Mutations in the STAT1-interacting domain of the hepatitis C virus core protein modulate the response to antiviral therapy

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Abstract. RNA viruses, such as hepatitis C virus (HCV), have markedly error-prone replication, resulting in high rates of mutagenesis. In addition, the standard treatment includes ribavirin, a base analog that is likely to cause mutations in different regions of the HCV genome, resulting in deleterious effects on HCV itself. The N-terminal region of the core protein is reported to block interferon (IFN) signaling by interaction with the STAT1-SH2 domain, resulting in HCV resistance to IFN therapy. In this study, mutations in the HCV core protein from IFN/ribavirin-treated patients were analyzed, with particular focus on the N-terminal domain of the HCV core which is reported to interact with STAT1. HCV PCR positive patients enrolled in this study were either undergoing pegylated IFN/ribavirin bitherapy and had completed 12 weeks of initial treatment or were treatment-naïve patients. The HCV core protein was cloned and sequenced from these patients and mutations observed in the STAT1-interacting domain of the core protein from treated patients were characterized using *in silico* interaction to depict the role of these mutations in disease outcomes. Our results suggest that the amino acids at positions 2, 3, 8, 16 and 23 of the HCV core protein are critical for core-STAT1 interaction and ribavirin-induced mutations at these positions interfere with the interaction, resulting in a better response of the treated patients. In conclusion, this study anticipates that HCV core residues 2, 3, 8, 16 and 23 directly interact with STAT1. We propose that IFN/ribavirin bitherapy-induced mutations in the STAT1-interacting domain of the HCV core protein may be responsible for the improved therapeutic response and viral clearance, thus amino acids 1-23 of the N-terminus of the core protein are an ideal antiviral target. However, this treatment may give rise to resistant variants that are able to escape the current therapy. We propose similar studies in responsive and non-responsive genotypes in order to gain a broader picture of this proposed mechanism of viral clearance.

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Introduction

Hepatitis C virus (HCV) is a major public health concern worldwide. Approximately 170 million people suffer from chronic HCV and are at risk of developing cirrhosis and hepatocellular carcinoma. In Pakistan alone, 10 million people are infected with HCV and 50% of them are infected with the 3a subtype. HCV is a small hepatotropic virus, and member of the *Flaviviridae* family, infecting 170-180 million people worldwide (1). Globally, 0.25-1.25 million new cases of HCV infection have been reported per year (2). The core protein is the first structural protein encoded by the HCV open reading frame (ORF), consisting of 191 amino acids in its immature form. It is one of the potential targets for specific drugs against HCV, as it is well conserved in all HCV genotypes and interacts with a number of cellular factors of the host immune system (3,4). Expression of the HCV core protein results in suppression of type I interferon (IFN) signaling leading to the reduction of phosphorylated STAT1 (P-STAT1). HCV core protein and STAT1 are reported to have a direct interaction involving residues in the N-terminal portion of the HCV core (amino acids 1-23) (5). Mutations in the N-terminal of the core protein are expected to modulate antiviral response in general, as well as response to conventional pegylated interferon (PEG-IFN) and ribavirin (PEG-IFN/ribavirin) combination therapy, eventually leading to sustained virological response.

Emerging HCV resistance to the current standard available treatment, PEG-IFN/ribavirin combination therapy, is of great concern, as is its low response and toxicity (6). Although new direct acting antivirals (DAAs) targeting HCV NS3-4A protease, namely telaprevir and boceprevir, have shown an increase in the sustained virological response (SVR) of up to 70% in patients infected with HCV genotype 1 (7), the conventional PEG-IFN/ribavirin treatment remains part of the therapy. Clinical studies have suggested that non-synonymous mutations are induced by ribavirin monotherapy and thus increase IFN sensitivity (8). The SVR has been shown to be markedly augmented by the addition of ribavirin to IFN monotherapy, with an increase in the response rate and a reduction in the relapse rate being observed (9). Mathematical

Table I. Demographic and follow-up data of hepatitis C virus (HCV) infected patients.

