Biochemical and Biophysical Research Communications 435 (2013) 147–152

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Both core and F proteins of hepatitis C virus could enhance cell proliferation in transgenic mice

Wen-Ta Hu [a,1], Hui-Chun Li [b,1], Shen-Kao Lee [a], Hsin-Chieh Ma [a], Chee-Hing Yang [a], Hung-Ling Chen [a], Shih-Yen Lo [a,c,*]

[a] Graduate Institute of Medical Biotechnology, Tzu Chi University, Hualien, Taiwan
[b] Department of Biochemistry, Tzu Chi University, Hualien, Taiwan
[c] Department of Laboratory Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 12 April 2013
Available online 27 April 2013

Keywords:
Hepatitis C virus
ARF/F/core+1 protein
Core protein
Cell proliferation
WNT signaling pathway
Transgenic mice

ABSTRACT

The role of the protein encoded by the alternative open reading frame (ARF/F/core+1) of the Hepatitis C virus (HCV) genome in viral pathogenesis remains unknown. The different forms of ARF/F/core+1 protein were labile in cultured cells, a myc-tag fused at the N-terminus of the F protein made it more stable. To determine the role of core and F proteins in HCV pathogenesis, transgenic mice with either protein expression under the control of Albumin promoter were generated. Expression of core protein and F protein with myc tag (myc-F) could be detected by Western blotting analysis in the livers of these mice. The ratio of liver to body weight is increased for both core and myc-F transgenic mice compared to that of wild type mice. Indeed, the proliferating cell nuclear antigen protein, a proliferation marker, was up-regulated in the transgenic mice with core or myc-F protein. Further analyses by microarray and Western blotting suggested that β-catenin signaling pathway was activated by either core or myc-F protein in the transgenic mice.

These transgenic mice were further treated with either Diethynitrosamine (a tumor initiator) or Phenobarbital (a tumor promoter). Phenobarbital but not Diethynitrosamine treatment could increase the liver/body weight ratio of these mice. However, no tumor formation was observed in these mice. In conclusion, HCV core and myc-F proteins could induce hepatocyte proliferation in the transgenic mice possibly through β-catenin signaling pathway.

1. Introduction

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1]. HCV belongs to the genus *Hepacivirus* in the family *Flaviviridae*. The HCV genome is a single, positive-stranded RNA with a nucleotide length of about 9.6 kb. It encodes a polyprotein precursor of approximately 3000 amino acids, which is processed by host and viral proteases into at least 10 different proteins, which are arranged in the order of NH2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH [2]. In the +1 frame, an alternate reading frame (ARF) overlaps with the core protein-coding sequence and encodes the ARF protein (ARFP). HCV ARFPs could be resulted from either frameshifting, transcriptional slippage or internal initiation in the +1 open reading frame of core protein coding sequence [3–7]. Abolishing the ARFP production has no effect on HCV replication in cultured cells or uPA-SCID mice, suggesting that ARFP is probably not important for the HCV reproductive cycle [8,9]. However, detection of the specific anti-ARFP antibodies and the T-cell responses in HCV-infected patients, as reported by many independent laboratories, provided strong evidence that this protein is expressed in vivo [10–12].

Sub-cellular localization of HCV ARFP has been studied [6,13–15]. However, the functional significance of this protein in the viral pathogenesis remains unknown [16]. It has been demonstrated that the ARFP to possess regulatory functions in vitro and has been suggested to play a role in advanced liver disease and liver cancer [8,17–20].

To determine the possible oncogenic property of HCV F protein, transgenic mice with this protein were generated in this study. As HCV core protein has been demonstrated to have an oncogenic role [21], the transgenic mice with core protein were also generated as a positive control.

* Corresponding author. Address: 701, Section 3, Chung Yang Road, Tzu Chi University, Hualien, Taiwan. Fax: +886 3 8571917.
E-mail address: losylo@mail.tcu.edu.tw (S.-Y. Lo).
[1] The first two authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.04.059

