Biochemical and Biophysical Research Communications 378 (2009) 118–122

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

HCV envelope protein function is dependent on the peptides preceding the glycoproteins

Tao Bian, Yongdong Zhou, Shengli Bi, Wenjie Tan*, Yue Wang*

National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Yingxin Street 100, Xuanwu Distract, Beijing 100052, PR China

ARTICLE INFO

Article history:
Received 29 October 2008
Available online 20 November 2008

Keywords:
Hepatitis C virus
Envelope protein
Glycosylation

ABSTRACT

Although significant advances have been made on the studies of HCV glycoproteins (E1 and E2) recently, the role of the peptides preceding each glycoprotein remains unclear. We expressed E1 and E2 using two individual plasmids to form HCV pseudoparticles (HCVpp) in order to characterize the peptides preceding E1 and E2.

Our data show that 14 amino acids from the HCV core and 12 amino acids from the E1 C-terminus are required for E1 and E2 function, respectively. The lack of a long enough peptide preceding E1 or E2 will abolish HCVpp infectivity, and the presence of fewer than 14 amino acids ahead of E1 and 12 amino acids ahead of E2 may alter their glycosylation. Furthermore, the peptides preceding E1 and E2 may be interchanged or may be replaced by those from genotype 2a. Our findings may contribute to the future development of new anti-HCV drugs.

HCV is the major cause of chronic hepatitis, which can evolve into cirrhosis, liver failure, or hepatocellular carcinoma [1–4]. There is currently no vaccine for HCV [4]. The only available treatment is a combination of high doses of pegylated α-interferon (IFN) and the guanosine analog ribavirin, and the efficacy of this regimen is largely dependent on the viral genotype. Genotype 1 viruses, which are the most prevalent, possess high levels of innate resistance to IFN; however, the reservoir of resistance in the other genotypes continues to build owing to the highly variable nature of HCV [5]. Given that there are approximately 170 million cases of chronic HCV infection worldwide [5], there is a pressing need to develop new therapies and vaccination strategies. To facilitate the development of such strategies, it is necessary to expand our knowledge of the structural and functional features of HCV proteins.

HCV is an enveloped plus-strand RNA virus of the *Flaviviridae* family [6]. Its genome contains a long open reading frame of 9,030 to 9,099 nucleotides that is translated into a single polyprotein of 3,010–3,033 amino acids [6,7]. Co- and post-translational cleavages of the polyprotein generate at least 10 different proteins, including two glycoproteins, E1 and E2 [6–8]. E1 and E2 are believed to be type I transmembrane proteins, with an N-terminal glycosylated ectodomain and a C-terminal hydrophobic anchor. E1 and E2 interact non-covalently to form a heterodimer that is believed to be the prebudding form of the HCV glycoprotein oligomer [9–13].

During translation, the nascent E1 and E2 polypeptides are targeted to the host endoplasmic reticulum (ER) membrane for modification by N-linked glycosylation [9,13]. E1 and E2 possess up to five and 11 potential glycosylation sites, respectively, which are anchored in the ER membrane by their TM domains (TMDs) [9–14]. In addition to serving as a membrane anchor, the TMD possesses a signal sequence in its C-terminal half that plays a major role in the localization of E1 and E2 to the ER, and is potentially involved in the assembly of the envelope proteins [9,13].

Due to difficulties in propagating HCV by cell culture, studies aimed at determining the antigenicity and immunogenicity of E1 and E2, the receptor for HCV, and the early steps of viral entry have been based on *in vitro*-expressed HCV envelope glycoproteins [15–17]. Many of the versions of E1 and/or E2 that have been studied have been soluble or truncated; thus, our knowledge of E1 and E2 is incomplete. A major advance in our ability to study HCV E1 and E2 was the development of HCV pseudoparticles (HCVpp), which consist of unmodified HCV envelope glycoproteins assembled onto retroviral core particles [18,19]. HCVpp and neutralizing antibodies have been used to analyze the interaction between E1 and E2 and the assembly of functional HCV glycoprotein heterodimers, and to characterize HCV itself and other basic features of E1 and E2 [9,13]. Despite these advances, the effect of the flanking polyprotein sequences on the biological function of HCV E1 and

Abbreviations: HCV, hepatitis C virus; HCVpp, HCV pseudoparticles; E1, envelope protein 1; E2, envelope protein 2; IFN, interferon; TMD, transmembrane domain; ER, endoplasmic reticulum.

