

Table 1 Transgenic mouse lines constitutively expressing hepatitis C virus proteins

HCV gene	Genotype	Promoter	Protein expression	Phenotypes	References
Core	1b	HBV	Similar to patients	Steatosis, HCC, insulin resistance, oxidative stress	Moriya 1997 ²¹ & 1998 ²² Tsutsumi 2002 ²³ & 2003 ²⁴ Moriishi 2003 ¹⁶ & 2007 ²⁵ Shintani 2004 ²⁶ Miyamoto 2007 ¹⁵ Tanaka 2008 ^{27,28} Machida 2006 ²⁹
Core	1b	EF-1a	Similar to patients	Steatosis, adenoma, HCC, oxidative stress	Pasquinelli 1998 ³⁰ Koike 1995, ³¹ Koike 1997 ³²
Core, E2 truncated	1b	MUP	(–)	None	Lerat 2003 ³³
E1-E2	1b	HBV	Abundant	None in the liver	Naas 2005 ³⁴
Core-E1-E2	1b	Albumin	Similar to patients	Steatosis, HCC, oxidative stress	Kamegaya 2005 ³⁵
Core-E1-E2	1a	CMV	Similar to patients	Steatosis, HCC	
Core or structural proteins	1b	Alb	Low	Larger tumor development with DEN treatment	
Structural proteins	1b	MUP	Low	None	Kawamura 1998 ³⁶
Structural proteins	1b	MHC	Low in the liver	Hepatitis	Honda 1999 ³⁷
Entire polyprotein	1b	Albumin	Only mRNA detectable	Steatosis, HCC	Lerat 2003 ³³
Entire polyprotein	1a	A1-antitrypsin		Steatosis, intrahepatic T cell recruitment	Alonzi 2004 ³⁸
NS3/4A	1a	MUP		None (modulation of immunity)	Frelin 2006 ³⁹
NS5A	1a	apoE		None (resistance to TNF)	Majumder 2002 ⁴⁰

HBV, hepatitis B virus; EF, elongation factor; MUP, major urinary protein; Alb, albumin; CMV, cytomegalovirus; MHC, major histocompatibility complex; AT, anti-trypsin; apo E, apolipoprotein E.

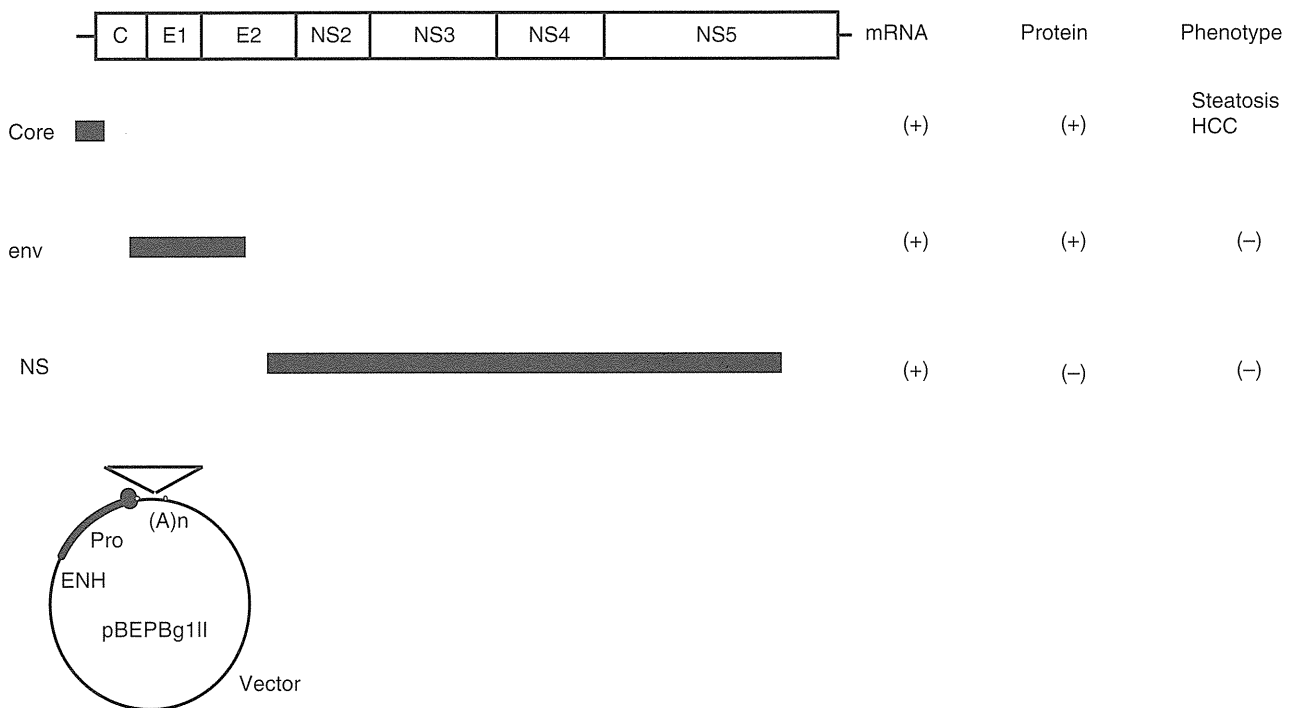


Figure 2 Transgenic mouse lines carrying the hepatitis C virus (HCV) genome.^{21,22,31,32,42} Three different kinds of transgenic mouse lines, carrying the core gene, envelope genes or non-structural genes of HCV, respectively, were established under the control of the same regulatory elements. Among these mouse strains, only the transgenic mice carrying the HCV core gene develop hepatocellular carcinoma (HCC) after an early phase with hepatic steatosis in two independent lineages. The mice transgenic for the envelope genes or non-structural genes do not develop HCC. env, envelope genes; NS, nonstructural genes.

OXIDATIVE STRESS AND INTRACELLULAR SIGNALING IN HCV-ASSOCIATED PATHOGENESIS

IT IS DIFFICULT to elucidate the mechanism underlying the development of HCC, even for our simple model in which only the core protein is expressed in otherwise normal liver. There is a notable feature in the localization of the core protein in hepatocytes; while the core protein predominantly exists in the cytoplasm associated with lipid droplets, it is also present in the mitochondria and nuclei.^{14,22} On the basis of this finding, the pathways related to these two organelles, the mitochondria and nuclei, were thoroughly investigated.

One effect of the core protein is an increased production of oxidative stress in the liver. We would like to draw particular attention to the fact that the production of oxidative stress is increased in our transgenic mouse model in the absence of inflammation in the liver. This reflects a state of an overproduction of reactive oxygen species (ROS) in the liver, or predisposition to it, which is staged by the HCV core protein without any interven-

ing inflammation.^{44,45} The overproduction of oxidative stress results in the generation of deletions in the mitochondrial and nuclear DNA, an indicator of genetic damage. In addition, analysis of the antioxidant system revealed that some antioxidative molecules are not increased despite the overproduction of ROS in the liver of core gene transgenic mice. These results suggest that HCV core protein not only induces overproduction of ROS but also attenuates some of the antioxidant system, which may explain the mechanism underlying the production of a strong oxidative stress in HCV infection compared to other forms of hepatitis.