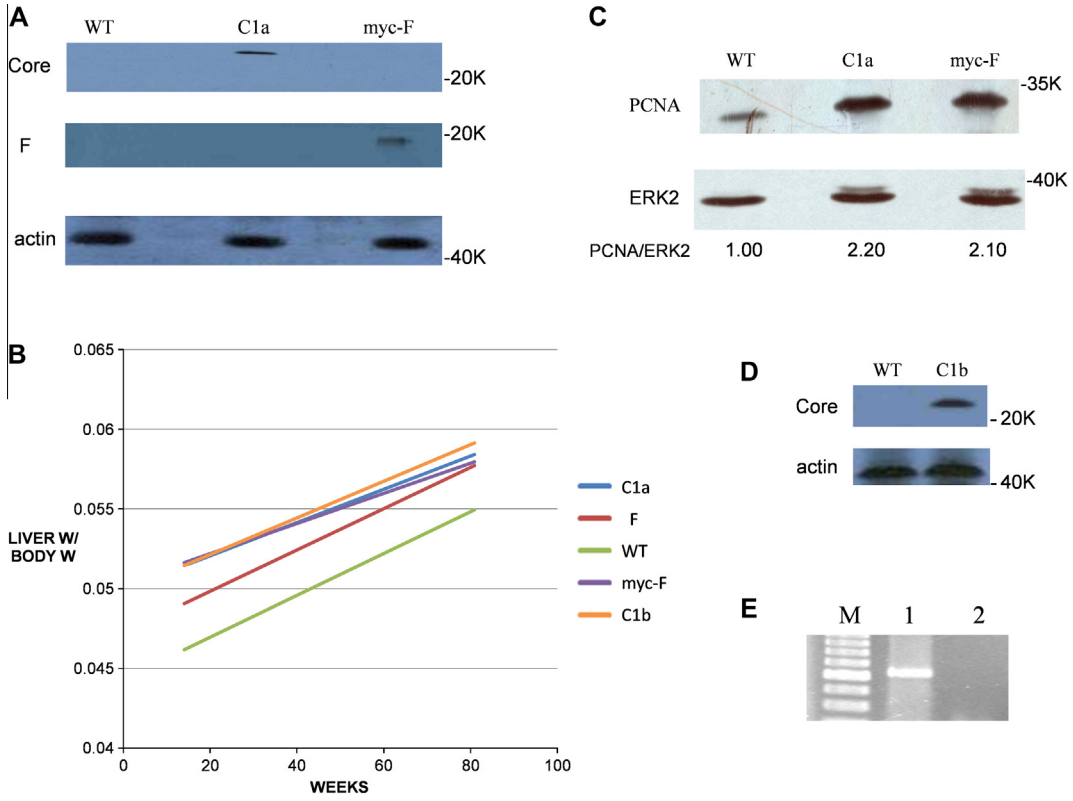

Fig. 1. Both core and F proteins could induce hepatocyte proliferation in the transgenic mice. (A) Protein samples from the livers of 53 week-old of wild-type mice or the transgenic mice with core or myc-F protein were used for Western blotting analysis using antibodies against core (upper panel), against F (middle panel), or against actin (lower panel) as a loading control. (B) The liver/body weight ratio of wild-type mice or the transgenic mice with different core or F proteins. (C) Protein samples from the livers of 53 week-old of wild-type mice or the transgenic mice with core or myc-F protein were used for Western blotting analysis using antibodies against PCNA (upper panel) as a marker of cell proliferation, or ERK2 (lower panel) as a loading control. (D) Protein samples from the livers of 53 week-old of wild-type mice or the transgenic mice with core 1b were used for Western blotting analysis using antibodies against core (upper panel), or against actin (lower panel) as a loading control. (E) The mRNAs extracted from 53 week-old of transgenic mice with F protein (lane 1) or wild-type mice (lane 2) were used for RT-PCR. Then, the products were analyzed by electrophoresis.

