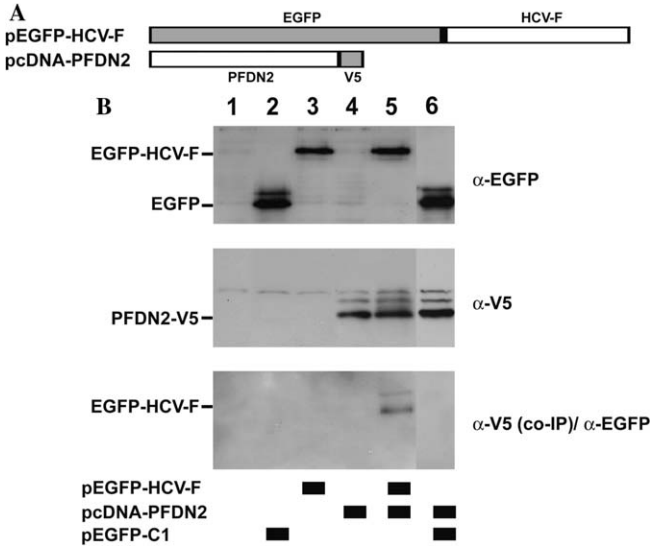

Fig. 1. Coimmunoprecipitation experiments for HCV F and PFD2 interaction. (A) The genomic structures of the protein coding regions in pEGFP-HCV-F and pcDNA-PDFN2. Shaded bars, EGFP and V5 coding regions; empty bars, HCV F and PFD2 coding regions. (B) pEGFP-HCV-F, pcDNA-PDFN2, and pEGFP-C1 were used to express EGFP-HCV-F, PDF2-V5, and EGFP respectively in HEK293 cells. Different combinations of the plasmids were used for cotransfection. The plasmids used in lanes 1–6 were marked by solid squares in the bottom. The transfected cells were harvested and half of the cell lysate was subjected to Western blot analysis using anti-EGFP antibody (upper panel) or anti-V5 antibody (middle panel). The remaining half of the cell lysate was subjected to coimmunoprecipitation experiments (lower panel), wherein anti-V5 antibody was used for immunoprecipitation and anti-EGFP antibody was used for detection.

Subsequently, cells were cotransfected with pEGFP-HCV-F and pcDNA-PFDN2. Coexpression of the two proteins (EGFP-HCV-F and PFD2-V5) was found in over 90% of the positive cells. When the two proteins were co-expressed, it was shown that the subcellular localization of EGFP-HCV-F protein was similar to that of EGFP, as it resided largely in the nuclei (Fig. 2B, green fluorescence). On the other hand, in the presence of EGFP-HCV-F protein, the subcellular localization of PFD2-V5 protein was drastically altered (Fig. 2B, red fluorescence). The protein was mostly co-localized with EGFP-HCV-F in the nucleus as shown by the merge of the two colors of fluorescence under confocal microscopy (Fig. 2B, yellow fluorescence, B-2 to -5). Occasionally, co-localization occurred in the peri-nuclear area (Fig. 2B, B-1). The alteration of subcellular localization of PFD2-V5 protein as well as the merge of the fluorescence confirmed the interaction between the two proteins.

Expression of HCV F protein interferes with the interaction between PFD1 and PFD2 in the yeast three-hybrid system

Eukaryotic PFD is a heterohexameric chaperone composed of six subunits (PFD1 to PFD6) [17,18]. Direct interaction between PFD1 and PFD2 has been clearly