Thus, in the absence of inflammation, the core protein induces oxidative stress overproduction, which may, at least in part, contribute to hepatocarcinogenesis in HCV infection. If inflammation were added to the liver with the HCV core protein, the production of oxidative stress would be escalated to an extent that cannot be scavenged anymore by a physiological antagonistic system. This suggests that the inflammation in chronic HCV infection would have a characteristic different in its quality from those of other types of

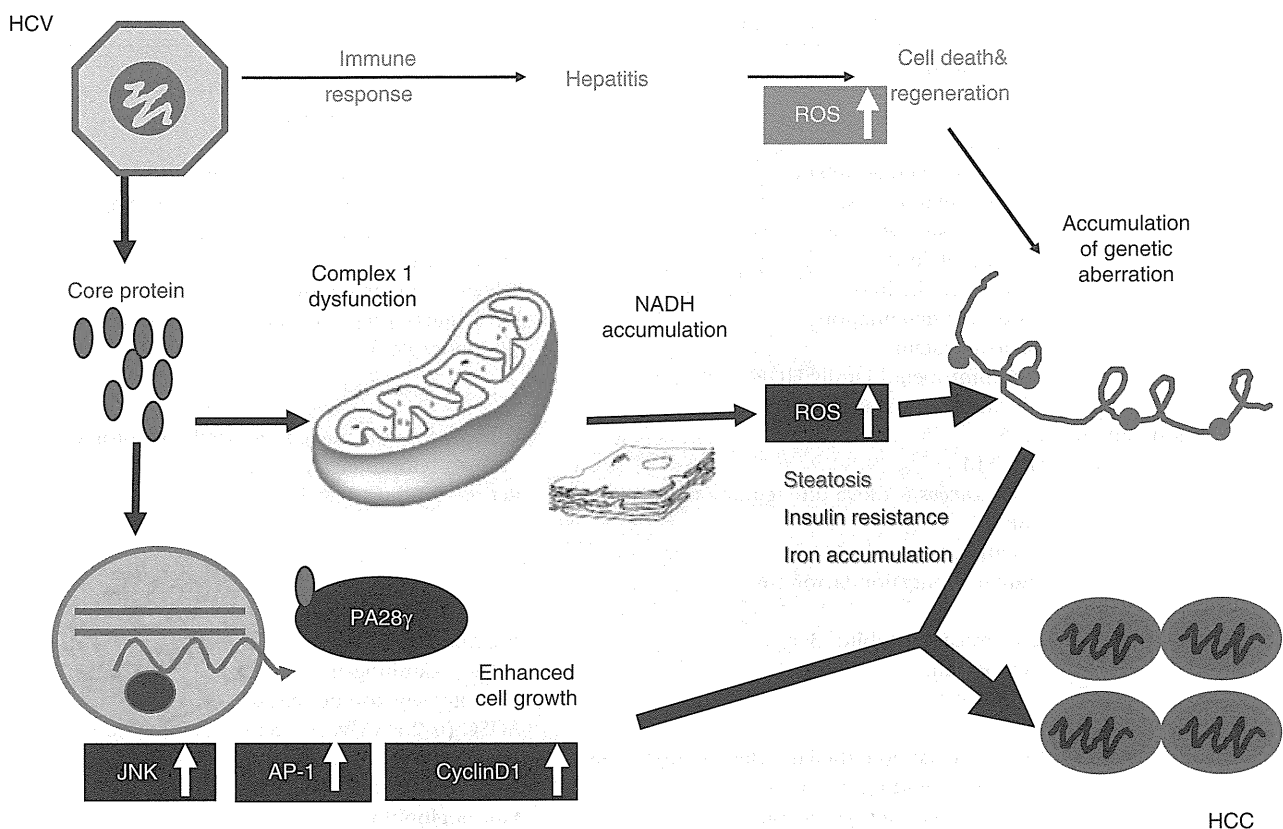


Figure 3 Mechanism of hepatocarcinogenesis in hepatitis C virus (HCV) infection. The core protein is localized in the nuclei of hepatocytes in addition to cytoplasm and may interact with nuclear factors, thereby deregulating the cell growth and death. The core protein may also affect the cell growth by its accumulating lipid in the hepatocytes, because lipid such as triglycerides are now known to be ligands for some nuclear receptors. Accumulated lipid may also cause genetic aberrations through its alteration to peroxy lipid. AP-1, activating protein-1; HCC, hepatocellular carcinoma; JNK, c-Jun N-terminal kinase; NADH, nicotinamide adenine dinucleotide; PA28, proteasome activator 28; ROS, reactive oxygen species.

hepatitis, such as autoimmune hepatitis. The basis for the overproduction of oxidative stress may be ascribed to the mitochondrial dysfunction.^{22,44} The dysfunction of the electron transfer system of the mitochondrion is suggested in association with the presence of the HCV core protein.⁴⁶

Other pathways in hepatocarcinogenesis would be the alteration of the expression of cellular genes and modulation of intracellular signaling pathways. For example, tumor necrosis factor (TNF)- α and interleukin (IL)-1 β have been found transcriptionally activated.²³ The mitogen-activated protein kinase (MAPK) cascade is also activated in the liver of the core gene transgenic mouse model. The MAPK pathway, which consists of three routes, c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK), is involved

in numerous cellular events including cell proliferation. In the liver of the core gene transgenic mouse model prior to HCC development, only the JNK route is activated. In the downstream of the JNK activation, transcription factor activating protein (AP)-1 activation is markedly enhanced.^{23,24} Far downstream, both the mRNA and protein levels of cyclin D1 and cyclin-dependent kinase (CDK)4 are increased. Thus, the HCV core protein modulates the intracellular signaling pathways and gives advantage to cell proliferation to hepatocytes (Fig. 3).

METABOLIC ASPECTS OF HCV INFECTION

STEATOSIS IS FREQUENTLY observed in chronic hepatitis C patients, and is significantly associated

Table 2 Cellular genes differentially expressed in hepatitis C virus core transgenic mouse³⁶

Liver	Upregulated	Downregulated
Lipid metabolism	NPC1	Stearoyl-CoA desaturase
	Catalase	Sterol-carrier protein X
	Very long chain acyl-CoA	α -Enolase carnitine acetyltransferase
	Dehydrogenase	Gal- β 1,4(3) GlcNAc- α 2,3-Sialyltransferase
	Carboxylesterase	Very long chain acyl-CoA synthetase
	Selenoprotein P	Liver transferrin
	Carbonic anhydrase	4-Hydroxyphenylpyruvate dioxygenase
	Adipose differentiation	LAF1 transketolases-adenosylmethionine synthetase
	Related protein	Apolipoprotein A-II
	Bilirubin/phenol family UDP	
Transcription and cell proliferation	Glucuronosyltransferase	
	Int-6	Human guanine nucleotide regulatory protein
	GCN5L1	alpha-fetoprotein
	<i>Homo sapiens</i> 8.2 kDa differentiation factor	Retinol binding protein
	USF1	
	Initiation factor eIF-4A1	
	Human elongation factor-1- δ	
Inflammation	Sui1	
	α -1 Protease inhibitor 3	α -2-Macroglobulin
	Hemopexin	LMW prekininogen
Others		Complement component C3
	Microvascular endothelial differentiation gene 1	AHSG (α -2 HS-glycoprotein) homolog
	Diazepam-binding inhibitor	Vitronectin
	Argininosuccinate synthetase	Epithelin 1 and 2
	Skeletal muscle- α tropomyosin	Murinoglobulin
	Ampd3 gene	
	DNA-binding protein	

with increased fibrosis and progression rate of fibrosis of the liver.⁴⁷ A comprehensive analysis of gene expression in the liver of core gene transgenic mice, in which steatosis develops from early in life, revealed that a number of genes related to lipid metabolism are significantly up- or downregulated (Table 2).

The composition of fatty acids that are accumulated in the liver of core gene transgenic mice is different from that in fatty liver due to simple obesity. Carbon 18 mono-unsaturated fatty acids (C18:1) such as oleic or vaccenic acids are significantly increased. This is also the case in the comparison of liver tissues from hepatitis C patients and simple fatty liver patients due to obesity.⁴⁵ The mechanism of steatogenesis in hepatitis C was investigated using this mouse model. There are at least three pathways for the development of steatosis. One is the frequent presence of insulin resistance in hepatitis C patients as well as in the core gene transgenic mice, which occurs through the inhibition of tyrosine-phosphorylation of insulin receptor substrate (IRS)-1.²⁶

Insulin resistance increases the peripheral release and hepatic uptake of fatty acids, resulting in an accumulation of lipid in the liver. The second pathway is the suppression of the activity of microsomal triglyceride transfer protein (MTP) by HCV core protein.⁴⁸ This inhibits the secretion of very low density protein (VLDL) from the liver, yielding an increase of triglycerides in the liver. The last one involves the sterol regulatory element-binding protein (SREBP)-1c, which regulates the production of triglycerides and phospholipids. In HCV core gene transgenic mice, SREBP-1c is activated, while neither SREBP-2 nor SREBP-1a is upregulated.²⁵ This corroborates the results in *in vitro* studies^{49,50} and a chimpanzee study.⁵¹ In humans, McPharson *et al.* have reported that there was no significant difference in the level of SREBP-1c mRNA in the liver tissues of chronic hepatitis C patients and normal subjects.⁵² However, the number of samples in that study was small, and a larger number must be examined to draw a conclusion in human patients.