Patients undergoing interferon/ribavirin treatment								Untreated patients			
Patient code	Age (Yrs)	Gender	Base line viral titer/ALT	12-week viral titer/ALT	24-week viral titer/ALT	36-week viral titer/ALT	48-week viral titer/ALT	Patient code	Age (Yrs)	Gender	Viral titer/ml ALT level U/l
PT1	42	Female	$5x10^9$/110	$2x10^5$/58	$3x10^5$/80	-	-	P1	32	Female	$5.6x10^6$/58
PT2	40	Male	$5x10^8$/110	$3x10^5$/56	$3x10^5$/80	-	-	P2	44	Female	$1x10^6$/98
PT3	39	Female	$7x10^6$/96	$2x10^5$/49	<4000/48	<4000/43	<4000/43	P3	40	Male	$5.6x10^6$/62
PT4	45	Male	$9x10^7$/127	$4x10^4$/50	<4000/50	<4000/46	<4000/46	P4	37	Male	$1.7x10^5$/78
PT5	41	Male	$4x10^8$/90	$4x10^4$/45	<4000/42	<4000/40	<4000/40	P5	29	Male	$8x10^6$/90
PT6	39	Female	$8x10^7$/98	$7x10^4$/47	<4000/47	<4000/38	<4000/38	P6	32	Female	$5x10^6$/68
PT7	40	Male	$6x10^8$/94	$4x10^6$/72	<4000/48	<4000/36	<4000/42	P7	37	Male	$9x10^4$/98
PT8	43	Male	$9x10^6$/105	$9x10^5$/68	<4000/48	<4000/42	<4000/38	P8	37	Female	$2x10^6$/127

The HCV core gene was amplified from the patient sera. In the treated patients, the core gene was amplified after the completion of 12 weeks of therapy and viral titer and alanine transaminase (ALT) levels were recorded at an interval of 12 weeks.

model applications have revealed viremic decay following combination therapy (10).

In this study we propose a possible mechanism of PEG-IFN/ribavirin-induced SVR. We suggest that PEG-IFN/ribavirin therapy-induced amino acid changes in the N-terminus of the HCV core are associated with viral clearance or persistence.

Materials and methods

Patient demographics. Patients with a positive PCR test for HCV, confirmed by Atta-ur-Rahman School of Applied Biosciences (ASAB) Diagnostics, were enrolled for this study under the approval of the Internal Review Board (IRB) of ASAB, National University of Sciences and Technology, Pakistan and a patient consent form was duly signed for each patient. All patients were infected with genotype 3a, the most prevalent genotype in Pakistan. The genotype of the patients was determined using the method described by Ohno *et al* (11). The HCV patients selected for this study were from two different groups. The patients in group A were receiving treatment with pegylated interferon α-2a (PEG-IFN α2a) 180 μg/week and ribavirin 800 mg/day for 24 weeks. In group B, patients that were recently diagnosed and had viral titer and alanine transaminase (ALT) levels relatively close to those of group A were selected (Table I). The follow-up for the treated patients was carried out for 24 weeks following the completion of treatment. The viral loads and ALT levels of the patients were measured at 12-week intervals (Table I). Viral RNA was quantified using a Bio-Rad RoboGene HCV amplification kit (Bio-Rad, Hercules, CA, USA), whereas Microlab 300 (Merck, Germany) was employed for ALT measurements. Sequencing of the isolated virus core gene was performed after 12 weeks of PEG-IFN/ribavirin bitherapy. To compare the mutations induced by ribavirin, the HCV core gene was also cloned and sequenced from HCV-infected patients without liver complications and who had not received any treatment.

PCR amplification cloning and sequencing of HCV core gene. For PCR amplification of the core gene, viral RNA was extracted from patient serum by using an RNA extraction kit (Qiagen, Hamburg, Germany) according to the manufacturer's instructions. The primers used for cDNA synthesis and PCR amplification were: 5'-AAA GAA TTC GCC ACC ATG CTA GAG TGG CGG AAT ACG TCT GGC C-3' (sense) and 5'-CCC GCG GCC GCT TAA CTG GCT GCT GGA TGA ATT AAG C-3' (antisense). Purified PCR products were cloned in PCRII TOPO Cloning vector (Invitrogen, Singapore) as instructed by the manufacturer. Two clones from each patient were subjected to sequencing using a CEQ 8000 genetic analysis system (Beckman Coulter, Miami, FL, USA) as described previously (12). Sequences from the present patients were aligned with reference isolates (Fig. 1). The aligned sequences of HCV core (>3000 sequences) from the European database (http://euhcvdb.ibcp.fr/euHCVdb/) were analyzed to check the conservation of the residues involved in core-STAT1 interactions in the N-terminus of the core gene.