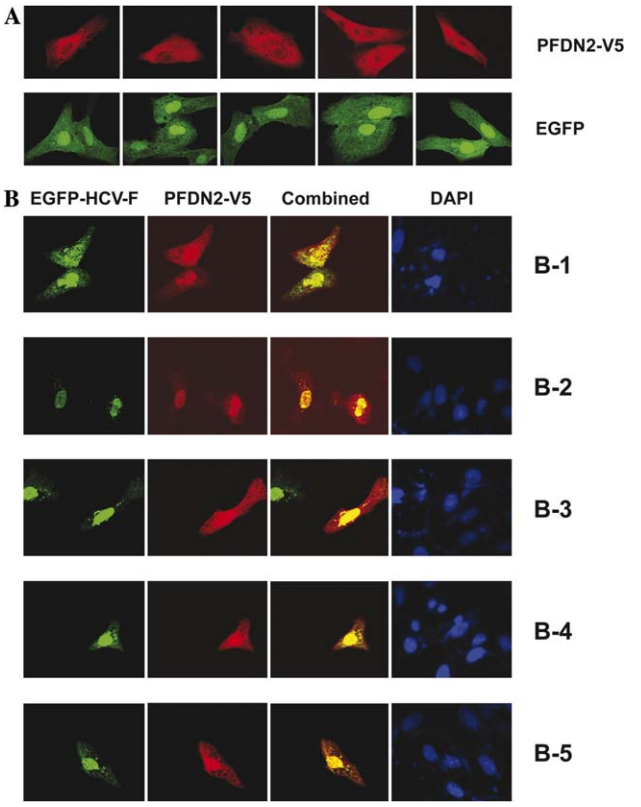

Fig. 2. Immunofluorescence analysis to verify the interaction between HCV F and PFD2. (A) Vero cells were transfected with pcDNA-PDFN2 or pEGFP-C1 to express PDF2-V5 (upper panel, rhodamine-conjugated secondary antibody) or EGFP (lower panel, FITC-conjugated secondary antibody) respectively. (B) Vero cells were cotransfected with pcDNA-PDFN2 and pEGFP-HCV-F. PDF2-V5 was detected using rhodamine-conjugated antibody. EGFP-HCV-F was detected using FITC-conjugated antibody. Nuclei were visualized by DAPI staining. Confocal microscopy was used to obtain the merging pictures. B-1 to B-5 were five representative examples of the same experiments.

demonstrated previously [23]. Since HCV F protein interacted with PFD2, we asked whether expression of HCV F protein interfered with the interaction between PFD1 and PFD2. Four plasmids, pLexA-PFDN2, pB42AD-PFDN1, pYES-NLS-HCV-F, and pYES-NLS were constructed for expression of PFD2, PFD1, HCV F protein (in-frame with the B42 nuclear localization signal), and only the peptide of B42 nuclear localization signal (as a control) (Fig. 3A). Cotransfection was performed using pLexA-PFDN2, pB42AD-PFDN1 and either pYES-NLS-HCV-F or pYES-NLS. Interaction between PFD1 and PFD2 occurred in the presence of pYES-NLS but not pYES-NLS-HCV-F. This result indicated that expression of HCV F interfered with the interaction between PFD1 and PFD2.

Expression of HCV F interferes with the formation of tubulin cytoskeleton

Because PFD complex is a chaperone involving in accurate tubulin folding, the effect of HCV F expression on tubulin cytoskeleton formation was examined. A plasmid, pcDNA-Xpress-HCV-F, was constructed for this experiment. This plasmid expressed the HCV F protein in-frame with a shorter peptide (Xpress epitope) for immunofluorescence analysis (Fig. 4). The Xpress-HCV-F protein localized to the nuclei or peri-nuclear regions in Vero cells. Tubulin cytoskeleton was demonstrated by coimmunofluorescent staining for α-tubulin. In normal cells, α-tubulin fibers were distributed all over the cytoplasm with enhanced accumulation of the fibers in the peri-nuclear regions (Fig. 4). In cells expressing Xpress-HCV-F protein, the peri-nuclear accumulation of the α-tubulin fibers significantly diminished. As a result, the contour of the nuclei

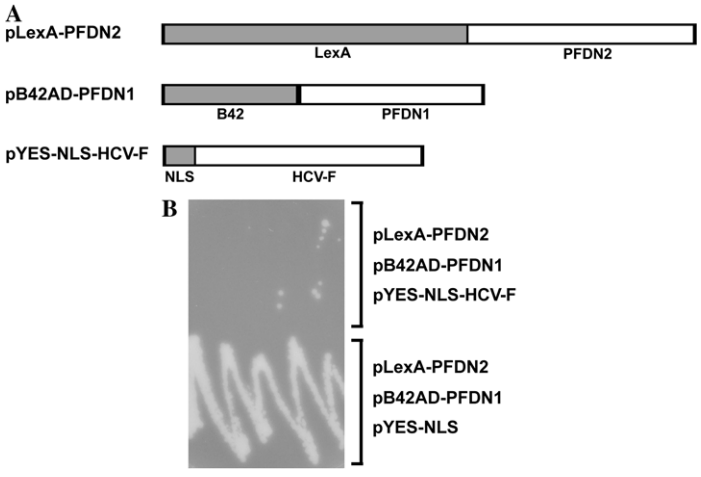

Fig. 3. The yeast three-hybrid experiments. (A) Genomic structures of the protein coding regions in pLexA-PFDN2, pB42AD-PFDN1, and pYES-NLS-HCV-F. Empty bars, coding sequences of PFD2, PFD1, and HCV F; shaded bars, coding sequences of LexA, B42, and NLS of B42. (B) Cotransfection of pLexA-PFDN2, pB42AD-PFDN1 and either pYES-NLS-HCV-F (upper panel) or pYES-NLS (lower panel) into EGY48 yeast cells. The cotransformants were selected in SD/-His/-Trp/-Ura medium and then streaked on an SD/Ga./Raf/-His/-Trp/-Ura/-Leu plate.