PROTEASOME ACTIVATOR 28 γ AND HCV

INTERESTINGLY, WE FOUND recently that a protein interacting with the core protein, proteasome activator (PA)28 γ , is indispensable for the core protein to exert its function for the development of steatosis, insulin resistance and HCC.^{15,25} The pathogenic mechanisms underlying the core protein-induced diseases are summarized in Figure 3. Steatosis is defined as an accumulation of lipid droplets, the majority of which are triglycerides. Biosynthesis of triglycerides is mainly regulated by SREBP-1c. Transcription of SREBP-1c is controlled by a heterodimer of nuclear hormone receptors, liver X receptor (LXR)- α and retinoid X receptor (RXR)- α . Indeed, it has been reported that many genes regulated by SREBP were induced during the early stage of HCV infection in the livers of chimpanzees.⁵³ Our study has demonstrated that the core protein enhances the binding activity of the LXR- α -RXR- α complex to the *srebp-1c* promoter in a PA28 γ -dependent manner, resulting in upregulation of SREBP-1c and its regulating genes.²⁵ The activation may be mediated by the direct interaction between the core protein and RXR- α ⁵⁴ or by suppression of a co-repressor such as Sp110b, a negative regulator of RAR- α , by sequestering it in the cytoplasm through interaction with the cytoplasmic core protein.⁵⁵ Another mechanism is thought to be suppression of lipid secretion. Reduced serum levels of cholesterol and apolipoprotein B have been reported in patients with severe hepatitis C and the core gene transgenic mice.⁵⁶ As stated before, the MTP regulates the assembly and secretion of VLDL consisting of apolipoprotein B, cholesterol and triglycerides. In the core gene transgenic mice, MTP-specific activity is significantly decreased.⁴⁸ Therefore, the downregulation of MTP may be involved in the development of the steatosis cooperating with upregulation of SREBP-1c, although the precise role of HCV core protein is still unclear. Recently, it has been reported that the assembly and budding of HCV occur around the accumulated lipid droplets within the endoplasmic reticulum.⁵⁷ Furthermore, increases in saturated and monounsaturated fatty acids enhance HCV RNA replication.⁵⁸ These data suggest that regulation of lipid metabolism by the core protein plays crucial roles in the HCV life cycle. Obesity and hepatic steatosis often result in insulin resistance. However, 1- to 2-month-old core gene transgenic mice, which do not exhibit apparent steatosis and obesity, already exhibit insulin resistance due to a decrease in insulin sensitivity in the liver.^{15,26} Moreover, the core gene transgenic mice have been shown to exhibit overt diabetes when fed a high-fat diet,

while control mice do not.²⁶ Binding of insulin to the insulin receptor triggers tyrosine phosphorylation of the IRS proteins, leading to the following signal transductions to increase glucose uptake and inhibit the net production of glucose in the liver. An inflammatory cytokine, TNF- α , is known to impair the insulin-signaling pathway through inhibition of tyrosine phosphorylation of IRS. In fact, the overproduction of TNF- α has been reported to reduce the phosphorylation of IRS-1 and Akt in the core gene transgenic mice despite the absence of hepatic steatosis.^{15,26} Moreover, in the latter study, hyperinsulinemia was cured by depletion of TNF- α , suggesting that upregulation of TNF- α contributes to the core protein-induced insulin resistance.²⁶ Our previous study has indicated that the core protein-induced overexpression of TNF- α is also dependent on the presence of PA28 γ .¹⁵

In relation to lipid metabolism, the core protein has also been found to interact with RXR- α .⁵⁹ RXR- α is one of the nuclear receptors, which forms a homodimer or heterodimers with other nuclear receptors including peroxisome proliferator-activated receptor (PPAR)- α , and plays a pivotal role in the regulation of the expression of genes relating to lipid metabolism, cell differentiation and proliferation. In fact, the core protein of HCV activates genes that have an RXR- α -responsive element as well as those with a PPAR- α -responsive element, in both mice and cultured cells.⁵⁵ Based on these results, we then examined the expression and function of PPAR- α in the liver of core gene transgenic mice.

PPAR- α AND “FATTY ACID SPIRAL” IN HCV-ASSOCIATED HEPATOCARCINOGENESIS

PEROXISOME PROLIFERATOR-ACTIVATED receptor- α is one of the PPAR genes, and plays a central role, as a heterodimer with RXR- α , in regulating fatty acid transport and catabolism. It is also known as a molecular target for lipid-lowering fibrate drugs.⁶⁰ On the other hand, a prolonged administration of PPAR- α agonists causes HCC in rodents. Currently, there is little evidence that the low-affinity fibrate ligands are associated with human cancers, but it is possible that chronic activation of high-affinity ligands could be carcinogenic in humans.⁵⁶

The level of PPAR- α protein was increased in the liver of core gene transgenic mice as early as 9 months old. PPAR- α protein is accumulated with age in the nuclei of hepatocytes together with cyclin D1 protein. However, the level of PPAR- α mRNA was not increased at any age.