Molecular modeling of HCV core gene and its in silico interaction with STAT1. The HCV core gene consensus sequence of all 16 clones from the untreated patients was submitted to the I-TASSER online web server (13) for molecular modeling and the model with the highest C-value was selected for further analysis. The model was refined with energy minimization by subjecting it to ionized water box and physiological concentrations. The AMBER 99 force field was used to minimize its energy after protonation of the system by fixing its charges and lone pairs. The minimized model was extracted from the solvent system and was docked with the STAT1 protein (pdb id 1yvl). The protein interaction between core and STAT1 was studied using the HADDOCK web server (14). Based on previous studies, residues 1-23 of the core were selected as the active site and residues 577-684 of STAT1 were selected as passive residues for this interaction. Different contact types, including ionic cutoff 4.5, hydrophobic cutoff 4.5, hydrogen bonds and disulfide cutoff 2.5, were evaluated between the

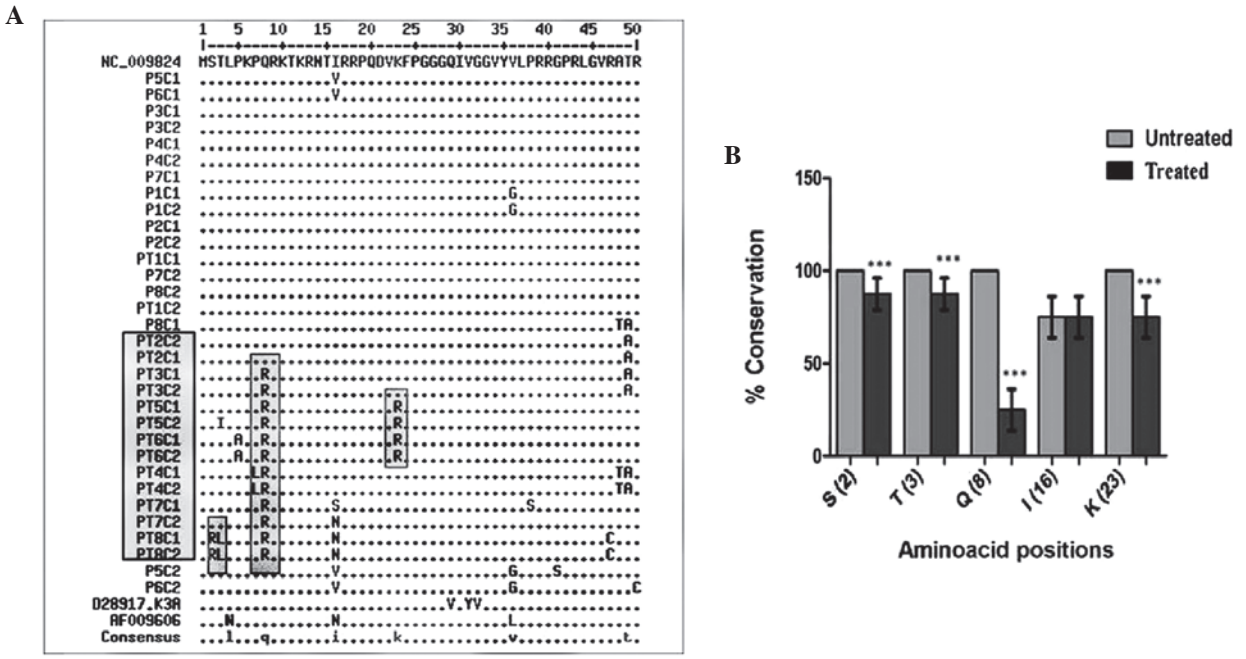

Figure 1. (A) Core sequence alignment of residues 1-50. AF009606 is the reference sequence H77 (1a), NC_009824 (NZL1,3a) and D28917.k3a respectively represent reference strains for the 3a subtype. Letter P represents the untreated patient number and C1 and C2 represent clone 1 and clone 2 from same patient. The letters PT are used for patients undergoing treatment. Mutations observed at residues 2, 3, 8 and 23 result in loss of core-STAT1 interaction and are highlighted with grey rectangles. All treated patients with the exception of PT1 and PT2 had resolved hepatitis C virus (HCV) infection. (B) Mutations in the HCV core STAT1-interacting residues that were frequently mutated in the HCV cores of treated patients. Letters S, T, Q, I and K represent the amino acids predominantly present at these positions in the cores from untreated patients. ***Significant changes (P<0.001) in these residues vs. those in the HCV cores of untreated patients. P<0.05 was considered statistically significant.

core and STAT1 using the default bond angles used in the Molecular Operating Environment (MOE). The sequence separation used was 4 residues apart. Histidine was selected as it is basic in character whereas methionine was characterized as hydrophobic in nature. The mutations observed at core residues 2, 3, 8, 16 and 23 (Fig. 1) were incorporated into the *in silico* interaction model. In order to investigate the further significance of the observed mutations in the core STAT1-interacting domain (amino acids 2, 3, 8, 16, 23) two way ANOVA was applied on mutational data from treated and untreated core residues. