Analysis of mutations in the core and NS5A genes of hepatitis C virus in non-responder and relapser patients after treatment with Peg-IFN- α and ribavirin

Kattareeya Kumthip¹ · Pattranuch Chusri¹ · Chansom Pantip² · Satawat Thongsawat³ · Amornrat O'Brien¹ · Niwat Maneekarn¹

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Abstract Mutations in several regions of HCV genome are shown to correlate with response to interferon (IFN) treatment. Persistence of HCV infection and poor susceptibility to treatment might be contributed by mutations arising within HCV genome which enable the virus to escape from host immune response/IFN treatment. This study investigated mutations in core and NS5A genes of HCV from non-responder and relapser patients after treatment with Peg-IFN- α and ribavirin. Viral RNA was extracted from patient sera and core and NS5A genes were amplified by RT-PCR. Nucleotide sequences of the core and NS5A genes were determined by direct sequencing, and converted to amino acid sequences. Nucleotide and amino acid sequences in the core region, ISDR, PKRBD, and V3 regions within NS5A after treatment were highly conserved when comparing to their corresponding sequences obtained before treatment. Interestingly, when comparing the virus from relapsers to those from non-responders, the number of mutations after treatment in N-terminal region of NS5A of virus from relapsers was significantly higher than those from non-responders ($P < 0.05$). Amino acid mutations at the N-terminus of NS5A of the virus in relapsers might help the virus to survive and somehow relapse after the cessation of the treatment.

Keywords Core · Hepatitis C virus · Mutation · NS5A · Peg-IFN- α · Ribavirin

Introduction

Hepatitis C virus (HCV), a member of the *Hepacivirus* genus of the family *Flaviviridae*, is an enveloped virus with a positive single-stranded RNA genome of approximately 9600 nucleotides encoding structural proteins (core, envelope 1, envelope 2 and p7) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) [22]. Infection with HCV is highly prevalent throughout the world, and the disease often progresses to chronic hepatitis, eventually leading to liver cirrhosis and hepatocellular carcinoma [27]. Although the combination of pegylated interferon-alpha (Peg-IFN- α) with ribavirin is the treatment of choice for HCV-infected patients, only approximately 50 % of the patients achieve a sustained virological response (SVR) [5, 6]. It has been reported that numerous factors are associated with the responses to IFN treatment and both host and viral factors are known to contribute to the responses to the treatment [12, 21, 23].

It has been suggested that mutations in the core and NS5A proteins correlate with the response to IFN treatment [1, 4, 11, 14, 20]. A correlation between a greater number of mutations in the IFN- α sensitivity determining region (ISDR; amino acid residues 2209–2248) and SVR to IFN- α therapy in HCV genotype 1b infected patients has been described [4, 11, 14]. Moreover, a significant correlation of amino acid substitution in the core region and the response to IFN and ribavirin combination therapy has been described [1, 11, 20]. It has been shown that substitutions of amino acid residues 70 and/or 91 in the core region were significantly associated with a non-virological response

✉ Niwat Maneekarn
niwat.m@cmu.ac.th

¹ Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

² Research Institute for Health Sciences, Chiang Mai University, Chiang Mai 50200, Thailand

³ Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

(NVR) [1] as well as a mutation which occurred in the core region was predictive of an NVR to IFN in Japanese patients who were infected with HCV 1b [11, 20]. These studies suggest that amino acid mutations in the core and NS5A regions of HCV appear to be important factors that correlate with the response to IFN treatment.

Additionally, it was hypothesized that amino acid sequence variation could play a central role in IFN resistance and HCV strains carrying a resistant type of ISDR were selected during IFN treatment [7, 9]. HCV, like other RNA viruses, has a high degree of genetic variability which is due to the lack of proofreading activity of the viral RNA-dependent RNA-polymerase [16, 17]. The persistence of HCV infection and poor susceptibility to treatment might be contributed by the viral protein interacting with host's antiviral protein as well as mutations arising within the HCV genome which enable the virus to escape from the host's immune response [23, 30]. Furthermore, antiviral therapy may select for mutations which allow the virus to survive during IFN treatment. Here, we hypothesized that the HCV strains in non-responders may carry IFN resistant inherited genes that conferred resistance to IFN-treatment. For relapsers, at the end of treatment, HCV was disappeared from their blood circulation, which behaved like the HCV from responders, but it reappeared in blood circulation again at 6 months after completion of the treatment. We also hypothesized that some of HCV in relapsers may undergo amino acid substitution during the treatment and gained amino acid residue(s) that conferred IFN-resistant genotype to the virus and therefore be able to survive and to replicate to high level that can be detected at 6 months after completion of the treatment. To evaluate these hypotheses, we aimed to investigate nucleotide and amino acid variabilities in the core and NS5A genes of HCV obtained at before and at 6 months after the end of treatment in the virus from non-responders and relapsers.