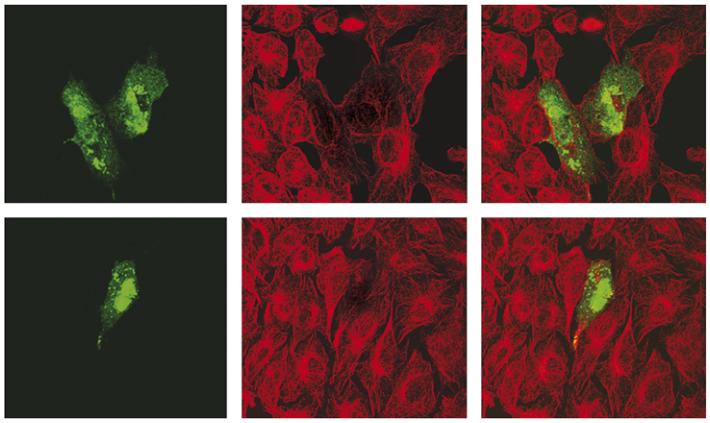

Fig. 4. Immunofluorescence analysis of α-tubulin in HCV F expressing Vero cells. Vero cells were transfected with pcDNA-Xpress-HCV-F. The Xpress-HCV-F protein was visualized by use of FITC-conjugated secondary antibody (left panel). α-Tubulin was visualized by use of rhodamine-conjugated secondary antibody (middle panel). Confocal microscopy was used to merge the two pictures (right panel).

could not be clearly seen. This result indicated that HCV F protein interfered with the organization of tubulin cytoskeleton.

Expression of HCV F protein resulted in reduced amounts of non-denatured α-tubulin

A plasmid, pcDNA-HCV-F, encoding the HCV F protein alone was constructed. This plasmid was transfected alone (Fig. 5, lane 3) or cotransfected with pcDNA-PFDN2 (Fig. 5, lane 5) into HEK293 cells. Two plasmids, pEGFP-C1 (Fig. 5, lane 2) and pcDNA-PFDN2 (Fig. 5, lane 4), were also transfected individually into cells as controls. Transfection efficiency was monitored by cotransfection with pCMVβ. It was found that in denaturing condition, the amounts of α-tubulin were the same for all different sets of transfected cells in the gel electrophoresis experiment. In contrast, under non-denaturing condition, the amounts of α-tubulin reduced significantly in cells expressing HCV F protein alone (Fig. 5, lane 3). The amount of native α-tubulin completely restored to the normal level in cells cotransfected with pcDNA-PFDN2 (Fig. 5, lane 5). Similar experiments were performed using pcDNA-Xpress-HCV-F to replace pcDNA-HCV-F (Fig. 5, lanes 6–10). The results were the same except that the amount of non-denatured α-tubulin was not completed restore to a normal level in cells co-expressing PFD2-V5. The data indicated that in cells expressing HCV F, some α-tubulin molecules could not polymerize successfully. These poorly assembled molecules possibly formed a faint background smear in the non-denaturing gel and were thus undetectable. In contrast, in the denaturing gel, all α-tubulin molecules were denatured and became monomers, whereby single bands with equal densities were shown. Co-expression of PFD2-V5 completely (or partly) reversed the effect of HCV F on α-tubulin, possibly because the excessive amount of PFD2-V5 helped to bind HCV F protein, thereby decreasing the interaction between F protein and native cellular PFD2. As a result, the function of PFD was restored.

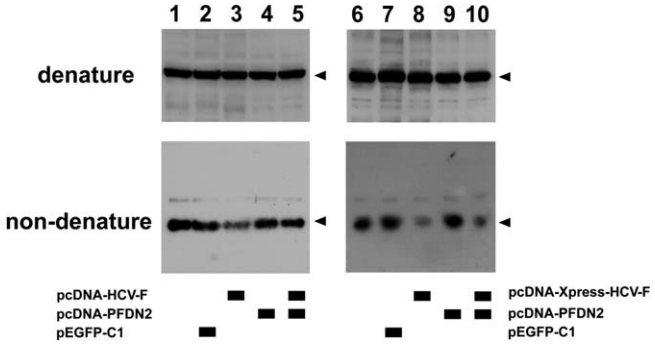

Fig. 5. Denaturing and non-denaturing gel electrophoresis experiments. HEK293 cells were transfected with pEGFP-C1, pcDNA-PFDN2 and either pcDNA-HCV-F (left panels) or pcDNA-Xpress-HCV-F (right panels) in different combination. The plasmids used in lanes 1–10 were marked at the bottom with solid squares. Transfection efficiency was monitored by cotransfection of pCMVβ (in a ratio of 1/10 in the transfected DNA mixture). The cells were harvested 3 days later. One-third of them were subjected to denaturing gel electrophoresis (upper panel), one-third of them were subjected to non-denaturing gel electrophoresis (lower panel), and the remaining cells were subjected to β-galactosidase assay. Western blot was performed by use of the mouse monoclonal TU-16 antibody for detection (solid triangles).