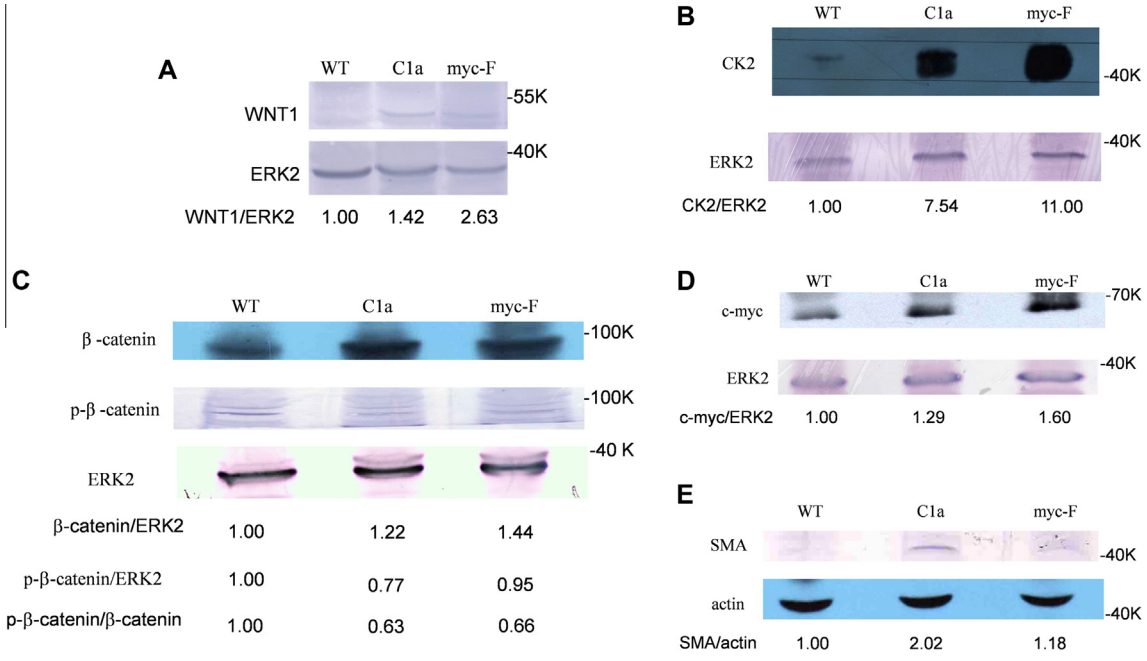

Fig. 2. Protein samples from the livers of 53 week-old of wild-type mice or the transgenic mice with core or myc-F protein were used for Western blotting analysis using antibodies against WNT1 (A), CK2 β (B), β-catenin (C), c-myc (D), or SMA (E). The protein amount of ERK2 or actin was used as a loading control.

2. Materials and methods

2.1. Plasmid construction

The expression plasmids for HCV ARF/F/ core +1 protein used in this study were derived from the plasmid p90/HCV FL-long pU (GI: 2316097) which contains the full-length sequence of the HCV-H isolate. To isolate the cDNA fragments that contain the indicated protein coding sequences, PCR reactions using specific primers were performed. Oligo primers used for PCR are available upon request. After PCR, the DNA fragments were cloned into the pcDNA3, pcDNA3.1-V5-His A (Invitrogene, USA), or pcDNA3-myc vector [22] for transient expression in mammalian cells using the same approach described previously [23,24]. The DNA coding fragment for core 1a was also derived from HCV-H isolate while core 1b was initially isolated from a local patient with HCV genotype 1b (this plasmid was provided by Dr. K.S. Jeng, Academia Sinica, Taipei, Taiwan). The expression vector for core or F protein in transgenic mice was under the control of liver-specific albumin promoter [25].

All the expression plasmids were verified by sequencing.

2.2. Cell culture

HuH7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, USA). All cultured cells were maintained at 37 °C with 5% CO_2.

2.3. Immunoprecipitation

Our previous procedures were followed for pulse-chase and immunoprecipitation to determine the half-lives of various HCV ARF/F/core+1 proteins [26]. The antibody used for the analysis was home-made rabbit anti-F polyclonal antibody.

2.4. Transgenic mice

Transgenic mice with different recombinant proteins were generated in FVB/N mice by Level Biotechnology Inc. (Taipei, Taiwan). After the establishment of transgenic mice, these mice were housed in the Laboratory Animal Center of Tzu Chi University. The research methods were approved by the Animal Care and Use Committee of Tzu-Chi University.

2.5. Western blotting analysis

Our previous procedures were followed for Western blotting analysis [17,23]. The primary antibodies used for the analyses in this study were mouse anti-F monoclonal antibody (a gift kindly provided by Dr. J.H. Ou, University of Southern California, USA), mouse anti-PCNA monoclonal antibody (Abcam, UK), mouse anti-β-catenin monoclonal antibody (Santa Cruz, USA), mouse anti-myc (4A6) monoclonal antibody (Millipore, USA), mouse anti-actin monoclonal antibody (Santa Cruz), mouse anti-CK2 β monoclonal antibody (Santa Cruz), rabbit anti-core polyclonal antibody [27], rabbit anti-p-β-catenin polyclonal antibody (Santa Cruz), rabbit anti-WNT1 polyclonal antibody (Abcam) and rabbit anti-Erk2 polyclonal antibody (Santa Cruz).

2.6. Microarray analysis

The microarray analysis was performed in NRPGM Microarray & Gene Expression Analysis Core Facility (National Yang-Ming University, Taipei, Taiwan) using Affymetrix Chips Mouse 430 2.0 (USA).