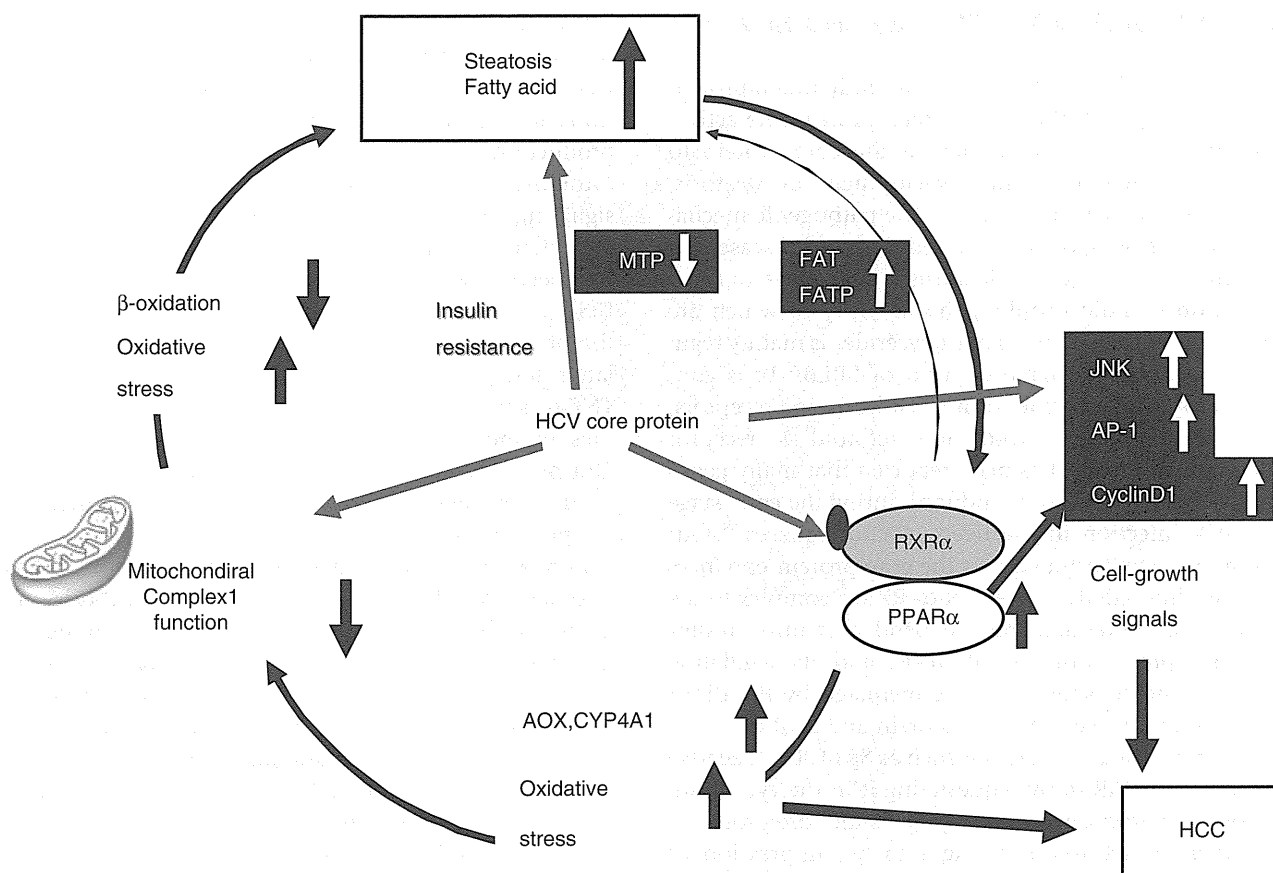


Figure 4 Hepatitis C virus (HCV) core protein causes “fatty acid spiral”. In HCV infection, the core protein induces steatosis through several pathways, leading to “fatty acid spiral” in the presence of the mitochondrial complex 1 dysfunction and peroxisome proliferator-activated receptor- α (PPAR- α) activation, both of which are also caused by the core protein. These intracellular alterations would contribute to hepatocarcinogenesis by inducing oxidative stress overproduction and cell-growth signal activation. In such a sense, the core protein of HCV is not a classical type oncoprotein, but rather seems to contribute to hepatocarcinogenesis by modulating intracellular metabolism and signaling. AOX, acyl-CoA oxidase; AP-1, activating protein-1; CYP, cytochrome P450; FAT, fatty acid translocase; FATP, fatty acid transport protein; HCC, hepatocellular carcinoma; JNK, c-Jun N-terminal kinase; MTP, microsomal triglyceride transfer protein; RXR- α , retinoid X receptor- α .

By the pulse-chase experiment, the stability of nuclear PPAR- α turned out to be increased in the presence of the core protein. In line with the increase of PPAR- α protein, target genes of PPAR- α were activated in the liver of core gene transgenic mice; these genes include cyclin D1, CDK4, acy-CoA oxidase, and peroxisome thiolase.²⁷ However, in general, the activation of PPAR- α leads to improvement but not aggravation of steatosis. Then, what is the function of PPAR- α activation that is observed in the core gene transgenic mice?

To clarify the role of PPAR- α activation in pathogenesis of steatosis and HCC, we mated core gene transgenic mouse with PPAR- α knockout (KO) mouse, and studied the phenotype. PPAR- α KO mice have reduced

expressions of target genes of PPAR- α , and have mild steatosis in the liver as expected.²⁸ It was unanticipated, however, that steatosis was absent in PPAR- α -null or -heterozygous core gene transgenic mice but present in PPAR- α -intact core gene transgenic mice at the age of 9 or 24 months.²⁷ 8-Hydroxy deoxyguanosine (8-OHdG) and peroxy lipids, both of which are markers for oxidative stress, were decreased in PPAR- α KO core gene transgenic mice. Mitochondrial dysfunction in the core gene transgenic mice, which contributes to an overproduction of oxidative stress,^{46,61} was also improved in PPAR- α KO core gene transgenic mice.

Finally, PPAR- α KO core gene transgenic mice did not develop HCC at the age of 24 months, while approxi-

mately one-third of PPAR- α -intact core gene transgenic mice did. It should be noted that core gene transgenic mice that are heterozygous for the PPAR- α gene neither developed HCC.²⁸ When clofibrate, a peroxisome proliferator, was administered for 24 months to PPAR- α -heterozygous mice, either with or without the core gene, HCC developed in a higher rate in the core-gene⁺ mice with a greater PPAR- α activation. It should be noted that steatosis was present only in core-gene⁺ PPAR- α -heterozygous mice. In summary, steatosis and HCC developed in PPAR- α -intact but not in PPAR- α -heterozygous or PPAR- α -null core gene transgenic mice, indicating that not the presence but the persistent activation of PPAR- α would be important in hepatocarcinogenesis by HCV core protein. In general, PPAR- α acts to ameliorate steatosis, but with the presence of mitochondrial dysfunction, which is also provoked by the core protein, the core-activated PPAR- α may exacerbate steatosis. A persistent activation of PPAR- α with “strong” ligands such as the core protein of HCV could be carcinogenic in humans, although the low-affinity fibrate ligands are not likely associated with human cancers.

Figure 4 illustrates our current hypothesis for the role of lipid metabolism in HCV-associated hepatocarcinogenesis. Immune-mediated inflammation should also play a pivotal role in hepatocarcinogenesis in HCV infection. However, in HCV infection, the core protein induces steatosis through the above-mentioned pathways, leading to “fatty acid spiral” in the presence of the mitochondrial complex 1 dysfunction and PPAR- α activation, both of which are caused by the core protein. These intracellular alterations would contribute to hepatocarcinogenesis by inducing oxidative stress overproduction and cell-growth signal activation. In such a sense, the core protein of HCV is not a classical type oncoprotein, but rather seems to contribute to hepatocarcinogenesis by modulating intracellular metabolism and signaling.

CONCLUSION

THE RESULTS OF our studies on transgenic mice have indicated a carcinogenic potential of the HCV core protein *in vivo*; thus, HCV would be directly involved in hepatocarcinogenesis. In research studies of carcinogenesis, the theory by Kinzler and Vogelstein⁶² has gained a wide popularity. They have proposed that the development of colorectal cancer is induced by the accumulation of a complete set of cellular gene mutations. They have deduced that mutations in the

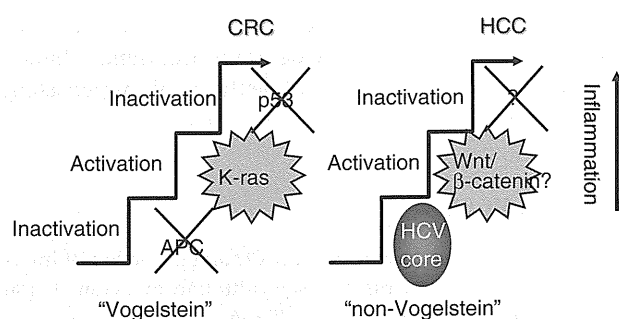


Figure 5 The role of hepatitis C virus (HCV) in hepatocarcinogenesis. Multiple steps are required in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that genetic mutations accumulate in hepatocytes. However, in HCV infection, some of these steps may be skipped in the development of hepatocellular carcinoma (HCC) in the presence of the core protein. The overall effects achieved by the expression of the core protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations, required for carcinogenesis. By considering such a “non-Vogelstein-type” process for the induction of HCC, a plausible explanation may be given for many unusual events happening in HCV carriers. APC, adenomatous polyposis coli; CRC, colorectal cancer.

adenomatous polyposis coli gene for inactivation, those in K-ras for activation and those in the p53 gene for inactivation accumulate, which cooperate toward the development of colorectal cancer.⁶² Their theory has been extended to the carcinogenesis of other cancers as well, called “Vogelstein-type” carcinogenesis (Fig. 5).

On the basis of the results we obtained for the induction of HCC by the HCV core protein, we would like to introduce a different mechanism for the hepatocarcinogenesis in HCV infection. We allow multi-stages in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that many mutations accumulate in hepatocytes. Some of these steps, however, may be skipped in the development of HCC in HCV infection to which the core protein would contribute (Fig. 5). The overall effects achieved by the expression of the viral protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations, required for carcinogenesis.

By considering such a “non-Vogelstein-type” process for the induction of HCC, a plausible explanation may be given for many unusual events happening in HCV carriers.⁴² Now it does not seem so difficult as before to determine why HCC develops in persistent HCV infection at an outstandingly high incidence.

Our theory may also give an account of the non-metastatic and multicentric de novo occurrence characteristics of HCC, which would be the result of persistent HCV infection.