* Corresponding authors. Fax: +86 10 63510565.
E-mail addresses: tanwj28@yahoo.cn (W. Tan), euy-tokyo@umin.ac.jp (Y. Wang).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.11.024

E2 remain unknown. Many studies have examined the function of the TMDs located at the C-termini of E1 and E2. Their results indicate that E1 and E2 are cleaved from the polyprotein by a host signal peptidase at the junction of their TMDs and the first amino acid of the next protein [9,13]; however, little is known about the role of the proteins that lie adjacent to the HCV TMDs.

In this report, we constructed two plasmids encoding E1 and E2 in order to study the role of the peptides located ahead of E1 and E2, using HCVpp. Our data show that the peptides preceding E1 and E2 are crucial for HCV glycoprotein function.

Materials and methods

Cell culture. Huh-7 human hepatocellular carcinoma cells and 293T human embryo kidney cells (American Type Culture Collection, Manassas, VA) were grown in Dulbecco's modified essential medium (Invitrogen) supplemented with 10% fetal bovine serum.

Plasmid construction. To study the characteristics of the upstream peptides required for full E1 and E2 function, two separate plasmids encoding E1 and E2 were constructed (Fig. 1A, middle and bottom). The sequences encoding E1 (polyprotein residues 192–383) and E2 (residues 384–746) (HCV genotype 1b, NCBI Accession No. L02836) were amplified by PCR and then ligated into pcDNA3.1(−)neo (Invitrogen) using standard procedures. The inserts were sequenced to confirm their identities.

To determine the exact number of preceding amino acids required to produce functional E1, the 15- (FLLALLSCLTTPASA), 14- (LLALLSCLTTPASA), 13- (LALLSCLTTPASA), 12- (ALLSCLTTPASA), and 0-(M) most C-terminal residues from the HCV core were added by PCR to produce the plasmids C15E1, C14E1, C13E1, C12E1, and atgE1, respectively. To determine the exact number of preceding amino acids required to produce functional E2, the 14- (KVLIVML LFAGVDG), 12- (LIVMLLFAGVDG), 11- (IVMLLFAGVDG), 10- (VMLL FAGVDG), and 0-(M) most C-terminal residues of E1 were added by PCR to produce the plasmids E1(14)E2, E1(12)E2, E1(11)E2, E1(10)E2, and atgE2, respectively. To confirm that the peptides preceding E1 and E2 are sequence-dependent, the relevant peptides for E1 and E2 were exchanged (the last 12 amino acids from E1 and the last 14 amino acids from the core, respectively) to produce E1(12)E1 and C14E2. To confirm that the peptides preceding E1 and E2 are genotype-specific, the relevant peptides for E1 and E2 were replaced by the last 14 amino acids (LLALLS*CITIPVS*A) from another core (genotype 2a, NCBI Accession NO. AF238485) and the last 12 amino acids (*VVILLMT*AGVD*A*) from another E1, respectively, to produce 2aC14E1 and 2aE1(12)E2. The amino acids that differ from those in the original sequences are shown in italics.