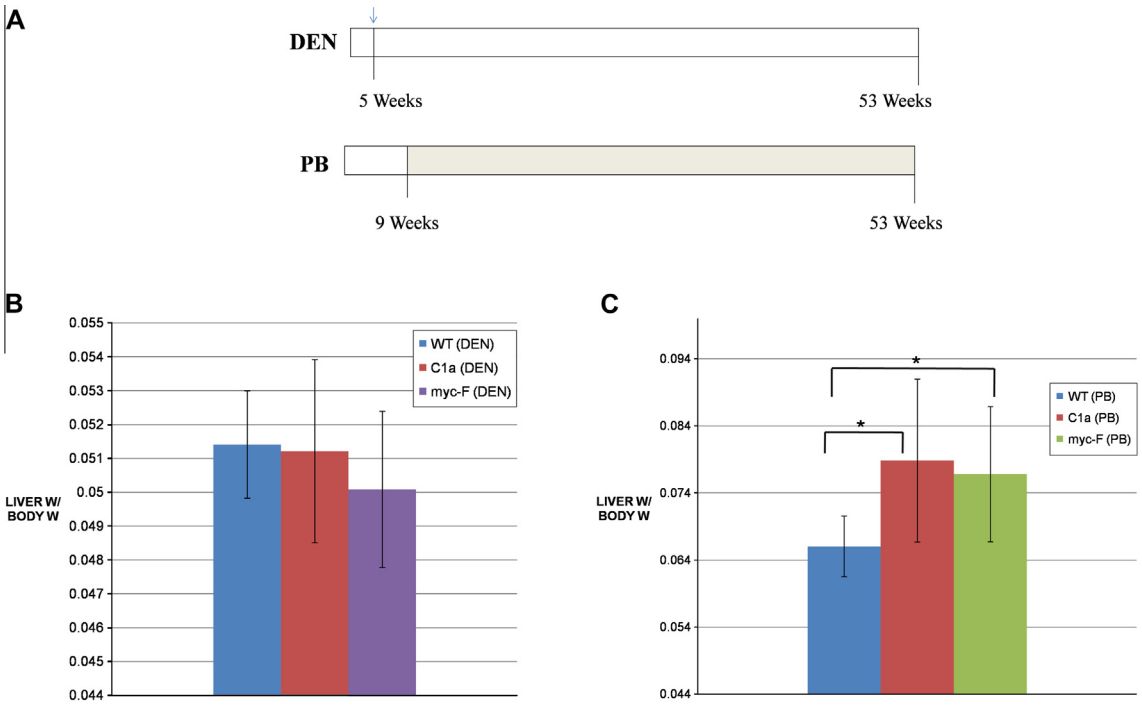

Fig. 3. PB but not DEN treatment could further increase the liver/body weight ratio of the transgenic mice with core or myc-F protein. (A) The timeline for the treatment of DEN or PB in the wild-type mice or the transgenic mice with core or myc-F protein. Five-week-old mice were injected 200 mg/kg DEN once and sacrificed at the end of 53 weeks. Nine-week-old mice were treated with 0.07% PB in water till their sacrifice at the end of 53 weeks. (B) The liver/body weight ratio of the wild-type mice or the transgenic mice with core or myc-F protein treated with DEN. (C) The liver/body ratio of the wild-type mice or the transgenic mice with core or myc-F protein treated with PB.

Table 1
Change of the liver/body weight ratio of the mice after the treatment of DEN or PB.

	DEN (+)/DEN (−)	PB (+)/PB (−)
WT	0.09%	34.53%
Core 1a	−9.76%	39.19%
myc-F	−9.81%	38.32%

3. Results

3.1. The HCV ARF/F/core+1 proteins are labile in cultured cells

To study the role of core and F proteins in HCV pathogenesis, transgenic mice with core or F protein were generated under the control of the liver-specific albumin promoter. Unlike core protein, authentic F protein expression could not be detected in Western blotting analysis (data not shown). Failure to detect F protein expression in the transgenic mice is not surprising because it is a labile protein in HuH7 cells and in vitro [14,15,28]. To find an ARF/F/core+1 fusion protein with a longer half-life, the stability of different forms of ARF/F/core+1 protein fused with different tags was compared in cultured cells. Among these recombinant fusion proteins, F proteins with a myc-tag at their N-terminus make them more stable(data not shown).

3.2. The liver/body weight ratio of the transgenic mice with core or F protein is higher than that of wild type mice

Plasmids encoding F proteins with a myc-tag at its N-terminus in the presence or absence of the N-terminal 10 amino acids overlapping with core protein were constructed for expression in the transgenic mice. Due to unknown reasons, only the transgenic mice with F protein in the absence of the N-terminal 10 amino acids overlapping with core protein were generated successfully (myc-F). Expression of HCV core or myc-F protein in the transgenic mice could be detected in Western blotting analysis (Fig. 1A). The liver/body weight ratio of the transgenic mice with core or myc-F protein is higher than that of wild type mice (Fig. 1B). Increased cell proliferation, increased liver inflammation or increased liver cell size could result in higher liver/body ratio in the transgenic mice with core or myc-F protein. Level of serum transaminases (e.g., ALT, AST) was routinely checked to monitor the liver damage in these transgenic mice. The levels of transaminases were not elevated in the transgenic mice with core or myc-F protein when compared with those in wild type mice (data not shown). The size of liver cells remained unchanged in the transgenic mice with core or myc-F protein compared with those in wild type mice when hematoxylin and eosin staining was performed (data not shown). These results indicated that both HCV core and myc-F proteins could enhance cell proliferation in the transgenic mice. Indeed, the cell proliferation marker PCNA gene expression was activated by either core or myc-F protein (Fig. 1C). However, no tumor formation was observed in these transgenic mice even up to 80 weeks(data not shown).