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Sequence analysis. For sequence comparison, H77 genotype 1a was taken as a reference strain for amino acid positioning. NZL1 and K3a isolates of GT3a were taken as references for sequence analysis (Fig. 1). Notably, as compared with reference isolates and clones from untreated patients, few major differences were observed in the N-terminal region of the core. Residues 2, 3, 8, 16 and 23 were frequently mutated in treated patients as compared with untreated patients (Fig. 1; Table II) and significant differences (P<0.001) were recorded at positions 2, 3, 8 and 23. Comprehensive analysis of the aligned core sequences reported in the European database showed that residues 2, 3, 8 and 23 are well conserved across all genotypes (Table III). Position 16 was not well conserved and the mutations observed had no effect on core-STAT1 interactions.

Table II. *In vivo* mutations observed in the hepatitis C virus core proteins from treated patients and their effects on STAT1 interaction.

Core residues	Mutations	Contact alteration
2	S/R	No contacts observed
3	T/L,I	No contacts observed
7	P/L	Contact established with Val 642 and Ile 647 of STAT1
8	R/Q	No contacts observed
16	S/N,I	Contact conserved either N/I with Asp 627
23	K/R	No contact observed

Contact report is based on the results achieved from the i*n silico* interaction of the core with STAT1 using the HADDOCK web server.

In silico characterization of the STAT1 binding domain of the core protein. Sequence variations observed in the present study were mostly found in the N-terminus of the protein, a region which has been previously shown to interfere with IFN signaling by interacting with the STAT1-SH2 domain (15). We therefore investigated how these amino acid changes potentially affect core-STAT1 interactions. For this purpose, an *in silico* approach based on prediction of molecular docking was used. Although the structure of STAT1 is known, the HCV core protein structure has not been reported. We therefore

Table III. Variability and contact report of hepatitis C virus core residues involved in the core-STAT1 interaction.

Position in core	Actual residue	Mutated residues	% variability	Contact report core-STAT1 interaction
2	S	N=1/3498	-	No contact observed
		G=1/3498	-	No contact observed
3	T	A=3	A=0.001	No contact observed
		R=2		No contact observed
		M=1		No contact observed
7	P	R=1	L=0.001	No contact observed
		L=4	-	Hydrophobic bonding between leucine 7 to valine 642 and isoleucine 647
8	Q			Hydrogen bonding between arginine at position 8 and asparagine 646
		R=7	R=0.002	No contact observed
		P=11	P=0.003	No contact observed
		H=2	P=0.001	Hydrogen bonding between histidine 8 to tyrosine 634
		K=2	P=0.001	Hydrogen bonding between lysine 8 to asparagine 650.
		S=1	P=0.001	No contact observed
23	K	R=10	R=0.002	No contact observed
		E=4		No contact observed
		S=31		No contact observed

%variability was calculated by comparison of 3,498 core sequences from the European HCV database. Number indicated with mutated residue is the number of times the mutation reported in 3498 sequences.