Materials and methods

Patients and treatments

This study was conducted with the approval of the Research Ethics Committee for Human Rights related to human experimentation, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (No. 6/2543). The serum samples were collected from chronic hepatitis C patients who visited Chiang Mai University Hospital during August 2003 to September 2010. The presence of HCV in the blood was determined by using Amplicor HCV test (Roche Diagnostics, Basel, Switzerland) and determination of HCV RNA level was determined by reverse transcription-polymerase chain reaction (RT-PCR) using Amplicor HCV

Monitor test (Roche Diagnostics, Basel, Switzerland). The HCV viral load during treatment was monitored by the branched DNA (bDNA) assay (Quantiplex HCV RNA 2.0 assay; Chiron Corp, Berkeley, CA, USA). The HCV genotype was initially identified by reverse hybridization assay (InnoLiPA HCV II; Innogenetics, Gent, Belgium) and confirmed by direct sequencing of full-length HCV core gene. Then the nucleotide sequences were aligned with those of the HCV reference strains retrieved from the Los Alamos HCV sequence database (LANL; <http://hcv.lanl.gov/>). After determining HCV genotypes, these patients were treated with 180 µg/week of Peg-IFN-α2a (Pegasys; Hoffmann-La Roche, Nutley, NJ, USA) or 1.5 µg/kg/week of Peg-IFN-α2b (Pegintron; Schering-Plough, Innishannon, County Cork, Ireland) in combination with ribavirin (800–1200 mg/day). The patients infected with HCV genotypes 1 and 6 were treated for 48 weeks whereas those infected with genotype 3 were treated for 24 weeks according to the protocol of Thailand Consensus Recommendations for Management of Chronic Hepatitis C virus 2005.

Serum samples were collected from several time points including before, during, at the end of treatment, and at 6 months posttreatment follow-up. The pretreatment sera were tested for HCV viral load and the viral load was concomitantly monitored during the course of treatment. The sera collected at the end of treatment and at 6 months after completion of the treatment were tested for the presence of viral RNA in order to assign the clinical outcomes of response to the Peg-IFN-α and ribavirin treatment. The patients were classified into three groups (responders, non-responders, and relapsers) as described previously [14]. A total of 158 patients chronically infected with HCV who had completed the treatment course with combination of Peg-IFN-α and ribavirin were obtained. One-hundred and twenty-one out of 158 (76.6 %) were responders to treatment, whereas 11 (7 %) were non-responders and 26 (16.4 %) were relapsers as described previously [15]. Only 37 patients who failed to respond to Peg-IFN-α and ribavirin combination therapy (non-responders and relapsers) were included in this study.

Amplification of full length core and NS5A genes

Viral RNA genome was extracted from 140 µl of serum sample by using a commercial kit (QIAamp Viral RNA Mini Kit; Qiagen, Hilden, Germany) according to the manufacturer's instruction. cDNAs were synthesized by using SuperScript III reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) with antisense primer 3UTR1 (5'-ACATGATCTGCAGAGAGGCC-3') and then full length of core and NS5A genes were amplified from cDNA by using Phusion High-Fidelity DNA Polymerase

(Finnzymes; Espoo, Finland). List of primers used for amplifying core and NS5A genes were as previously described [14]. The amplified PCR products were purified by using QIA quick PCR Purification Kit (Qiagen, Hilden, Germany) and then subjected to direct nucleotide sequencing using a dye terminator sequencing kit (BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Amino acid sequences and statistical analysis

The nucleotide sequences obtained from direct sequencing of the purified PCR products were edited manually based on the sequencing trace profile to correct the sequences using the programs FinchTV (Geospiza) and BioEdit Sequence Alignment Editor (Tom Hall, Ibis Therapeutics), and then nucleotide sequences were translated into amino acid sequences with the BioEdit program. Multiple alignments of deduced amino acid sequences was performed with ClustalX (Conway Institute UCD Dublin). Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago IL., USA) to analyze the correlations between the variations of amino acid sequences in the core and NS5A genes and the clinical outcomes. The Wilcoxon matched-pairs test was used to determine the difference in the number of variations in the core and NS5A proteins between two groups of patients with different clinical outcomes in response to Peg-IFN- α and ribavirin treatment. The χ^2 and Fisher's exact probability tests were used to determine the correlation between specific point mutations that occurred in the core and NS5A proteins and clinical outcomes of the patients. A *P* value of <0.05 indicates a statistically significant difference.