The transgenic mice with core protein derived from HCV genotype 1b were also generated (Fig. 1D). The liver/body weight ratio of these transgenic mice is also higher than that of wild type mice (Fig. 1B). However, no tumor formation was observed in these transgenic mice(data not shown).

Expression of authentic F protein in the transgenic mice could not be detected by Western blotting analysis, though the mRNA expression could be detected by RT-PCR (Fig. 1E). The liver/body weight ratio of the transgenic mice with F protein is also slightly higher than that of wild type mice (Fig. 1B). As expected, no tumor formation was observed in these transgenic mice(data not shown).

3.3. WNT signaling pathway was activated by HCV core or myc-F protein in the transgenic mice

Both core and myc-F proteins could induce liver cell proliferation in the transgenic mice (Fig. 1). To determine which cellular genes were affected by core or myc-F protein, microarray analysis was performed using mRNAs derived from the 53 week-old wild type mice and the transgenic mice with either core or myc-F protein. Compared to the wild-type mice, gene expression, modulated by core or myc-F protein more than fourfold in the transgenic mice, was further analyzed by David Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/). The most significant pathway activated by core or myc-F protein was WNT signaling pathway (data not shown). Many ligands (WNT super-family), receptors and genes enhancing this signaling pathway were up-regulated while genes repressing this signaling pathway were down-regulated by core or myc-F protein(data not shown). It has been demonstrated that WNT-1 was up-regulated by core protein in cultured cells [29]. However, unlike other WNT members (e.g. WNT6, WNT7a, WNT9a) up-regulated more than four folds by core protein in the microarray analysis, WNT-1 was only up-regulated slightly (data not shown). To complement the information derived from the microarray analysis, Western blotting analysis was performed. Indeed, WNT-1 was up-regulated significantly by core or myc-F protein when determined by Western blotting analysis (Fig. 2A). Casein kinase II, a positive regulator of WNT pathway, was also analyzed by Western blotting. As shown in Fig. 2B, CKII β protein was significantly activated by core or myc-F protein. To further confirm the results from the microarray analysis, β-catenin and its phosphorylated form were determined by Western blotting analysis. The β-catenin protein was up-regulated by both core and myc-F protein while its phosphorylated form was reduced by either protein (Fig. 2C). The un-phosphorylated β-catenin protein could go to nucleus and activate many genes, e.g. c-myc [30]. As expected, the c-myc gene expression was activated by either core or myc-F protein in the transgenic mice (Fig. 2D).

3.4. SMA protein expression was enhanced by core but not myc-F protein in the transgenic mice

HCV core protein could promote liver fibrogenesis via up-regulation of CTGF with TGF-β. The fibrosis marker SMA, expressed in the fibroblasts and accumulated in the fibrotic tissue, was activated by core protein in HepG2 cells [31]. In agreement with the findings in the cultured cells, expression of SMA was also enhanced

Table 2
The liver/body weight ratio of the transgenic mice after the treatment of DEN or PB.

	Treatment	Liver/body weight ratio	Compared with Wt (*P*-value)
Wild type	DEN	0.051415 ± 0.001589	–
Core 1a	DEN	0.051216 ± 0.002703	$P > 0.05$
myc-F	DEN	0.050092 ± 0.002309	$P > 0.05$
Wild type	PB	0.066041 ± 0.004543	–
Core 1a	PB	0.078847 ± 0.012163	$P < 0.05$
myc-F	PB	0.076820 ± 0.010091	$P < 0.05$

by core protein in the transgenic mice. On the other hand, expression of SMA protein was not enhanced by myc-F protein in the transgenic mice (Fig. 2E).