Discussion

Although several lines of evidence indicate that HCV F protein is indeed produced during chronic HCV infection, the function of this protein is not clear. In this study, we showed that the F protein interacted with PFD2, a subunit of the PFD complex. The interaction perturbed normal function of the PFD complex, resulting in disturbance of tubulin cytoskeleton (or microtubule) organization. It is believed that cytoskeletal components play a crucial role in viral infection [24,25]. Many different molecules in the cytoskeleton system play important roles in regulating cellular functions. At different stages of viral infection, from cell entry to virion secretion, cytoskeleton components

are mostly involved. For example, energy-consuming cytoskeleton components and factors regulating assembly or disassembly of the cytoskeleton are needed for intracellular virion trafficking for many different viruses [25]. On the other hand, cytoskeleton deregulation has been observed in viral infections, while the roles of such actions in the virus life cycle were not clear [24,26,27].

The cytoskeleton is made up of three kinds of protein filaments: actin filaments (or microfilaments), intermediate filaments, and microtubules. Microtubules are composed of α- and β-tubulin heterodimers, forming hollow tubes of 13 protofilaments [28]. They are polarized structures with a fast-growing plus end and a slow-growing minus end. Usually, the minus ends are located at the microtubules organizing centers near the nucleus, while the plus ends extend to the cell periphery [25,28]. In the present study, expression of HCV F protein greatly diminished the density of microtubules in the peri-nuclear area, suggesting that the growth of minus ends at the organizing centers were impeded.

In a recent report, it was shown that HCV replication complex-mediated RNA synthesis required intact microtubule and actin polymerization [32]. Therefore, interruption of normal microtubule formation likely resulted in decreased HCV replication. At this time, it is not clear why HCV should generate F protein to exert a seemingly unfavorable cellular effect. One possible explanation for this contradiction was that expression of HCV F protein deterred high level of HCV replication, avoiding massive apoptosis of the host cells. To date, several viral proteins of HCV have been shown to induce apoptosis of hepatocytes [29–31]. A relatively lower level of HCV replication was thus helpful for the virus to achieve virus persistence. Furthermore, since HCV F constitutes only a small fraction of the core gene products compared with the core protein, the replication inhibition effect likely occurs only when a large amount of HCV genomes are accumulated. In this view, the HCV F protein serves as a modulator to prevent cytolysis of the host cells. Alternatively, perturbing the formation of microtubules maybe necessary to accomplish important steps in virus life cycle, such as efficient viral protein transport. For example, in herpes simplex virus infection, microtubule reorganization facilitates the nuclear localization of VP22, a major virion tegument protein [26]. In that instance, the microtubule reorganization is characterized by the loss of obvious microtubule organizing centers, similar to what exerted by HCV F protein. Presently, it is unknown whether viral protein trafficking in HCV infection involves microtubule reorganization.

In this study, tagged HCV F proteins were exploited throughout the experiments except for the non-denaturing gel electrophoresis. This is due to the lack of a commercially available, specific antibody. The EGFP-HCV-F fusion protein was predominantly localized in the nuclei mostly due to the fact that EGFP itself was localized in the nuclei. The nuclear localization appeared to be advantageous in the co-localization experiment as the PDF2-V5 protein could be observed being dragged into the nuclei by the EGFP-HCV-F protein. On the other hand, the Xpress-HCV-F protein was localized to the peri-nuclear as well as the nuclear area. In previous studies, the HCV F protein has been found in the cytoplasm (membrane associated), peri-nuclear area, and nuclei [6,8,15]. In our study, P16 was found in the nucleus of human hepatocytes [8]. Since this protein is only 16 kDa, the subcellular localization of this protein might be dependent on its interacting partners as well as the variations of its amino acid sequence.

In summary, we have identified a cellular protein, PFD2, which interacts with HCV F protein. The interaction perturbs normal function of the PFD complex, resulting in aberrant organization of tubulin cytoskeleton. This molecular event likely contributes to viral persistence in chronic HCV infection.

Acknowledgments

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