GenBank nucleotide accession numbers

Nucleotide sequences analyzed in this study are available in the GenBank database under the following accession numbers. Twenty-one pretreatment sequences of full-length HCV core gene were GQ913857, GQ913858, GQ913859, GQ913860, HM041976, HM041977, HM041993, HM041996, HM042017, HM042018, HM042019, HM042020, HM042024, HM042027, HM042030, KM246350, KM246360, KM246364, KM246366, KM246368, and KM246373 and 15 pretreatment sequences of full-length HCV NS5A gene were GQ913867, GQ913868, GQ913869, GQ913870, HM042041, HM042042, HM042057, HM042078, HM042080, HM042081, HM042087, KM246382, KM246387, KM246389, and KM246392. Nucleotide sequences of HCV core gene obtained at 6 months after the end of treatment from corresponding individual patients

were KM246348, KM246349, KM246351 to KM246359, KM246361, KM246362, KM246363, KM246365, KM246367, KM246369, KM246370, KM246371, KM246372, and KM246374. Post-treatment nucleotide sequences of HCV NS5A were KM246375 to KM246381, KM246383, KM246384, KM246385, KM246386, KM246388, KM246390, KM246391, and KM246393.

Results

Clinical characteristics of the patients

From a total of 158 patients with HCV infection who had completed the treatment course with combined Peg-IFN- α and ribavirin, 37 patients who failed to respond to the treatment including 11 non-responders and 26 relapsers were enrolled in this study. Since the final clinical outcomes of non-responders and relapsers were unsuccessful treatment, the viral RNA was detected in their blood at 6 months after completion of the treatment and therefore these two groups were considered as treatment failure groups. Although the 37 patients were positive for HCV RNA at both time points, the full-length core and NS5A genes of the viruses from 16 patients could not be successfully amplified at 6 months after completion of the treatment. It was probably due to low level of HCV RNA in their serum samples. Finally, we have got HCV from 21 patients analyzed in this study; 8 patients were non-responders including 3 patients with genotype 1a, 2 with 1b, and 3 with 3a and 13 patients were relapsers including 1 patient with genotype 1a, 3 with 1b, 5 with 3a, 1 with 3b, and 3 with 6f. The non-responders had a mean age of 52.5 ± 8.3 years (range from 39 to 61 years) while relapsers had a slightly higher mean age of 53.1 ± 8.9 years (range from 40 to 76 years). Majority of non-responders and relapsers were male (6/8; 75 % and 9/13; 69.2 %, respectively). Moreover, all of non-responders (100 %) exhibited high levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while 92.3 % of relapsers showed high levels of both ALT and AST. Most of the patients also showed high viral load (>500,000 IU/ml) before treatment; 87.5 % for non-responders and 84.6 % for relapsers (Table 1).

Analysis of amino acid variability in the core and NS5A genes before and after Peg-IFN- α and ribavirin treatment

To investigate whether the treatment with Peg-IFN- α and ribavirin could induce amino acid variability in the core and NS5A proteins, we compared amino acid sequences of HCV obtained at before treatment and at 6 months after

Table 1 Baseline characteristics of the 21 patients who failed to respond to Peg-IFN- α and ribavirin combination therapy

Characteristics	Non-responders	Relapsers
Number of patients	8	13
Age (years) ^a	52.5 $\pm$ 8.3	53.1 $\pm$ 8.9
Gender (M/F) ^b	6/2	9/4
Patients with high level of ALT ^c	8 (100 %)	12 (92.3 %)
Patients with high level of AST ^d	8 (100 %)	12 (92.3 %)
HCV RNA > 500,000 IU/ml	7 (87.5 %)	11 (84.6 %)