3.5. PB but not DEN treatment could further increase the liver/body weight ratio of the transgenic mice with core or myc-F protein

Both core and myc-F proteins could enhance liver cell proliferation though no tumor formation was observed in the transgenic mice (Fig. 1B). It is well known that one tumor initiator in cooperation with one tumor promoter (e.g., DEN, as a tumor initiator, and PB, as a tumor promoter) induces tumor formation, but neither one of them alone could induce tumor formation [32,33]. Therefore, another factor (e.g., a tumor initiator or a tumor promoter) is probably needed to induce tumor formation in the transgenic mice with core or myc-F protein. When treated with DEN (Fig. 3A), the liver/body weight ratio of mice with or without the transgenic gene was not increased comparing with that of non-treated ones (Table 1). On the contrary, the liver/body weight ratio of mice with or without the transgenic gene with PB treatment was increased significantly comparing with that of non-treated ones (Fig. 3A and Table 1). When the transgenic mice with core or myc-F protein were treated with DEN, the liver/body weight ratio did not increase comparing with that of wild type mice (Fig. 3B and Table 2). On the other hand, when the transgenic mice with core or myc-F protein were treated with PB, the liver/body weight ratio was increased comparing with that of wild type mice (Fig. 3C and Table 2). However, neither DEN nor PB treatment induced tumor formation in the transgenic mice with core or myc-F protein(data not shown).

4. Discussion

It has been demonstrated that HCV core protein induced the formation of hepatocellular carcinoma in the transgenic mice [21]. However, no tumor formation was observed in this study in the transgenic mice with core proteins from different HCV genotypes, even in the presence of DEN or PB(data not shown). It is possible that the mouse strain and/or the promoter used to express the transgenes make the differences. It is interesting to point out that core protein is more often to cause diseases in C57BL/6 mice under the control of hepatitis B viral promoters [34]. Therefore, it is not surprising that core protein did not induce tumor formation in this study.

From the results of the microarray analysis, WNT signaling pathway was the major pathway activated by core and myc-F proteins (data not shown). Western blotting analysis showed that HCV core and myc-F proteins up-regulated WNT1 and CKII proteins, which in turn activated β-catenin (Fig. 2A–C). As expected, c-myc protein, regulated by β-catenin, was also up-regulated (Fig. 2D). It is known that RB1, p53 and WNT pathways were commonly affected in HCCs of different etiology [35]. WNT-1 was reported previously to be up-regulated by core protein in HuH7 cells [29]. The oncogene c-myc expression has also been demonstrated to be activated by core or F protein in cultured cells [19,36,37]. In agreement with these reports, the WNT signaling pathway was activated by core and myc-F proteins in our transgenic mice. This could cause liver cell proliferation of the transgenic mice with core or myc-F protein (Fig. 1B and C). How core or myc-F protein activates the WNT signaling pathway needs further investigation.

The c-myc protein is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation [38]. Protein kinase CK2 also plays a central role in the control of a variety of pathways in cell proliferation, transformation, apoptosis and senescence [39]. Both core and myc-F proteins could have oncogenic potential through up-regulation of c-myc and CK2 proteins (Fig. 2).

Both core and myc-F proteins could induce liver cell proliferation (Fig. 1). PB (as a tumor promoter), but not DEN treatment, could further cooperate with core or myc-F protein to increase the liver/body weight ratio (Table 2). Therefore, core and myc-F proteins may play a role to induce cell transformation, both act similarly as a tumor initiator. This is supported by the identification of WNT signaling pathway activated by core and myc-F proteins using microarray analysis and Western blotting analysis(data not shown and Fig. 2). On the other hand, core and myc-F proteins could perform different functions, e.g. core, but not myc-F protein, could increase SMA gene expression (Fig. 2E).

Similar to myc-F protein, the authentic F protein, though labile, could still increase the liver/body weight ratio to a less extent (Fig. 1B). Therefore, myc-F protein acted as a stabilized form of F protein in the transgenic mice just like they were in cultured cells(-data not shown). In conclusion, both HCV core and F proteins could enhance liver cell proliferation in the transgenic mice possibly through the activation of WNT signaling pathway.

Acknowledgments

We thank Dr. C.M. Rice for providing the plasmid p90/HCV FL-long pU, Drs. K.S. Jeng and T.F. Tsai for the expression vector in transgenic mice. This work was supported by grants from the National Science Council of Taiwan (NSC 97-3112-B-320-001 and NSC 98-2320-B-320-001) and from the Tzu Chi University (TCRPP1 00016 and TCRPP101017) to Dr. Shih-Yen Lo.