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Steatosis, liver injury, and hepatocarcinogenesis in hepatitis C viral infection

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In addition to the link with development of hepatocellular carcinoma (HCC), hepatitis C virus (HCV) infection is associated with several hepatic and extrahepatic manifestations. A role of hepatic steatosis in the pathogenesis of chronic hepatitis C has been shown, implying hepatitis C as a metabolic disease. Furthermore, recent epidemiological studies have suggested a linkage between insulin resistance and chronic HCV infection. In addition to the data indicating the presence of lipid metabolism disturbance and insulin resistance in the cohort of chronic hepatitis C patients, we found evidence showing the association between these two conditions and HCV infection using mice transgenic for the HCV core gene. These mice develop HCC late in life after the phase of hepatic steatosis and insulin resistance. The nonappearance of both steatosis and HCC in HCV core gene transgenic mice that are null for the proteasome activator 28 γ implies a close relationship between lipid metabolism disturbance and hepatocarcinogenesis. Also, the core protein is shown to bind with retinoid X receptor (RXR)- α , resulting in the upregulation of some lipid metabolism enzymes, including cellular retinol binding protein II and acyl-CoA oxidase. In addition, the persistent activation of peroxisome proliferator activated receptor (PPAR)- α has recently been found in the liver of HCV core gene transgenic mice, yielding dramatic changes in lipid metabolism and hepatocyte proliferation, including HCC development. These results would provide a clue for further understanding of the role of lipid metabolism in pathogenesis of HCV infection, including liver injury and hepatocarcinogenesis.

Key words: lipid metabolism, transgenic mouse, oxidative stress, intracellular signal transduction, peroxisome proliferator activated receptor

Introduction

Worldwide, approximately 170 million people are persistently infected with hepatitis C virus (HCV), which induces a spectrum of chronic liver diseases from chronic hepatitis to cirrhosis and, eventually, to hepatocellular carcinoma (HCC).¹ HCV has been given increasing attention because of its wide and deep penetration in the community, tied with a very high incidence of HCC in persistent HCV infection. Once liver cirrhosis is established in hosts persistently infected with HCV, HCC develops at a yearly rate of approximately 7%,² resulting in the development of HCC in nearly 90% of HCV-associated cirrhotic patients in 15 years. In addition, the outstanding features in the mode of hepatocarcinogenesis in HCV infection, i.e., development of HCC in a multicentric fashion and at a very high incidence, are not common in other malignancies except for hereditary cancers such as familial polyposis of the colon. Knowledge of the mechanism underlying HCC development in persistent HCV infection, therefore, is imminently required for the prevention of HCC.

In addition to the link with development of HCC, HCV infection is associated with several hepatic and extrahepatic manifestations.³ A role of hepatic steatosis in the pathogenesis of chronic hepatitis C has been shown, implicating hepatitis C as a metabolic disease.⁴ Moreover, recent epidemiological studies have suggested a linkage between insulin resistance and chronic HCV infection.⁵ In addition to the epidemiological data indicating the presence of lipid metabolism disturbance and insulin resistance in the cohort of chronic hepatitis C patients, detailed analyses on the relationship between

Oxidative stress overproduction and intracellular signaling pathway activation are the major pathways in the core-induced liver pathology

It is difficult to elucidate the mechanism underlying the development of HCC, even for our simple model in which only the core protein is expressed in otherwise normal liver. There is a notable feature in the localization of the core protein in hepatocytes; while the core protein predominantly exists in the cytoplasm associated with lipid droplets, it is also present in the mitochondria and nuclei.^{10,17} On the basis of this finding, the pathways related to these two organelles, the mitochondria and nuclei, were thoroughly investigated.

One effect of the core protein is an increased production of oxidative stress in the liver. We would like to draw particular attention to the fact that the production of oxidative stress is increased in our transgenic mouse model in the absence of inflammation in the liver. This finding reflects a state of overproduction of reactive oxygen species (ROS) in the liver,¹⁸ or predisposition to it, which is staged by the HCV core protein without any intervening inflammation.^{19,20} The overproduction of oxidative stress results in the generation of deletions in mitochondrial and nuclear DNA, an indicator of genetic damage. In addition, analysis of antioxidant system revealed that some antioxidative molecules are not increased despite the overproduction of ROS in the liver of core gene transgenic mice: hemoxygenase-1 and glutathione peroxidase are not augmented whereas catalase and glutathione S-transferase levels are increased and enhanced by iron overloading (Moriya et al., manuscript in preparation). These results suggest that HCV core protein not only induces overproduction of ROS but also attenuates some of the antioxidant systems, which may explain the mechanism underlying the production of a strong oxidative stress in HCV infection compared to other forms of hepatitis.

In the absence of inflammation, thus, the core protein induces oxidative stress overproduction, which may, at least in part, contribute to hepatocarcinogenesis in HCV infection. If inflammation were added to the liver with the HCV core protein, the production of oxidative stress would be escalated to an extent that can no longer be scavenged by a physiological antagonistic system. This idea suggests that the inflammation in chronic HCV infection would have a characteristic difference in its quality from those of other types of hepatitis, such as autoimmune hepatitis. The basis for the overproduction of oxidative stress may be ascribed to the mitochondrial dysfunction.^{10,19} The dysfunction of the electron transfer system of the mitochondrion is suggested in association with the presence of the HCV core protein.²¹

Other pathways in hepatocarcinogenesis would be the alteration of the expression of cellular genes and modulation of intracellular signaling pathways. For example, tumor necrosis factor (TNF)- α and interleukin-1 β have been found to be transcriptionally activated.²² The mitogen-activated protein kinase (MAPK) cascade is also activated in the liver of the core gene transgenic mouse model. The MAPK pathway, which consists of three routes, c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK), is involved in numerous cellular events including cell proliferation. In the liver of the core gene transgenic mouse model before HCC development, only the JNK route is activated. Downstream of JNK activation, transcription factor activating protein (AP)-1 activation is markedly enhanced.^{20,21} At far downstream, both the mRNA and protein levels of cyclin D1 and CDK4 are increased. Thus, the HCV core protein modulates the intracellular signaling pathways and gives an advantage for cell proliferation to the hepatocytes. Interestingly, we found recently that a protein interacting with the core protein, proteasome activator 28 γ (PA28 γ), is indispensable for the core protein to exert its function for the development of steatosis, insulin resistance, and HCC.^{23,24}

Lipid metabolism and HCV infection

Steatosis is frequently observed in chronic hepatitis C patients and is significantly associated with increased fibrosis and progression rate of fibrosis of the liver.⁶ A comprehensive analysis of gene expression in the liver of core gene transgenic mice, in which steatosis develops from early in life, revealed that a number of genes related to lipid metabolism are significantly upregulated or downregulated (Table 1).

The composition of fatty acids that are accumulated in the liver of core gene transgenic mice is different from that in fatty liver resulting from simple obesity. Carbon-18 monounsaturated fatty acids (C18:1) such as oleic or vaccenic acids are significantly increased; this is also the case in the comparison of liver tissues from hepatitis C patients and patients with simple fatty liver due to obesity.²⁰ The mechanism of steatogenesis in hepatitis C was investigated using this mouse model. There are at least three pathways for the development of steatosis. One is the frequent presence of insulin resistance in hepatitis C patients as well as in the core gene transgenic mice, which occurs through the inhibition of tyrosine phosphorylation of insulin receptor substrate (IRS)-1.²⁵ Insulin resistance increases the peripheral release and hepatic uptake of fatty acids, resulting in an accumulation of lipid in the liver. The second pathway is the suppression of the activity of

Table 1. Cellular genes differentially expressed in hepatitis C virus (HCV) core transgenic mouse liver

	Upregulated	Downregulated
Lipid metabolism	NPC1 Catalase Very long chain acyl-CoA dehydrogenase Carboxylesterase selenoprotein P Carbonic anhydrase Adipose differentiation-related protein Bilirubin/phenol family UDP glucuronosyltransferase	Stearoyl-CoA desaturase Sterol-carrier protein X Alpha-enolase carnitine acetyltransferase Gal beta 1,4(3) GlcNAc alpha 2,3-sialyltransferase Very long chain acyl-CoA synthetase Liver transferrin 4-Hydroxyphenylpyruvate dioxygenase LAF1 transketolase s-Adenosylmethionine synthetase Apolipoprotein A-II
Transcription and cell proliferation	Int-6 GCN5L1 <i>H. sapiens</i> 8.2k-Da differentiation factor USF1 Initiation factor eIF-4AI Human elongation factor-1-delta Sui1	Human guanine nucleotide regulatory protein Alpha-fetoprotein Retinol binding protein
Inflammation	Alpha-1 protease inhibitor 3 Hemopexin	Alpha-2-macroglobulin LMW prekininogen Complement component C3 AHSG(alpha 2 HS-glycoprotein) homologue
Others	Microvascular endothelial differentiation gene 1 Diazepam-binding inhibitor Argininosuccinate synthetase Skeletal muscle alpha-tropomyosin Ampd3 gene DNA-binding protein	Vitronectin Epithelin 1 and 2 Murinoglobulin

microsomal triglyceride transfer protein (MTP) by HCV core protein²⁶; this inhibits the secretion of very low density protein (VLDL) from the liver, yielding an increase of triglycerides in the liver. The last pathway involves sterol regulatory element-binding protein (SREBP)-1c, which regulates the production of triglycerides and phospholipids. In HCV core gene transgenic mice, SREBP-1c is activated, whereas neither SREBP-2 nor SREBP-1a is upregulated.²⁷