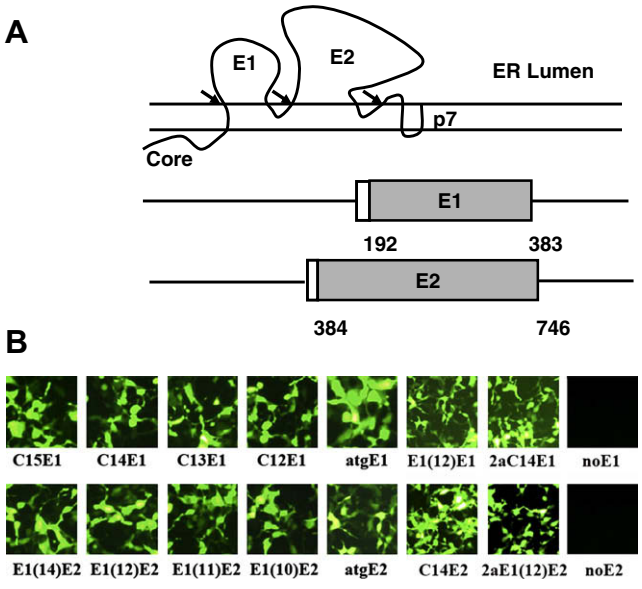

Fig. 1. HCV structural proteins shown anchored in the ER, the E1 and E2 constructs used in this study, and the expression efficiency of the glycoprotein-encoding plasmids. (A) Upper, processing of the HCV structural proteins (from the N- to the C-terminus): core, E1, E2, and P7; arrows show the host peptidase cleavage sites. Middle and bottom, the E1 and E2 constructs used in this study; the empty boxes ahead of each glycoprotein represent the C-terminal peptides of the upstream proteins. (B) The expression efficiency of the glycoprotein-encoding plasmids confirmed by immunocellular staining.

Human immunodeficiency virus (HIV)-HCV pseudotype particle production and infectivity assay. HCVpp were produced by transfecting human 293T cells with four vectors: an E1 expression plasmid; an E2 expression plasmid; pCMVAR9 (kindly provided by Dr. Didier Trono, Salk Institute, La Jolla, CA), which encodes the gag-pol protein of HIV [20]; and pCS-CpG (kindly provided by Dr. Masayo Takahashi, Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan), which encodes green fluorescent protein (GFP) and the ψ sequence required for gag-pol binding [21]. After 72 h, viral particles were harvested from the supernatants of the transfected cells after filtration through a 45-μm membrane and were used for infection assays.

Huh-7 cells were seeded at 50,000 cells per well in a 24-well plate the day prior to infection. For infection, the medium was removed, and 500 μl of the filtered supernatant and 4 ng/ml Polybrene (Sigma, St. Louis, MO) were added. After overnight incubation, the inoculum was removed and replaced with DMEM supplemented with 5% fetal bovine serum. At 72 h post-infection, the medium was removed, and the cells were harvested for FACS analysis.

p24 ELISA. To monitor the expression efficiency of different combinations of E1 and E2 in the production of HCVpp, a commercially available HIV type 1 p24 antigen enzyme-linked immunosorbent assay (ELISA) kit (Aalto Bio Reagents, Dublin, Ireland) was used to quantify the p24 content in the samples.

Immunoprecipitation and Western blotting. To assess the expression characteristics of HCV E1 and E2, the supernatants from the 293T cells were harvested for immunoprecipitation and Western blot analysis. The cells were washed twice with PBS and then lysed in 400 μl of lysis buffer [1]. The cell extracts were centrifuged and incubated with 200 μl of supernatant and 20 μl of HCV 1b-infected patient serum (informed consent was obtained before the samples were collected) overnight at 4 °C. We recovered the immunoprecipitates by centrifugation and washed them four times with immunoprecipitation buffer. SDS-PAGE was performed to separate the bound proteins. For Western blotting, the corresponding patient's serum was used as the primary antibody.

Results

Efficient expression of the E1 and E2 constructs

To confirm the expression of the E1 and E2 constructs, the plasmids encoding C15E1, C14E1, C13E1, C12E1, atgE1, E1(12)E1, 2AC14E1, E1(14)E2, E1(12)E2, E1(11)E2, E1(10)E2, atgE2, C14E2, and 2AE1(12)E2 were transfected into 293T cells. Forty-eight hours later, the cells were harvested for immunocellular staining. HCV 1b-infected patient serum was used as the primary antibody; the expression of each aforementioned version of HCV E1 and E2 was checked using a FITC-conjugated anti-human antibody. As shown in Fig. 1B, all of the constructs-expressed HCV E1 and E2 with high efficiency.

Infectivity of HCVpp generated from a Chinese patient infected with HCV 1b

The 293T cells were cotransfected with four plasmids: E1, E2, pCMVAR9, and pCS-CpG. At 72 h post-transfection, the culture medium was collected, filtered, and used to infect Huh-7 cells; after 72 h, the infected cells were counted by FACS analysis. As shown in Fig. 2A, the HCVpp harboring E1 and E2 generated from a Chinese patient who was infected with HCV 1b infected as many as 24.5 ± 3.3% of the Huh-7 cells, indicating that our HCVpp were infectious.