References

[1] M. Levrero, Viral hepatitis and liver cancer: the case of hepatitis C, Oncogene 25 (2006) 3834–3847.
[2] S.M. Lemon, C.M. Walker, M.J. Alter, M. Yi, Hepatitis C Virus, In: D.M. Knipe, P.M. Howley (Eds.), Fields' Virology, Lippincott Williams & Wilkins, Philadelphia, 2007, pp. 1253–1304.
[3] M. Baril, L. Brakier-Gingras, Translation of the F protein of hepatitis C virus is initiated at a non-AUG codon in a +1 reading frame relative to the polyprotein, Nucleic Acids Res. 33 (2005) 1474–1486.
[4] Z. Xu, J. Choi, T.S. Yen, W. Lu, A. Strohecker, S. Govindarajan, D. Chien, M.J. Selby, J. Ou, Synthesis of a novel hepatitis C virus protein by ribosomal frameshift, EMBO J. 20 (2001) 3840–3848.
[5] N. Vassilaki, P. Mavromara, Two alternative translation mechanisms are responsible for the expression of the HCV ARFP/F/core+1 coding open reading frame, J. Biol. Chem. 278 (2003) 40503–40513.
[6] N. Vassilaki, H. Boleti, P. Mavromara, Expression studies of the HCV-1a core+1 open reading frame in mammalian cells, Virus Res. 133 (2008) 123–135.
[7] M. Wolf, M. Dimitrova, T.F. Baumert, C. Schuster, The major form of hepatitis C virus alternate reading frame protein is suppressed by core protein expression, Nucleic Acids Res. 36 (2008) 3054–3064.
[8] N. Vassilaki, P. Mavromara, The HCV ARFP/F/core+1 protein: production and functional analysis of an unconventional viral product, IUBMB Life 61 (2009) 739–752.
[9] L.K. McMullan, A. Grakoui, M.J. Evans, K. Mihalik, M. Puig, A.D. Branch, S.M. Feinstone, C.M. Rice, Evidence for a functional RNA element in the hepatitis C virus core gene, Proc. Natl. Acad. Sci. USA 104 (2007) 2879–2884.
[10] A.D. Branch, D.D. Stump, J.A. Gutierrez, F. Eng, J.L. Walewski, The hepatitis C virus alternate reading frame (ARF) and its family of novel products: the alternate reading frame protein/F-protein, the double-frameshift protein, and others, Semin. Liver Dis. 25 (2005) 105–117.
[11] A. Budkowska, A. Kakkanas, E. Nerrienet, O. Kalinina, P. Maillard, S.V. Horm, G. Dalagiorgou, N. Vassilaki, U. Georgopoulou, M. Martinot, A.A. Sall, P. Mavromara, Synonymous mutations in the core gene are linked to unusual serological profile in hepatitis C virus infection, PLoS One 6 (2011) e15871.
[12] F. Komurian-Pradel, A. Rajoharison, J.L. Berland, V. Khouri, M. Perret, M. Van Roosmalen, S. Pol, F. Negro, G. Paranhos-Baccala, Antigenic relevance of F protein in chronic hepatitis C virus infection, Hepatology 40 (2004) 900–909.
[13] M. Ratinier, S. Boulant, S. Crussard, J. McLauchlan, J.P. Lavergne, Subcellular localizations of the hepatitis C virus alternate reading frame proteins, Virus Res. 139 (2009) 106–110.
[14] J. Roussel, A. Pillez, C. Montpellier, G. Duverlie, A. Cahour, J. Dubuisson, C. Wychowski, Characterization of the expression of the hepatitis C virus F protein, J. Gen. Virol. 84 (2003) 1751–1759.