In relation to lipid metabolism, the core protein has also been found to interact with retinoid X receptor (RXR)- α .²⁸ RXR- α is one of the nuclear receptors, which forms a homodimer or heterodimers with other nuclear receptors, including PPAR (peroxisome proliferator-activated receptor)- α , and plays a pivotal role in the regulation of the expression of genes relating to lipid metabolism, cell differentiation, and proliferation. In fact, the core protein of HCV activates genes that have an RXR- α -responsive element as well as those with a PPAR- α -responsive element, both in mice and in cultured cells.²⁸ Based on these results, we, then, examined the expression and function of PPAR- α in the liver of core gene transgenic mice.

PPAR- α activation in HCV-associated hepatocarcinogenesis

PPAR- α , one of the PPAR genes, plays a central role as a heterodimer with RXR- α in regulating fatty acid transport and catabolism. It is also known as a molecular target for lipid-lowering fibrate drugs.²⁹ On the other hand, prolonged administration of PPAR- α agonists causes HCC in rodents. Currently, there is little evidence that the low-affinity fibrate ligands are associated with human cancers, but it is possible that chronic activation of high-affinity ligands could be carcinogenic in humans.²⁹

The level of PPAR- α protein was increased in the liver of core gene transgenic mice as early as 9 months of age. PPAR- α protein is accumulated with age in the nuclei of hepatocytes together with cyclin D1 protein. However, the level of PPAR- α mRNA was not increased at any age. By pulse-chase experiment, the stability of nuclear PPAR- α was increased in the presence of the core protein. In line with the increase of PPAR- α protein, target genes of PPAR- α were activated in the liver of core gene transgenic mice; these genes include

cyclin D1, cyclin-dependent kinase (CDK)-4, acyl-CoA oxidase, and peroxisome thiolase.³⁰ However, in general, the activation of PPAR- α leads to improvement but not aggravation of steatosis. Then, what is the function of PPAR- α activation that is observed in the core gene transgenic mice?

To clarify the role of PPAR- α activation in pathogenesis of steatosis and HCC, we mated a core gene transgenic mouse with a PPAR- α knockout (KO) mouse and studied the phenotype. PPAR- α KO mice have reduced expression of target genes of PPAR- α , and have mild steatosis in the liver, as expected.³¹ It was unanticipated, however, that steatosis was absent in PPAR- α -null or -heterozygous core gene transgenic mice but present in PPAR- α -intact core gene transgenic mice at the age of 9 or 24 months.³⁰ 8-Hydroxy deoxyguanosine (8-OHdG) and peroxy lipids, both of which are markers for oxidative stress, were decreased in PPAR- α KO core gene transgenic mice. Mitochondrial dysfunction in the core gene transgenic mice, which contributes to overproduction of oxidative stress,¹⁹ was also improved in PPAR- α KO core gene transgenic mice.

Finally, PPAR- α KO core gene transgenic mice did not develop HCC at the age of 24 months, whereas about one-third of PPAR- α -intact core gene transgenic mice did. It should be noted that core gene transgenic mice that are heterozygous for the PPAR- α gene also did not develop HCC.³² When clofibrate, a peroxisome proliferator, was administered for 24 months to PPAR- α -heterozygous mice, either with or without the core gene, HCC developed in a higher rate in the core gene (+) mice with greater PPAR- α activation. It should be noted that steatosis was present only in core gene (+) PPAR- α -heterozygous mice. In summary, steatosis and HCC developed in PPAR- α -intact but not in PPAR- α -heterozygous or PPAR- α -null core gene transgenic mice, indicating that not the presence but the persistent activation of PPAR- α would be important in hepatocarcinogenesis by HCV core protein. In general, PPAR- α acts to ameliorate steatosis, but with the presence of mitochondrial dysfunction, which is also provoked by the core protein, the core-activated PPAR- α may exacerbate steatosis. Persistent activation of PPAR- α with "strong" ligands such as the core protein of HCV could be carcinogenic in humans, although the low-affinity fibrate ligands are not likely associated with human cancers.

HCV core protein causes "fatty acid spiral"

Figure 2 illustrates our current hypothesis for the role of lipid metabolism in HCV-associated hepatocarcinogenesis. Immune-mediated inflammation should also play a pivotal role in hepatocarcinogenesis in HCV

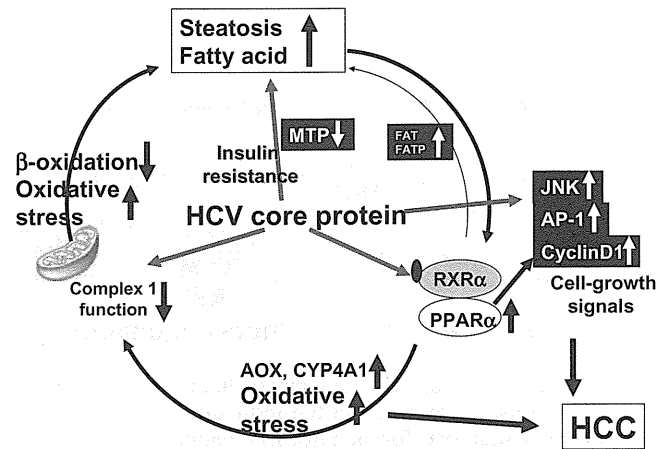


Fig. 2. "Fatty acid spiral" by HCV core protein. In HCV infection, the core protein induces steatosis via several pathways, leading to "fatty acid spiral" in the presence of the mitochondrial complex 1 dysfunction and PPAR- α activation, both of which are also caused by the core protein. These intracellular alterations would contribute to hepatocarcinogenesis by inducing oxidative stress overproduction and cell-growth signal activation. In such a sense, the core protein of HCV is not a classical type oncoprotein, but rather seems to contribute to hepatocarcinogenesis by modulating intracellular metabolism and signaling. *HCV*, hepatitis C virus; *HCC*, hepatocellular carcinoma; *ROS*, reactive oxygen species; *JNK*, c-Jun N-terminal kinase; *ERK*, extracellular signal-regulated kinase; *AP-1*, activating protein-1; *RXR α* , retinoid X receptor- α ; *PPAR- α* , peroxisome proliferator activated receptor- α ; *AOX*, acyl-CoA oxidase; *CYP*, cytochrome P450; *MTP*, microsomal triglyceride transfer protein; *FAT*, fatty acid translocase; fatty acid transport protein

infection. However, in HCV infection, the core protein induces steatosis through the aforementioned pathways, leading to "fatty acid spiral" in the presence of the mitochondrial complex 1 dysfunction and PPAR- α activation, both of which are caused by the core protein. These intracellular alterations would contribute to hepatocarcinogenesis by inducing oxidative stress overproduction and cell-growth signal activation. In such a sense, the core protein of HCV is not a classical-type oncoprotein, but rather seems to contribute to hepatocarcinogenesis by modulating intracellular metabolism and signaling.