The peptides located ahead of HCV E1 are essential for E1 function

Little is known of the role played by the peptides located upstream of E1, except for the peptide that serves to anchor the protein in the ER during synthesis. To assess whether the sequence at the C-terminus of the core is necessary for E1 function, we kept all of the conditions unchanged in the four-plasmid transfection unit except for the plasmid encoding E1 with a preceding peptide of variable length. Specifically, we deleted the entire sequence ahead of E1 (atgE1) and then attempted to generate HCVpp using this plasmid; interestingly, no infectivity was observed (Fig. 2A). To determine the exact length of the peptide required for E1 function, we shortened the length to between 12 and 15 amino acids. As shown in Fig. 2A, E1 with upstream peptide 14–15 amino acids long was able to form infectious HCVpp, whereas the infectivity of the HCVpp was significantly reduced when the length was shortened to 13 amino acids and was abolished when the length was shortened to 12 amino acids. To eliminate the possibility that the changes were caused by the transfection and packaging efficiency, we monitored the level of p24 in the supernatant; the level of p24 was similar and stable in all cases (data not shown). To further investigate why 14 amino acids from the HCV core C-terminus are necessary for E1 function, we monitored the expression of each of the above constructs in 293T cells. Immunoprecipitation and Western blotting revealed that C15E1, C14E1, and C13E1 were fully glycosylated, whereas C12E1 and atgE1 were poorly glycosylated (Fig. 3). As only the fully glycosylated form of E1 was associated with HCVpp, the loss of infectivity may be explained, at least in part, by poor glycosylation. Together, these data indicate that at least 14 amino acids from the HCV core C-terminus are crucial for E1 function and that this domain is associated with E1 glycosylation.

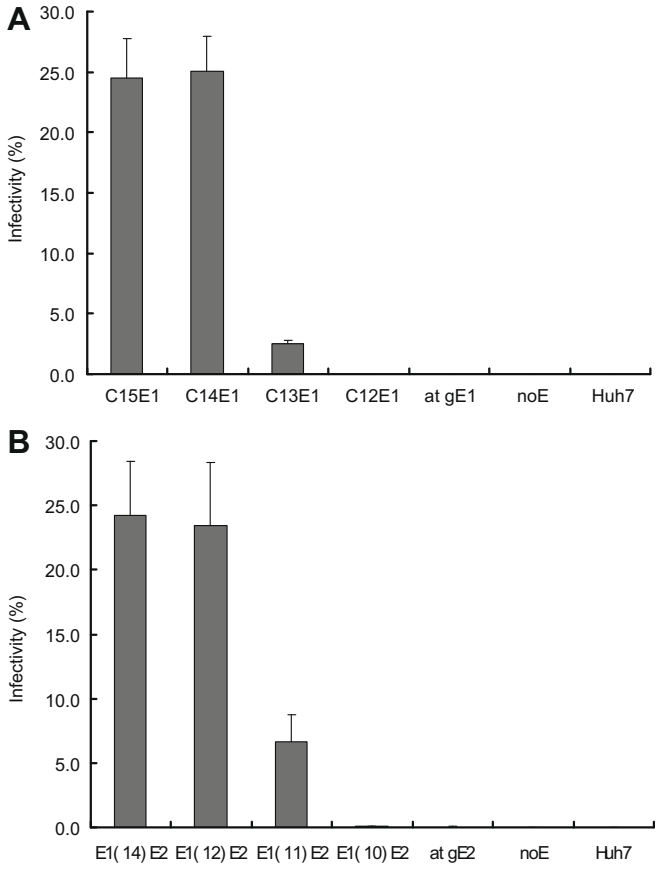

Fig. 2. Infection assay using E1 and E2 with preceding peptides of variable length. (A) We selected a functional E2 and combined it with C15E1, C14E1, C13E1, C12E1, and atgE1, respectively, to form HCVpp. We then assayed the function of each E1. Infectivity is expressed as the number of infected cells/number of cells counted (%). No E1 and naive Huh-7 cells were used as negative controls for FACS analysis ($n = 5$). (B) We selected a functional E1 and combined it with E1(14)E2, E1(12)E2, E1(11)E2, E1(10)E2, and atgE2, respectively, to form HCVpp, similar to (A). We then compared the function of each E2 by an infection assay. No E2 and naive Huh-7 cells were used as negative controls ($n = 3$).