^a Age is expressed as mean $\pm$ standard deviation (SD)^b M/F male to female ratio^c ALT alanine aminotransferase (normal range 7–42 U/L)^d AST aspartate aminotransferase (normal range 3–37 U/L)

finish the treatment in the same patients. Amino acid sequence changes occurred after treatment compared to those of before treatment sequences were counted for variability. Of these 21 patients, the amino acid sequences in the core region at before and after Peg-IFN- α and ribavirin treatment were 100 % identical in the 13 patients while the other 8 patients had only 1 or 2 amino acid changes (mean of 0.4 ± 0.6) (Table 2 and Fig. 1). In contrast, a higher variability of amino acid sequences was observed in the NS5A region; ranging from 2 to 13 amino acid changes were detected in NS5A proteins among patients who failed to respond to therapy (5.9 ± 4.0). However, there was no amino acid changes in the ISDR

regions, except for 1 patient who had only 1 amino acid change. Also, there were only few amino acid changes in the PKRBD and V3 regions; 6 patients that had only 1 or 2 amino acid changes in PKRBD and 5 patients had 1–3 amino acid changes in V3 (Table 2 and Fig. 1). These data demonstrated a high degree of amino acid conservation in the core, ISDR, PKRBD, and V3 of NS5A of HCV and treatment with Peg-IFN- α and ribavirin for 24–48 weeks had not much selective pressure on the viruses from these two groups of patients, non-responders and relapsers.

Comparison of mutations in the core and NS5A genes of the viruses obtained from non-responders and relapsers after Peg-IFN- α and ribavirin treatment

We have hypothesized that during treatment with Peg-IFN- α and ribavirin the viruses in relapsers who initially responded well to the treatment, i.e. the virus disappeared at the end of treatment but relapsed at 6 months afterward, may have higher amino acid variations that enable the virus to escape from the host's immune response or to gain a greater ability to resist to the treatment. To test this hypothesis, we evaluated the number of amino acid variation occurring after the treatment (at 6 months posttreatment followed-up) in the core and NS5A regions of the viruses from relapsers compared to those from non-responders. As shown in Table 2 and Fig. 1, there was no significant difference in amino acid variation in the core region between non-responders and relapsers after Peg-

Table 2 Number of nucleotide and amino acid mutations occurred within the core and NS5A sequences of HCV isolated at post-treatment in non-responder and relapser groups compared to those at the pretreatment

Clinical outcomes	Core	NS5A	N-terminus NS5A	C-terminus NS5A	ISDR	PKRBD	V3
Amino acid substitution							
Non-responders ^a	0.6 $\pm$ 0.7	4.5 $\pm$ 4.3	0.6 $\pm$ 0.8*	4.3 $\pm$ 4.6	0.17 $\pm$ 0.4	0.6 $\pm$ 0.8	0.3 $\pm$ 0.8
Relapsers ^b	0.4 $\pm$ 0.6	6.6 $\pm$ 2.6	3.5 $\pm$ 2.8*	3.1 $\pm$ 2.3	0	0.3 $\pm$ 0.5	1.0 $\pm$ 1.3
NR + RL ^c	0.4 $\pm$ 0.6	5.9 $\pm$ 4.0	2.4 $\pm$ 2.6	3.6 $\pm$ 3.3	0.07 $\pm$ 0.2	0.4 $\pm$ 0.6	0.7 $\pm$ 1.1
Nucleotide substitution							
Non-responders	4.1 $\pm$ 3.7	20.6 $\pm$ 21.0	8.0 $\pm$ 10.4	12.6 $\pm$ 11.2	1.8 $\pm$ 1.8	4.8 $\pm$ 5.7	1.6 $\pm$ 1.8
Relapsers	3.4 $\pm$ 3.5	32.6 $\pm$ 16.1	18 $\pm$ 10.1	14.6 $\pm$ 9.2	1.7 $\pm$ 2.1	4.1 $\pm$ 4.6	2.0 $\pm$ 1.9
NR + RL	3.7 $\pm$ 3.5	27.8 $\pm$ 18.5	14 $\pm$ 11.1	13.8 $\pm$ 9.7	1.8 $\pm$ 1.9	4.4 $\pm$ 4.9	1.8 $\pm$ 1.8

The number of amino acid variation is expressed as mean $\pm$ SD

Core; amino acid (aa) 1–191, NS5A; aa 1973–2419, N-terminal part of NS5A; aa 1973–2208, C-terminal part of NS5A; aa 2209–2419, ISDR; interferon sensitivity-determining region; aa 2209–2248, PKRBD; protein kinase R binding domain; aa 2209–2274, V 3; variable region 3; aa 2353–2379

* Significant difference exist as determined by Kruskal–Wallis test, $P < 0.05$ ^a n = 8 for core gene and n = 6 for NS5A gene^b n = 13 for core gene and n = 9 for NS5A gene^c NR + RL = non-responders and relapsers (Treatment failure group)

Fig. 1 Number of amino acid variations in the core and NS5A proteins of HCV obtained from non-responders and relapsers.