The HCV protein may allow some steps in multistep hepatocarcinogenesis to be skipped

The results of our studies on transgenic mice have indicated a carcinogenic potential of the HCV core protein *in vivo*; thus, HCV would be directly involved in hepatocarcinogenesis. In research studies of carcinogenesis, the theory outlined by Kinzler and Vogelstein³³ has gained wide popularity. They have proposed that the

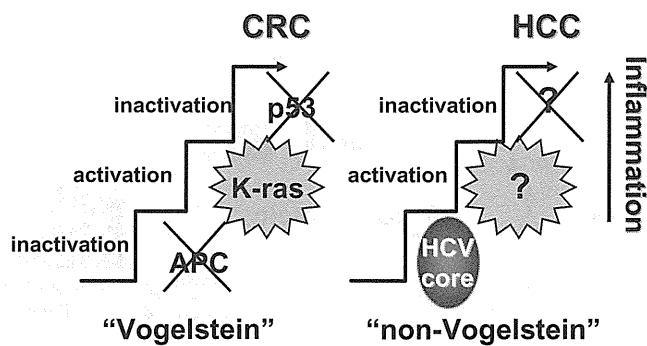


Fig. 3. Mechanism of HCV-associated hepatocarcinogenesis. Multiple steps are required in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that genetic mutations accumulate in hepatocytes. However, in HCV infection, some of these steps may be skipped in the development of HCC in the presence of the core protein. The overall effects achieved by the expression of the core protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations required for carcinogenesis. By considering such a “non-Vogelstein-type” process for the induction of HCC, a plausible explanation may be given for many unusual events happening in HCV carriers

development of colorectal cancer is induced by the accumulation of a complete set of cellular gene mutations. They have deduced that mutations in the APC gene for inactivation, those in *K-ras* for activation, and those in the *p53* gene for inactivation accumulate, which cooperate toward the development of colorectal cancer.³³ Their theory has been extended to the carcinogenesis of other cancers as well, called “Vogelstein-type” carcinogenesis (Fig. 3).

On the basis of the results we obtained for the induction of HCC by the HCV core protein, we would like to introduce a different mechanism for hepatocarcinogenesis in HCV infection. We do allow multistages in the induction of all cancers; it would be mandatory for hepatocarcinogenesis that many mutations accumulate in hepatocytes. Some of these steps, however, may be skipped in the development of HCC in HCV infection to which the core protein would contribute (see Fig. 3). The overall effect achieved by the expression of the viral protein would be the induction of HCC, even in the absence of a complete set of genetic aberrations required for carcinogenesis.

By considering such a “non-Vogelstein-type” process for the induction of HCC, a plausible explanation may be given for many unusual events happening in HCV carriers.³⁴ Now it does not seem so difficult as before to determine why HCC develops in persistent HCV infection at an outstandingly high incidence. Our theory may also give an account of the nonmetastatic and multicentric de novo occurrence characteristics of HCC, which would be the result of persistent HCV infection.

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Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan

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Background & Aims: To evaluate the efficacy and safety of telaprevir in combination with peginterferon- α 2b (PEG-IFN) and ribavirin (RBV) in patients with chronic hepatitis C.

Methods: In a multi-center randomized clinical trial in Japan, on patients infected with HCV of genotype 1, 126 patients were assigned to telaprevir for 12 weeks along with PEG-IFN and RBV for 24 weeks (Group A), while 63 to PEG-IFN and RBV for 48 weeks (Group B).

Results: HCV RNA disappeared more swiftly in patients in Group A than B, and the frequency of patients without detectable HCV RNA at week 4 (rapid virological response (RVR)) was higher in Group A than B (84.0% vs. 4.8%, $p < 0.0001$). Grade 3 and 4 skin disorders, including Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms, as well as Grade 3 anemia (< 8.0 g/dl), occurred more frequently in Group A than B (skin disorders, 11.9% vs. 4.8%; anemia, 11.1% vs. 0.0%). The total RBV dose was smaller in Group A than B (47.0% vs. 77.7% of the target, $p < 0.0001$). Despite these drawbacks, sustained virological response (SVR) was achieved more frequently in Group A than B (73.0% vs. 49.2%, $p = 0.0020$).

Conclusions: Although the triple therapy with telaprevir-based regimen for 24 weeks resulted in more adverse events and less total RBV dose than PEG-IFN and RBV for 48 weeks, it was able to achieve higher SVR within shorter duration by carefully monitoring adverse events and modifying the RBV dose as required.

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Introduction

Over the world, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1]. Most individuals with persistent HCV infection can fulfill the life expectancy, while about 30% of them develop life-threatening liver disease such as decompensated cirrhosis and hepatocellular carcinoma [2,3].

Currently, interferon (IFN) is the only antiviral drug capable of terminating HCV infection. The present standard-of-care (SOC) therapy for patients infected with HCV of genotype 1, the most prevalent genotype over the world, is peginterferon (PEG-IFN) combined with ribavirin (RBV) for 48 weeks. However, sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after the completion of therapy, can be achieved in only 42–52% of the patients [4–6]. To cope with this grim situation, a number of direct acting antivirals (DAAs) have been designed and developed, represented by NS3/4A protease inhibitors and NS5B polymerase or NS5A inhibitors [7]. Among them, telaprevir has shown promising results, when combined with PEG-IFN and RBV, in the phase 2 [8,9] and 3 clinical trials [10,11], by improving SVR to ~70% in patients infected with HCV-1.

Previous trials with the triple therapy were conducted in Europe and the United States, respectively. Hence, Asians were under-represented, accounting only for 1.6–2.1% of studied patients, and distributions of genotypes 1a (44–67%) and 1b (27–55%) varied widely [8–10]. In view of ethnic differences in response to IFN-based treatments [12,13], as well as profiles of resistance to telaprevir difference between genotypes 1a and 1b [14], a multi-center, randomized, and treatment-controlled clinical trial was conducted for comparison of therapeutic efficacy between the triple therapy and SOC in patients infected with HCV-1b in Japan.

Patients and methods

Patients

From November 2008 through August 2010, 220 patients, who were infected with HCV-1 and had not received antiviral treatments before, were recruited at 41 institutions in Japan. They joined the study for finding differences in the

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Abbreviations: PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; SOC, standard of care; DAA, direct acting antiviral.



Table 1. Baseline characteristics of patients.

Features ^a	Group A: T12PR24 (n = 126)	Group B: PR48 (n = 63)
Men (%)	66 (52.4%)	33 (52.4%)
Age (years)	53.0 (20-65)	55.0 (20-65)
Weight (kg)	60.2 (40.7-87.5)	64.1 (42.1-84.9)
BMI (kg/m ²)	22.6 (16.2-31.1)	23.3 (17.9-30.8)
Hemoglobin (g/dl)	14.3 (12.1-17.1)	14.5 (12.3-17.5)
White blood cells (/mm ³)	5300 (2900-10,670)	5130 (2950-11,050)
Platelets (x10 ⁴ /mm ³)	19.2 (9.0-36.2)	20.2 (8.7-37.0)
ALT (IU/L)	36.5 (12-252)	45.0 (18-259)
AST (IU/L)	34.0 (18-170)	38.0 (17-142)
Total bilirubin (mg/dl)	0.70 (0.3-1.9)	0.80 (0.4-1.8)
Total cholesterol (mg/dl)	182 (111-299)	180 (116-263)
HCV RNA (log ₁₀ IU/ml)	6.7 (5.1-7.5)	6.9 (5.1-7.4)
HCV genotypes		
1a	2 (1.6%)	0 (0.0%)
1b	124 (98.4%)	63 (100.0%)

^aValues are the median with the range in parentheses, or number with the percentage in parentheses.

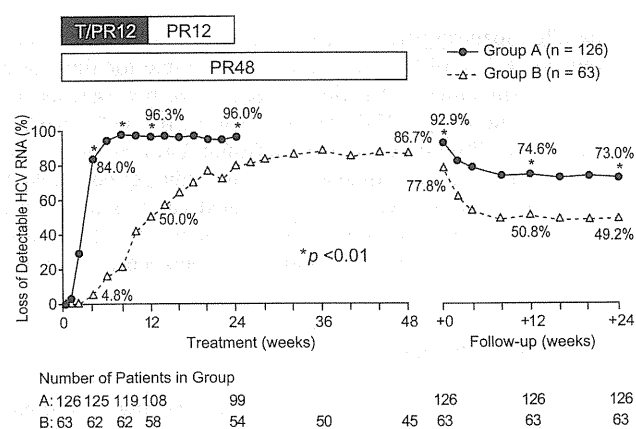


Fig. 1. Loss of detectable HCV RNA in patients in Groups A and B. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and weeks 12 and 24 in the follow-up period. An asterisk (*) indicates $p < 0.01$ differences. The number of patients at each time point is indicated below the graph.