The peptides located upstream of HCV E2 are essential for E2 function

To study the contribution of the residues located at the C-terminus of E1 to E2 function, we used a functional E1 construct and attempted to generate HCVpp using E2 flanked by 14, 12, 11, 10, and 0 amino acids from the E1 C-terminus. As shown in Fig. 2B, E1(14)E2 and E1(12)E2 formed HCVpp with fairly high infectivity, whereas infectivity decreased greatly when the peptide was shortened to 11 amino acids (E1[11]E2). At a length of 10 amino acids (E1[10]E2), the ability to form infectious HCVpp was completely abolished, which is similar to the result obtained with no peptide upstream of E2 (atgE2). Similarly, the level of p24 in the supernatant was maintained at a comparable and stable level (data not shown). Together, our data indicate that the residues located upstream of HCV E2 are essential for E2 function. Immunoprecipitation and Western blotting showed that E2 expressed from the E1(14)E2, E1(12)E2, and E1(11)E2 plasmids was fully glycosylated, but E2 expressed from the E1(10)E2 and E1(10)E2 plasmids was poorly glycosylated (Fig. 3). This may explain why E1(10)E2 and E1(10)E2 were unable to form infectious HCVpp.

The peptides preceding E1 and E2 may be exchanged

To assess whether the peptide preceding E1 is sequence-dependent, we substituted the peptide required for E1 with that required for E2 and attempted to generate HCVpp using the resulting construct (E1[12]E1). Interestingly, the E1 was able to form HCVpp with a level of infectivity equal to that of C14E1 (Fig. 4A). To assess whether the peptide preceding E2 is E2-dependent, we substituted the peptide required for E2 with that required for E1 (C14E2). C14E2 was capable of forming HCVpp with the same level of infectivity as HCVpp containing E1(12)E2 (Fig. 4A). Our data demonstrate that the peptides preceding E1 and E2 may be interchanged.

The peptides preceding E1 and E2 may be replaced by those from another genotype

To assess whether the peptides preceding the HCV glycoproteins E1 and E2 may be replaced by those from other genotypes, we replaced the peptides for 1b E1 (LLALLSCLTTPASA) and E2 (LIVMLLFAGVDG) with the respective peptides from the 2a strain. Both 2aC14E1 (LLALLS*CITIPV*SA, the amino acids in italics differ between the two sequences) and 2aE1(12)E2 (*VVILLMT*AGVD*A*) formed HCVpp with the same level of infectivity as that seen with 1b C14E1 and 1b E1(12)E2 (Fig. 4B).

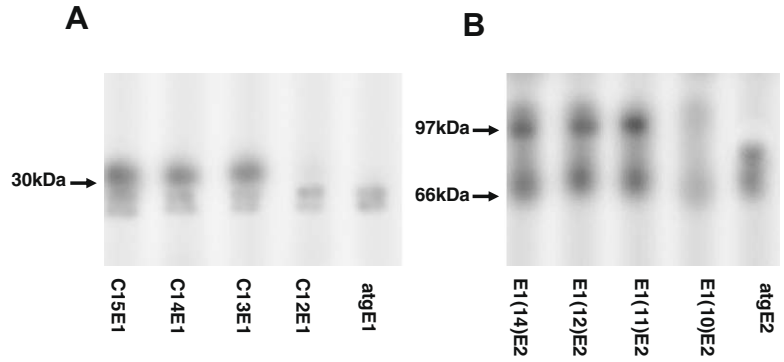

Fig. 3. Immunoprecipitation and Western blot analysis. At the time the HCVpp were collected, the 293T cells were also sampled for immunoprecipitation and Western blot analysis. The glycoproteins were immunoprecipitated using serum from an HCV-infected patient. The immunoprecipitates were analyzed by Western blotting under reducing conditions. The sizes of the molecular mass markers are given on the left side. (A), E1; (B), E2 ($n = 3$).