*Significant differences between HCV from non-responders and relapsers as determined by Kruskal–Wallis test, $P < 0.05$

IFN- α and ribavirin treatment. Only 0–2 amino acid substitutions (mean of 0.6 ± 0.7) were seen in the virus from non-responders and 0–1 substitution (mean of 0.4 ± 0.6) was seen in relapsers. However, the number of amino acid variation of the viruses from relapsers was higher in the full-length NS5A; relapsers had 6.6 ± 2.6 amino acid residue substitution while non-responders had 4.5 ± 4.3 . Interestingly, the significant difference of amino acid variations was observed in the N-terminal part of NS5A. After treatment, the number of amino acid substitutions in the viruses from relapsers (3.5 ± 2.8) were significantly greater than those from non-responders (0.6 ± 0.8) ($P < 0.05$). However, there was no specific amino acid substitution was significantly associated with particular clinical outcome. Furthermore, no significant differences in the number of amino acid substitution at the C-terminal region, ISDR, PKR, and V3 of NS5A between these two groups were observed (Table 2; Fig. 1).

Nucleotide sequence comparison at before and at 6 months followed-up after the end of treatment was also analyzed. Overall, nucleotide substitutions in the NS5A region (27.8 ± 18.5) of the treatment failure group (NR + RL) were much higher than those in the core region (3.7 ± 3.5) (Table 2). Again, nucleotide sequences in the ISDR and V3 regions were quite conserved (1.8 ± 1.9 and 1.8 ± 1.8 , respectively) compared to other regions within the NS5A. Considering nucleotide substitution between non-responders and relapsers, it was found that nucleotide substitution in the core and other regions within NS5A genes of the viruses from non-responders and relapsers were not significantly different, except for N-terminus of NS5A. Nucleotide substitution in the N-terminus of NS5A of the virus from relapsers was 18.0 ± 10.1 whereas non-responders was 8.0 ± 10.4 (Table 2; Fig. 2). The findings

were in good agreement with the observation in amino acid level.

Considering of genotype-related manner, a similar analysis was done for each HCV genotype. In the core region, there was no significant difference in amino acid variation in the core region between non-responders and relapsers in patients infected with HCV genotypes 1a, 1b, and 3a. Due to lack of non-responders from genotypes 3b and 6f, analysis of amino acid variation in these genotypes could not be done. Comparison of amino acid variations in the NS5A region of HCV genotypes 1a, 1b, and 3a is shown in Table 3. The results showed that the number of amino acid variations in the entire NS5A of HCV 1b and 3a in relapsers were higher than those in non-responders. Again, the similar observation was also found in the N-terminal region of NS5A of HCV genotypes 1b and 3a.

Discussion

The present study investigated amino acid variations of HCV obtained from non-responders and relapsers at before and after Peg-IFN- α and ribavirin treatment in the entire sequences of core and NS5A proteins. When the sequences of core protein of the viruses at before and after treatment were compared, a high degree of amino acid conservation was clearly observed. In addition, the high conservation of amino acid sequences in the core protein at before and after treatment was observed in several HCV genotypes, 1a, 1b, 3a, 3b, and 6f, included in this study. Many reports have described the correlation between mutations of several regions within NS5A, including ISDR, PKRBD, V3, and the sensitivity to IFN/ribavirin therapy [4, 11, 18, 20]. One mechanism has been proposed to explain the resistance of HCV to IFN is the binding of NS5A protein through the

Fig. 2 Number of nucleotide variations in the core and NS5A genes of HCV obtained from non-responders and relapsers

Table 3 Number of amino acid variations in NS5A of HCV from non-responders and relapsers in genotypes 1a, 1b, and 3a as shown by mean $\pm$ SD

HCV genotypes	Patients	Full-length NS5A	N-terminus NS5A	C-terminus NS5A
HCV 1a	Non-responders	40 $\pm$ 24.7	18 $\pm$ 14.1	22 $\pm$ 10.6
	Relapsers	21	12	9
HCV 1b	Non-responders	22 $\pm$ 8.4	6.0 $\pm$ 4.9	17 $\pm$ 3.5
	Relapsers	28 $\pm$ 16.2	14 $\pm$ 10.6	13 $\pm$ 5.7
HCV 3a	Non-responders	1.0 $\pm$ 0.7	1.0 $\pm$ 0.7	0
	Relapsers	33 $\pm$ 21.5	17 $\pm$ 11.9	16 $\pm$ 9.8