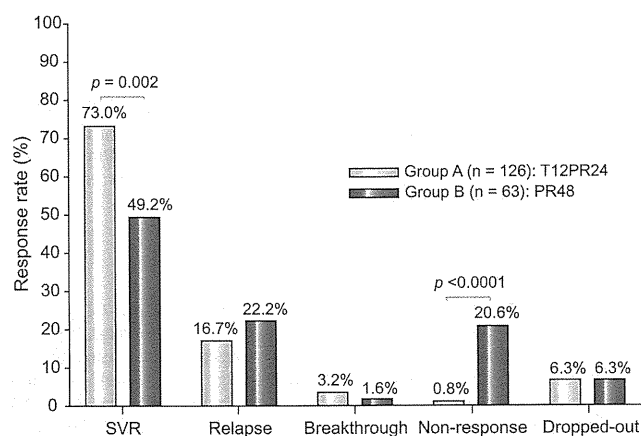


Fig. 2. Comparison of treatment responses between patients in Groups A and B. SVR, sustained virological response (HCV RNA negative 24 weeks after the completion of treatment); relapse, reappearance of HCV RNA in serum during follow-up period; breakthrough, reappearance of HCV RNA in serum during treatment period; non-response, HCV RNA continuously detectable in serum during treatment period.

treatment response and adverse events between the triple therapy involving telaprevir, PEG-IFN and RBV, and SOC with PEG-IFN and RBV. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of each institution. Each patient gave a written informed consent before participating in this study.

Study design

This prospective, multi-center, and randomized study was planned on Japanese patients with chronic hepatitis C who met inclusion and did not meet exclusion criteria. Main inclusion criteria were: (a) diagnosed with chronic hepatitis C, and had not received antiviral treatments before; (b) infected with HCV-1 confirmed by the sequence analysis in the NS5B region; (c) had HCV RNA levels $\geq 5.0 \log_{10}$ IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (d) Japanese aged from 20 to 65 years at the entry; (e) had the body weight between >40 and ≤ 120 kg; (f) were not pregnant and capable of contraception till 24 weeks after the treatment; and (g) agreed on the admission for

15 days since the treatment start. Main exclusion criteria were: (a) decompensated liver cirrhosis; (b) hepatitis B surface antigen; (c) hepatocellular carcinoma or other malignancy, or its history; (d) autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic hepatitis C; (e) depression or schizophrenia, or its history, or history of suicide attempts; (f) chronic renal disease or creatinine clearance ≤ 50 ml/min at the baseline; (g) hemoglobin <12 g/dl, neutrophil counts $<1500/\text{mm}^3$ or platelet counts $<100,000/\text{mm}^3$ at the baseline; and (h) pregnancy in progress or planned during the study period of either partner.

Patients were randomly assigned to either of the following two treatment groups in a 2:1 ratio, with stratification to balance sex and age: (1) the triple therapy with telaprevir, PEG-IFN, and RBV for 12 weeks, followed by PEG-IFN and RBV for an additional 12 weeks (Group A: T12PR24); and (2) SOC with PEG-IFN and RBV for 48 weeks (Group B: PR48). After the treatment was completed or discontinued, they were followed for ≥ 24 weeks for SVR evaluation. Patients were followed regularly for subjective symptoms and objective signs, as well as blood

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Table 2. Comparison of SVR stratified by demographic and virological factors as well as discontinuation of study drugs between two groups with different therapeutic regimens.

	A: T12PR24 n = 126	B: PR48 n = 63	Differences p value
Gender			
Men	50/66 (75.8%)	18/33 (54.5%)	0.0400
Women	42/60 (70.0%)	13/30 (43.3%)	0.0214
Age (years)			
≤49	35/41 (85.4%)	13/21 (61.9%)	0.0543
≥50	57/85 (67.1%)	18/42 (42.9%)	0.0125
HCV RNA (log ₁₀ IU/ml)			
≥7	18/26 (69.2%)	5/18 (27.8%)	0.0132
<7	74/100 (74.0%)	26/45 (57.8%)	0.0556
Discontinuation of study drugs			
Not discontinued	66/79 (83.5%)	27/46 (58.7%)	0.0030
All drugs discontinued	14/27 (51.9%)	4/17 (23.5%)	0.1143

counts and chemistry. HCV RNA levels were monitored at day -28, days 1 (pre-dose), 2, and 3, weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 (both groups), as well as weeks 26, 28, 32, 36, 40, and 48 (Group B), during the treatment period; they were monitored at weeks 2, 4, 8, 12, 16, 20, and 24 in the follow-up period (both groups).

HCV RNA and genotypes

HCV RNA was quantified using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of this assay was 1.2–7.8 log₁₀ IU/ml, and samples with no HCV RNA detected were reported as: <1.2 log₁₀ IU/ml (no HCV RNA detectable). Genotypes of HCV were determined by direct sequencing followed by phylogenetic analysis of the NS5B region [15].

Antiviral treatments

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) 750 mg was administered three times a day at an 8-h interval (q8h) after each meal. Peginterferon-α2b (PegIntron®, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 µg/kg (range: 1.250–1.739 µg/kg) once a week. Ribavirin (Rebetol®, MSD, Tokyo, Japan) 200–600 mg was administered after breakfast and dinner. The daily dose of RBV was adjusted to the body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ~≤80 kg; and 1000 mg for >80 kg.

RBV dose was diminished by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1000 mg) when hemoglobin decreased <12 g/dl, and by extra 200 mg when it lowered <10 g/dl. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dl at baseline or in those in whom it decreased by 1 g/dl within a week and below 13 g/dl. Dose modification of RBV in Group B was conducted in accordance with SOC. PEG-IFN dose was reduced to one half, when leukocyte counts decreased <1500/mm³, neutrophil counts <750/mm³ or platelet counts <8 × 10⁴/mm³; PEG-IFN was discontinued when they decreased <1000/mm³, 500/mm³ or 5 × 10⁴/mm³, respectively. The triple therapy was discontinued or interrupted when hemoglobin decreased <8.5 g/dl. In patients whose hemoglobin increased ≥8.5 g/dl within 2 weeks after the interruption, treatment was resumed with PEG-IFN and RBV 200 mg. The reduction of telaprevir dose was not permitted.

Statistical analysis

SVR was evaluated in the full analysis set. The difference in SVR between Groups A and B with the 2-sided 95% confidence interval (CI) was calculated with the adjustment for sex and age, and p value was evaluated by the Wald-test. Continuous variables between groups were compared by the Mann-Whitney test (U-test), and categorical variables by the Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC), and a p value <0.05 was considered significant.

Results

Patient cohorts

Of the 220 Japanese patients from whom an informed consent was obtained, 31 (14.1%) were found not eligible for the study entry. The remaining 189 patients were randomly assigned to T12PR24 (Group A [n = 126]) or PR48 (Group B [n = 63]). Overall, 114 out of the 126 (90.0%) patients in Group A and 54 out of the 63 (85.7%) in Group B completed the full study period. Table 1 compares baseline characteristics of studied patients in Groups A and B. There were no differences in demographic characters, hematology, biochemistry, or virology between the two groups of patients.

Loss of HCV RNA during treatment

Dynamics of HCV RNA during treatment was much different between Groups A and B. HCV RNA disappeared more frequently (98.4% vs. 79.4%, *p* <0.001) and swiftly (within 8 vs. 38 weeks) in patients in Group A than B. Time courses of the loss of HCV RNA are compared in Fig. 1. The loss of HCV RNA increased constantly, sharply, and swiftly in Group A. By contrast, in Group B, it gradually increased during the first 24 weeks of treatment. Rapid virological response at 4 weeks (RVR) occurred more frequently in Group A than B (84.0% vs. 4.8%, *p* <0.0001). HCV RNA was undetectable in >90% of patients in Group A, while it stayed undetectable in <80% of patients in Group B at the start of follow-up. After treatment completion, HCV RNA re-appeared in patients in both Groups A and B (16.7% vs. 22.2%, *p* = 0.4272).

Responses to treatments

Fig. 2 compares treatment responses between Groups A and B. SVR was achieved more frequently in Group A than B (73.0% vs. 49.2%, *p* = 0.0020). By contrast, non-response was less frequent in Group A than B (0.8% vs. 20.6%, *p* <0.0001). The difference in SVR between Groups A and B, adjusted for sex and age, was 23.8% (95% CI: 9.4–38.2%, *p* = 0.0012, Wald-test).