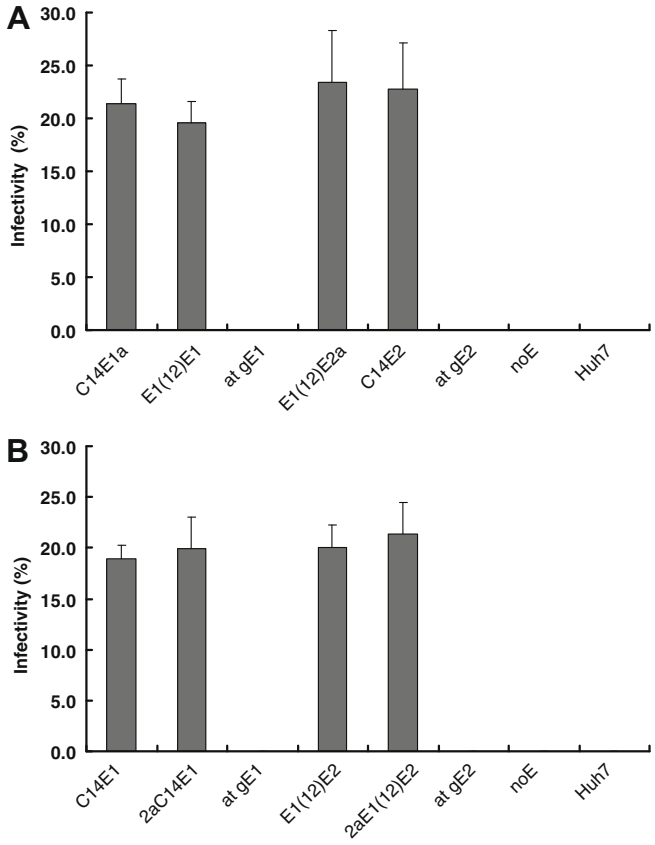

Fig. 4. The peptides required for E1 and E2 may be inter-exchanged and may be replaced by those from another genotype. (A) E1(12)E1, the last 12 amino acids from the E1 C-terminus were added ahead of E1; C14E2, the last 14 amino acids from the core C-terminus were added ahead of E2 ($n = 4$). (B) For 2aC14E1, the last 14 residues from the HCV 1b core C-terminus were replaced by the corresponding residues from genotype 2a. For 2aE1(12)E2, the last 12 amino acids from the HCV 1b E1 C-terminus were replaced by the corresponding residues from genotype 2a ($n = 3$).

Discussion

The HCV envelope glycoproteins present at the virion surface have been poorly characterized; however, the recent development of an HCVpp system [18,19] has improved our understanding of the TMDs of E1 and E2, and the cleavage and glycosylation of the HCV polyproteins [9,13]. Still, few studies have addressed the contribution of the peptides located just upstream of the glycoproteins to their function. In this study, we found that at least 14 amino acids from the core C-terminus were required for E1 function and that at least 12 amino acids from the E1 C-terminus were required for E2 function. We also found that these peptides influence the glycosylation of E1 and E2. Furthermore, the peptides upstream of E1 and E2 can be exchanged as well as replaced by those from another genotype.

HCVpp are believed to contain fully functional envelope glycoproteins and to mimic the function of native HCV particles; thus, they are the best model available today for studying the earliest steps of the HCV life cycle [9,13]. In this study, we used the infectivity of HCVpp to assess E1 and E2 function. During the synthesis of E1 and E2, the ectodomains of the glycoproteins target them to the ER lumen, where they are modified by N-linked glycosylation [9]. HCV envelope glycoproteins have been shown to assemble into oligomeric complexes and to form E1 and E2 heterodimers that are stabilized by non-covalent interactions [13]. Extensive characterization of these heterodimers has suggested that they are the prebudding form of the functional complex, which subsequently plays an active role in host cell entry [10,11]. Our data indicate that the HCV glycoproteins are anchored to the ER membrane by two flanking peptides: one located at the glycoprotein's own C-terminus, and the other at the C-terminus of the protein that lies upstream of the glycoprotein (Fig. 1A). E1 and E2 initially form a loop-like structure, which is subsequently glycosylated. The lack of a long enough upstream peptide may influence the anchoring, which in turn may affect the glycosylation, folding, and heterodimerization of the glycoproteins, thereby abolishing HCVpp infectivity.

The TMDs of the HCV glycoproteins possess unusual features [9–14]. The domains contain fewer than 30 residues and are composed of two hydrophobic stretches separated by a short segment containing one or two fully conserved charged residues [9–14]. Topological studies of the HCV envelope glycoproteins have shown the reorientation of the C-termini of the TMDs, leading to a single membrane-spanning domain [9,13,22–26]. Only 12–14 residues (i.e., less than half of the TMD in E1 and E2) correspond to most of the C-terminus, which implies that the ER anchor does not require the entire TMD. The last fourteen amino acids (C14) of the HCV 1b C-terminus (LLALLSCLTTPASA) differs from HCV 2a C14 at three residues (22%) (LLALLSC*ITIPVS*A, different amino acids are written in italics); similarly, the C-terminus of HCV 1b E1 (LIVMLLFAGVDG) differs from that of HCV 2a at 6 residues (50%) (*VVILLMT*AGVD*A*). These amino acid differences suggest that the peptides preceding E1 and E2 act as topological anchors and are not directly involved in the modification of the glycoproteins. We found no evidence that the charged residues within the TMDs are involved in this task.