PKRBD and ISDR with antiviral protein PKR which allows the synthesis of viral proteins and generation of viral progeny during IFN treatment [8]. Amino acid substitution in the ISDR may result in the inhibition of binding between NS5A and PKR, and therefore allows PKR to play its role in inhibiting viral protein synthesis and viral progeny production. Interestingly, the ISDR and PKRBD on NS5A of HCV included in the present study remained unchanged after Peg-IFN- α and ribavirin treatment in patients who failed to respond to the treatment. The low levels of amino acid variability present in ISDR/PKRBD support the concept that viruses obtained from non-responders and relapsers may preserve amino acid sequences that help retention of IFN resistance as well as carry a resistant type of ISDR/PKRBD during IFN treatment. The findings are in agreement with other investigators that have demonstrated the effect of IFN on amino acid mutation in the NS5A gene [3, 19, 28].

The relapsers are patients who respond well to IFN and ribavirin treatment, i.e. the virus is undetectable in their sera at the end of treatment, however, 6 months after the completion of treatment the virus re-appears in their sera, suggesting that the ultimate result of the treatment failed to eradicate the virus. The clinical outcome of relapsers at

6 months after the completion of treatment is more or less the same as of non-responders in the sense that the final clinical outcomes of both groups are not respond to IFN and ribavirin treatment, and the virus, therefore, remains circulating in the sera of these patients [14]. We hypothesized that the virus in the non-responder group may carry inherited gene characteristics at before treatment that confers resistance to IFN and ribavirin. Therefore, this type of virus can survive in the sera of non-responders even after exposure to IFN and ribavirin treatment. In contrast, the virus in the relapser group behave similar to the virus in the responder group by initially responding to IFN and ribavirin treatment. During the exposure to IFN and ribavirin, most of the viruses in the relapsers may be eradicated but some viral quasiespecies can survive by mutating their genome to gain IFN-resistant phenotype. This mutant virus therefore can survive at an undetectable level at the end of treatment. The virus may replicate and accumulate to high levels at 6 months after the completion of treatment. It has been reported that the NS5A protein is an essential component of HCV replication complex. The NS5A protein is organized into three discrete domains, of which the domain I and II (amino acid residues 1-342 of NS5A) are essential for RNA replication [25, 26].

Interestingly, the present study revealed that the number of amino acid variations in full-length NS5A, particularly at N-terminal part of NS5A (amino acid residues 1-236 of NS5A; codons 1973-2208) of HCV from relapsers were significantly greater than those from non-responders. The high amino acid variations and nucleotide mutations occurred in full-length NS5A particularly at N-terminal part of the virus from relapsers as well as the observation of high level of viral RNA at 6 months after completion of treatment may suggest the possibility that mutations arising within the HCV genome enable the virus to escape from the host's immune response and allow the virus to survive during Peg-IFN- α and ribavirin treatment. This mutating virus can survive and maintains in the blood circulation at an undetectable level at the end of treatment and may replicate to a high level at 6 months after the completion of treatment.

The mechanism responsible for HCV persistence at 6 months after the completion of treatment in relapsers is unclear. The presence of minus-strand HCV RNA, a replicative intermediate of HCV RNA genome, in peripheral blood mononuclear cells (PBMCs) is thought to be responsible for the replication site of HCV in an extra-hepatic site [13, 24, 29]. The persistence of HCV in PBMCs may protect the virus from the effects of IFN and ribavirin during the treatment. After the end of treatment, the virus which persists in the PBMCs may act as a reservoir and replicate to high level in a suitable environment [2, 10].

In conclusion, the present study analyzed nucleotide and amino acid sequence variabilities in the core and NS5A proteins of HCV obtained from non-responders and relapsers. The core, NS5A-ISDR, NS5A-PKRBD, and NS5A-V3 regions were highly conserved after the treatment with Peg-IFN- α and ribavirin. Interestingly, the number of nucleotide and amino acid variations in the N-terminal part of NS5A of the viruses from relapsers were significantly greater than those from non-responders. These data provide a better understanding of genetic variability in the core and NS5A of HCV in relation to the response to Peg-IFN- α and ribavirin combination therapy.

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