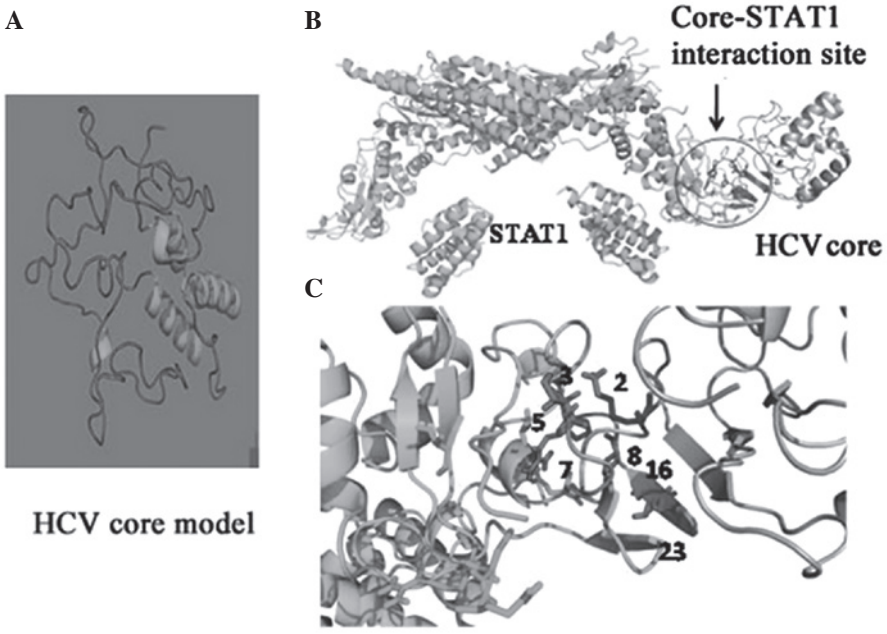

Figure 2. Hepatitis C virus (HCV) core and STAT1 interaction. (A) HCV core model obtained from I-TASSER. (B) HCV core-STAT1 interaction results obtained by the HADDOCK web server. STAT1 dimmer has been shown towards left and the core is at right side of the interaction model. (C) Magnified interaction area. Numbers show the positions repeatedly mutated in different isolates of the HCV core and the positions involved in the interaction.

started by determining a structural model for the HCV core protein (Fig. 2A). The characterization of interaction contacts between the HCV core and STAT1 were then determined (Fig. 2B; Table II) and the contact details of the interacting residues are provided in Table III. Based on our molecular modeling approaches, amino acids S, T, Q and K at positions 2, 3, 8 and 23, respectively, appear critical for core-STAT1 interaction. Changes in these residues, as observed in some of our clones, resulted in loss of contact between core and STAT1 (Fig. 2; Tables II and III). The follow-up data and the core-

STAT1 docking results clearly correlate the SVR observed in six out of eight patients that carried observed mutations. Follow-up of the untreated patients was not conducted, as they were recommended for treatment.

Discussion

The mechanism of viral persistence and clearance has not been well elucidated. Viral capsid proteins have been proposed previously as targets for anti-viral drugs, as they are well conserved across the 6 major genotypes (16). In the current study, the HCV core gene was amplified from the serum of patients that were undergoing PEG-IFN/ribavirin treatment for 12 weeks and from treatment-naïve patients. As the core quasispecies tends to be conserved during acute HCV infection (17), in the present study patients without liver complications and at a relatively early stage of disease were enrolled and thus a more conserved core gene was anticipated.