The development of anti-HCV strategies has been hampered by the lack of a convenient model system; indeed, most studies of the immunogenicity and antigenicity of HCV glycoproteins have been performed exclusively using transient expression systems. Thus, our knowledge of these glycoproteins is limited, which has directly affected our ability to produce an anti-HCV vaccine. In 2003, HCVpp were first established, and an HCV cell culture system was created in 2005. These two systems have allowed us to advance our knowledge of basic viral biology, neutralizing antibodies, HCV receptors, and the interaction of HCV with host cells. They may offer even more benefit by allowing us to pursue previously addressed research areas in new ways. In particular, the design and development of an anti-HCV vaccine should be reconsidered.

Recent anti-HCV strategies have focused on inhibiting the virus' replicative elements and glycosylation process, as well as on using immune modulators and non-specific hepatoprotective agents [3,27,28]. However, given our data showing the function of the TMDs of HCV E1 and E2, further study leading to the development of new anti-HCV drugs should include these domains.

Acknowledgments

This study was supported by the Hi-Tech Research and Development Program of China (2007AA02Z157, and 2007AA02Z455) and the National Natural Science Foundation of China (30571673 and 30671961).

References

[1] Y. Wang, N. Kato, A. Jazag, N. Dharel, M. Otsuka, H. Taniguchi, T. Kawabe, M. Omata, Hepatitis C virus core protein is a potent inhibitor of RNA silencing-based antiviral response, Gastroenterology 130 (2006) 883–892.
[2] G. Liao, Y. Wang, J. Chang, T. Bian, W. Tan, M. Sun, W. Li, H. Yang, J. Chen, X. Zhang, S. Bi, M. Omata, S. Jiang, Hepatitis B virus precore protein augments genetic immunizations of the truncated hepatitis C virus core in BALB/c mice, Hepatology 47 (2008) 25–34. Erratum in: Hepatology 47 (2008) 2149.
[3] M.P. Manns, G.R. Foster, J.K. Rockstroh, S. Zeuzem, F. Zoulim, M. Houghton, The way forward in HCV treatment—finding the right path, Nat. Rev. Drug Discov. 6 (2007) 991–1000. Review. Erratum in: Nat. Rev Drug Discov. 7 (2008) 102. Nat. Rev. Drug Discov. 7 (2008) 458.
[4] M. Houghton, S. Abrignani, Prospects for a vaccine against the hepatitis C virus, Nature 436 (2005) 961–966. Review.
[5] NIH Consensus Statement on Management of Hepatitis C: 2002. NIH Consensus and State-of-the-Science Statements vol. 19, No. 3, June 10–12, 2002.
[6] Q.L. Choo, G. Kuo, A.J. Weiner, L.R. Overby, D.W. Bradley, M. Houghton, Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome, Science 244 (1989) 359–362.
[7] F. Penin, J. Dubuisson, F.A. Rey, D. Moradpour, J.M. Pawlotsky, Structural biology of hepatitis C virus, Hepatology 39 (2004) 5–19.
[8] G.M. Lauer, B.D. Walker, Hepatitis C virus infection, N. Engl. J. Med. 345 (2001) 41–52.
[9] A. Goffard, J. Dubuisson, Glycosylation of hepatitis C virus envelope proteins, Biochimie 85 (2003) 295–301.
[10] A. Op De Beeck, C. Voisset, B. Bartosch, Y. Ciczora, L. Cocquerel, Z. Keck, S. Foung, F.L. Cosset, J. Dubuisson, Characterization of functional hepatitis C virus envelope glycoproteins, J. Virol. 78 (2004) 2994–3002.
[11] M. Flint, C. Logvinoff, C.M. Rice, J.A. McKeating, Characterization of infectious retroviral pseudotype particles bearing hepatitis C virus glycoproteins, J. Virol. 78 (2004) 6875–6882.