[15] Z. Xu, J. Choi, W. Lu, J.H. Ou, Hepatitis C virus f protein is a short-lived protein associated with the endoplasmic reticulum, J. Virol. 77 (2003) 1578–1583.
[16] A. Basu, R. Steele, R. Ray, R.B. Ray, Functional properties of a 16 kDa protein translated from an alternative open reading frame of the core-encoding genomic region of hepatitis C virus, J. Gen. Virol. 85 (2004) 2299–2306.
[17] H.C. Ma, T.W. Lin, H. Li, S.M. Iguchi-Ariga, H. Ariga, Y.L. Chuang, J.H. Ou, S.Y. Lo, Hepatitis C virus ARFP/F protein interacts with cellular MM-1 protein and enhances the gene trans-activation activity of c-Myc, J. Biomed. Sci. 15 (2008) 417–425.
[18] M.L. Tsao, C.H. Chao, C.T. Yeh, Interaction of hepatitis C virus F protein with prefoldin 2 perturbs tubulin cytoskeleton organization, Biochem. Biophys. Res. Commun. 348 (2006) 271–277.
[19] W.B. Wu, S.W. Shao, L.J. Zhao, J. Luan, J. Cao, J. Gao, S.Y. Zhu, Z.T. Qi, Hepatitis C virus F protein up-regulates c-myc and down-regulates p53 in human hepatoma HepG2 cells, Intervirology 50 (2007) 341–346.
[20] G. Dalagiorgou, N. Vassilaki, P. Foka, A. Boumlic, A. Kakkanas, E. Kochlios, S. Khalili, E. Aslanoglou, S. Veletza, G. Orfanoudakis, D. Vassilopoulos, S.J. Hadziyannis, J. Koskinas, P. Mavromara, High levels of HCV core+1 antibodies in HCV patients with hepatocellular carcinoma, J. Gen. Virol. 92 (2011) 1343–1351.
[21] K. Moriya, H. Fujie, Y. Shintani, H. Yotsuyanagi, T. Tsutsumi, K. Ishibashi, Y. Matsuura, S. Kimura, T. Miyamura, K. Koike, The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice, Nat. Med. 4 (1998) 1065–1067.
[22] H.C. Ma, Y.Y. Ku, Y.C. Hsieh, S.Y. Lo, Characterization of the cleavage of signal peptide at the C-terminus of hepatitis C virus core protein by signal peptide peptidase, J. Biomed. Sci. 14 (2007) 31–41.
[23] C.H. Yang, H.C. Li, J.G. Jiang, C.F. Hsu, Y.J. Wang, M.J. Lai, Y.L. Juang, S.Y. Lo, Enterovirus type 71 2A protease functions as a transcriptional activator in yeast, J. Biomed. Sci. 17 (2010) 65.
[24] W.Y. Wei, H.C. Li, C.Y. Chen, C.H. Yang, S.K. Lee, C.W. Wang, H.C. Ma, Y.L. Juang, S.Y. Lo, SARS-CoV nucleocapsid protein interacts with cellular pyruvate kinase protein and inhibits its activity, Arch. Virol. 157 (2012) 635–645.
[25] H.C. Lin, Y.F. Chen, W.H. Hsu, C.W. Yang, C.H. Kao, T.F. Tsai, Resveratrol helps recovery from fatty liver and protects against hepatocellular carcinoma induced by hepatitis B virus X protein in a mouse model, Cancer Prev. Res. (Phila) 5 (2012) 952–962.
[26] S.Y. Lo, M.J. Selby, J.H. Ou, Interaction between hepatitis C virus core protein and E1 envelope protein, J. Virol. 70 (1996) 5177–5182.
[27] H.C. Ma, C.H. Ke, T.Y. Hsieh, S.Y. Lo, The first hydrophobic domain of the hepatitis C virus E1 protein is important for interaction with the capsid protein, J. Gen. Virol. 83 (2002) 3085–3092.
[28] K. Yuksek, W.L. Chen, D. Chien, J.H. Ou, Ubiquitin-independent degradation of hepatitis C virus F protein, J. Virol. 83 (2009) 612–621.
[29] T. Fukutomi, Y. Zhou, S. Kawai, H. Eguchi, J.R. Wands, J. Li, Hepatitis C virus core protein stimulates hepatocyte growth: correlation with upregulation of wnt-1 expression, Hepatology 41 (2005) 1096–1105.
[30] F. Marks, U. Klingmuller, K. Muller-Decker, Signal transduction by receptors with seven transmembrane domains, in: J. Foltin (Ed.), Cellular Signal Processing, Garland Science, Taylor and Francis Group, LLC, New York, 2009, pp. 213–215.
[31] J.Y. Shin, W. Hur, J.S. Wang, J.W. Jang, C.W. Kim, S.H. Bae, S.K. Jang, S.H. Yang, Y.C. Sung, O.J. Kwon, S.K. Yoon, HCV core protein promotes liver fibrogenesis via up-regulation of CTGF with TGF-beta1, Exp. Mol. Med. 37 (2005) 138–145.
[32] V. Armuth, I. Berenblum, Tritiated thymidine as a broad spectrum initiator in transplacental two-stage carcinogenesis, with phorbol as promoter, Int. J. Cancer 24 (1979) 355–358.
[33] I. Berenblum, Carcinogenicity testing for control of environmental tumor development in man, Isr. J. Med. Sci. 15 (1979) 473–479.
[34] D. Kremsdorf, N. Brezillon, New animal models for hepatitis C viral infection and pathogenesis studies, World J. Gastroenterol. 13 (2007) 2427–2435.
[35] Y. Edamoto, A. Hara, W. Biernat, L. Terracciano, G. Cathomas, H.M. Riehle, M. Matsuda, H. Fujii, J.Y. Scoazec, H. Ohgaki, Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis, Int. J. Cancer 106 (2003) 334–341.
[36] R.B. Ray, L.M. Lagging, K. Meyer, R. Ray, Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype, J. Virol. 70 (1996) 4438–4443.
[37] R.B. Ray, L.M. Lagging, K. Meyer, R. Steele, R. Ray, Transcriptional regulation of cellular and viral promoters by the hepatitis C virus core protein, Virus Res. 37 (1995) 209–220.
[38] C.V. Dang, MYC on the path to cancer, Cell 149 (2012) 22–35.
[39] N.A. St-Denis, D.W. Litchfield, Protein kinase CK2 in health and disease: from birth to death: the role of protein kinase CK2 in the regulation of cell proliferation and survival, Cell Mol. Life Sci. 66 (2009) 1817–1829.