Notably, as compared with isolates from untreated patients (Fig. 1), few significant differences were observed in the N-terminal region of the cores from treated patients. Comprehensive analysis of the aligned core sequences reported in the European database showed that residues 2, 3, 8 and 23 are well conserved across all genotypes. Amino acid changes in this part of the protein are known to modulate viral assembly or core interactions with host factors (18). The N-terminal region of the core (amino acids 1-23) has been shown to block IFN signaling by interaction with the STAT1-SH2 domain that plays a significant role in HCV resistance to IFN therapy (19). *In silico* molecular docking was used to observe the potential effects of these changes on the core-STAT1 interaction. For this purpose, the HCV core protein structure was modeled (Fig. 2A) and the interaction contacts between the HCV core and STAT1 were determined (Fig. 2B). The contact details of the interacting residues are provided in Table II. Based on our molecular modeling approaches, amino acids S, T, Q and K at residues 2, 3, 8 and 23 appear critical for core-STAT1 interaction. Changes in these residues, as observed in the majority of our clones from treated patients, resulted in a loss of contact between the core and STAT1. Mutations at similar positions were rarely reported in the HCV database and these residues tend to be conserved among various genotypes.

Follow-up information (Table I) revealed that the core mutations observed in six patients at critical residues resulted in a loss of contact with STAT1, thus ensuring better antiviral response and facilitating viral clearance. However, two of the patients, patients 1 (PT1) and 2 (PT2), showed no mutation at these positions. These two patients were non-responders and discontinued therapy after six months (Table I). Notably, in patient 4 (PT4), the virus had counteracted the loss of the STAT1 interaction at position 8 by a P>L shift at core position 7 that resulted in the establishment of a new interaction with Val 642/Ile 647 of STAT1 (Table III). This new contact may modulate STAT1 signaling and a relapse may occur following the accumulation of the resistant variant. An early virological response reported for genotype 3a was not evident in the current study, possibly due to the small sample size; however, the identification of non-responders is not unusual for genotype 3a. We have recently recorded a significant difference in the mutation rate of HCV glycoprotein E2 in treated vs. untreated patients and have observed for the first time a glycosylation position shift in envelope protein E2 that results in antibody escape variants, giving the virus a chance to survive following the therapeutic response (unpublished data).

Previous reports have indicated that amino acid substitutions at position 70 and/or 91 in the HCV core protein region of patients infected with HCV-1b are pretreatment predictors of a poor virological response to PEG-IFN/ribavirin combination therapy and telaprevir/PEG-IFN/ribavirin triple therapy (20,21). In all patients included in this study, the core position 70 was occupied by arginine as reported for genotype 1b and should favor the treatment response, however the failure of patients 1 and 2 to respond to treatment suggest that there may be more than one predictor of therapeutic outcomes. Core residue 91 appears to be genotype-specific and thus may contribute to the genotype-specific antiviral response to IFN/ribavirin therapy. Another study, however, described that the ribavirin monotherapy-induced mutagenic effect, studied in the context of the NS3 and NS5B regions of HCV, was reduced in patients receiving PEG-IFN and ribavirin combination therapy, possibly due to the antiviral action of IFN (22). In the current study, since the ribavirin monotherapy was not included due to its absence from the general medical practices prevailing in Pakistan, there is a possibility that certain other error mutations may have been immediately eliminated by the concurrently administered IFN. This may account for the relatively small number of mutations observed in the current study, despite the presence of a mutagenic analog in the combination bitherapy.

In conclusion, this study suggests that IFN/ribavirin bitherapy-induced mutations in the STAT1-interacting domain of the HCV core protein may be responsible for the improved therapeutic response and viral clearance, at least in the GT3a genotype, the most prevalent genotype in Pakistan. However, this treatment may give rise to resistant variants that are able to escape the current therapy. In addition, this study indicates for the first time that residues 2, 3, 8, and 23 of the HCV core are critical for the core-STAT1 interaction and we propose these residues as a potential target for antiviral drug design.

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References

1. Alter MJ, Mast EE, Moyer LA and Margolis HS: Hepatitis C. Infect Dis Clin North Am 12: 13-26, 1998.
2. Chen SL and Morgan TR: The natural history of hepatitis C virus (HCV) infection. Int J Med Sci 3: 47-52, 1997.
3. Lin W, Kim SS, Yeung E, Kamegaya Y, Blackard JT, Kim KA, Holtzman MJ and Chung RT: Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. J Virol 80: 9226-9235, 2006.