[12] M. Lavie, A. Goffard, J. Dubuisson, Assembly of a functional HCV glycoprotein heterodimer, Curr. Issues Mol. Biol. 9 (2007) 71–86.
[13] C. Voisset, J. Dubuisson, Functional hepatitis C virus envelope glycoproteins, Biol. Cell. 96 (2004) 413–420.
[14] F. Helle, J. Dubuisson, Hepatitis C virus entry into host cells, Cell. Mol. Life Sci. 65 (2008) 100–112.
[15] G. Leroux-Roels, E. Depla, F. Hulstaert, L. Tobback, S. Dincq, J. Desmet, I. Desombere, G. Maertens, A candidate vaccine based on the hepatitis C E1 protein: tolerability and immunogenicity in healthy volunteers, Vaccine 22 (2004) 3080–3086.
[16] M. Yi, S. Kaneko, D.Y. Yu, S. Murakami, Hepatitis C virus envelope proteins bind lactoferrin, J. Virol. 71 (1997) 5997–6002.
[17] J.M. Heile, Y.L. Fong, D. Rosa, K. Berger, G. Saletti, S. Campagnoli, G. Bensi, S. Capo, S. Coates, K. Crawford, C. Dong, M. Wininger, G. Baker, L. Cousens, D. Chien, P. Ng, P. Archangel, G. Grandi, M. Houghton, S. Abrignani, Evaluation of hepatitis C virus glycoprotein E2 for vaccine design: an endoplasmic reticulum-retained recombinant protein is superior to secreted recombinant protein and DNA-based vaccine candidates, J. Virol. 74 (2000) 6885–6892.
[18] B. Bartosch, J. Dubuisson, F.L. Cosset, Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes, J. Exp. Med. 197 (2003) 633–642.
[19] M. Hsu, J. Zhang, M. Flint, C. Logvinoff, C. Cheng-Mayer, C.M. Rice, J.A. McKeating, Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles, Proc. Natl. Acad. Sci. USA 100 (2003) 7271–7276.
[20] L. Naldini, U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. Gage, I. Verma, D. Trono, *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector, Science 272 (1996) 263–267.
[21] H. Miyoshi, U. Blomer, M. Takahashi, F. Gage, I. Verma, Development of a self-inactivating lentivirus vector, J. Virol. 72 (1998) 8150–8157.
[22] L. Cocquerel, S. Duvet, J.C. Meunier, A. Pillez, R. Cacan, C. Wychowski, J. Dubuisson, The transmembrane domain of hepatitis C virus glycoprotein E1 is a signal for static retention in the endoplasmic reticulum, J. Virol. 73 (1999) 2641–2649.
[23] L. Cocquerel, A. Op de Beeck, M. Lambot, J. Roussel, D. Delgrange, A. Pillez, C. Wychowski, F. Penin, J. Dubuisson, Topological changes in the transmembrane domains of hepatitis C virus envelope glycoproteins, EMBO J. 21 (2002) 2893–2902.
[24] L. Cocquerel, J.C. Meunier, A. Pillez, C. Wychowski, J. Dubuisson, A retention signal necessary and sufficient for endoplasmic reticulum localization maps to the transmembrane domain of hepatitis C virus glycoprotein E2, J. Virol. 72 (1998) 2183–2191.
[25] Y. Ciczora, N. Callens, F. Penin, E.I. Pécheur, J. Dubuisson, Transmembrane domains of hepatitis C virus envelope glycoproteins: residues involved in E1E2 heterodimerization and involvement of these domains in virus entry, J. Virol. 81 (2007) 2372–2381.
[26] J. Dubuisson, S. Duvet, J.C. Meunier, A. Op De Beeck, R. Cacan, C. Wychowski, L. Cocquerel, Glycosylation of the hepatitis C virus envelope protein E1 is dependent on the presence of a downstream sequence on the viral polyprotein, J. Biol. Chem. 275 (2000) 30605–30609.
[27] J. Balzarini, Targeting the glycans of glycoproteins: a novel paradigm for antiviral therapy, Nat. Rev. Microbiol. 5 (2007) 583–597. Review.
[28] S.L. Tan, A. Pause, Y. Shi, N. Sonenberg, Hepatitis C therapeutics: current status and emerging strategies, Nat. Rev. Drug Discov. 1 (2002) 867–881.