4. Melén K, Fagerlund R, Nyqvist M, Keskinen P and Julkunen I: Expression of hepatitis C virus core protein inhibits interferon-induced nuclear import of STATs. J Med Virol 73: 536-547, 2004.
5. de Lucas S, Bartolome J and Carreno V: Hepatitis C virus core protein down-regulates transcription of interferon-induced antiviral genes. J Infect Dis 191: 93-99, 2005.
6. Hadziyannis S, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A and Ackrill AM; PEGASYS International Study Group: Peginterferon-α2a and ribavirin therapy in chronic hepatitis C: A randomized study of treatment duration and ribavirin dose. Annal Intern Med 140: 346-355, 2004.
7. Dore GJ, Matthews GV and Rockstroh J: Future of hepatitis C therapy: development of direct-acting antivirals. Curr Opin HIV AIDS 6: 508-513, 2011.
8. Asahina, Y, Izumi N, Enomoto N, Uchihara M, Kurosaki M, Onuki Y, Nishimura Y, Ueda K, Tsuchiya K, Nakanishi H, Kitamura T and Miyake S: Mutagenic effects of ribavirin and response to interferon/ribavirin combination therapy in chronic hepatitis C. J Hepatol 43: 623-629, 2005
9. Chung RT, Gale M Jr, Polyak SJ, Lemon SM, Liang TJ and Hoofnagle JH: Mechanisms of action of interferon and ribavirin in chronic hepatitis C: Summary of a workshop. Hepatology 47: 306-320, 2008.
10. Herrmann E, Lee JH, Marinos G, Modi M and Zeuzem S: Effect of ribavirin on hepatitis C viral kinetics in patients treated with pegylated interferon. Hepatology 37: 1351-1358, 2003.
11. Ohno T, Mizokami M, Wu RR, Saleh MG, Ohba KI, Orito E, Mukaide M, Williams R and Lau JY: New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 35: 201-207, 1997.
12. Waheed Y, Tahir S, Ahmad T and Qadri I: Sequence comparison and phylogenetic analysis of core gene of hepatitis C virus from Pakistani population. Afr J Biotechnol 9: 4561-4567, 2010.
13. Wu S, Skolnick J and Zhang Y: Ab initio modeling of small proteins by iterative TASSER simulations. BMC Biol 5: 17, 2007.
14. de Vries SJ, van Dijk M and Bonvin AM: The HADDOCK web server for data-driven biomolecular docking. Nat Protoc 5: 883-897, 2010.
15. Prevelige PE Jr: Inhibiting virus-capsid assembly by altering the polymerisation pathway. Trends Biotechnol 16: 61-65, 1998.
16. Klein KC, Dellos SR and Lingappa JR: Identification of residues in the hepatitis C virus core protein that are critical for capsid assembly in a cell-free system. J Virol 79: 6814-6826, 2005.
17. Tang X, Wagoner J, Negash A, Austin M, McLauchlan J, Hahn YS, Rosen HR and Polyak SJ: Functional characterization of core genes from patients with acute hepatitis C. J Infect Dis 201: 912-922, 2010.
18. Hourioux C, Ait-Goughoulte M, Patient R, Fouquenet D, Arcanger-Doudet F, Brand D, Martin A and Roingeard P: Core protein domains involved in hepatitis C virus-like particle assembly and budding at the endoplasmic reticulum membrane. Cell Microbiol 9: 1014-1027, 2007.
19. Lin W, Choe WH, Hiasa Y, Kamegaya Y, Blackard JT, Schmidt EV and Chung RT: Hepatitis C virus expression suppresses interferon signaling by degrading STAT1. Gastroenterology 128: 1034-1041, 2005.
20. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K and Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48: 372-380, 2005.
21. Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y and Kumada H: Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 52: 421-429, 2010.
22. Hofmann WP, Polta A, Herrmann E, Mihm U, Kronenberger B, Sonntag T, Lohmann V, Schönberger B, Zeuzem S and Sarrazin C: Mutagenic effect of ribavirin on hepatitis C nonstructural 5B quasispecies in vitro and during antiviral therapy. Gastroenterology 132: 921-930, 2007.