Effect of combined siRNA of HCV E2 gene and HCV receptors against HCV

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Abstract

Background/Aim: Hepatitis C virus (HCV) is a major threat as almost 3% of the world’s population (350 million individual) and 10% of the Pakistani population is chronically infected with this virus. RNA interference (RNAi), a sequence-specific degradation process of RNA, has potential to be used as a powerful alternative molecular therapeutic approach in spite of the current therapy of interferon-α and ribavirin against HCV which has limited efficiency. HCV structural gene E2 is mainly involved in viral cell entry via attachment with the host cell surface receptors i.e., CD81 tetraspanin, low density lipoprotein receptor (LDLR), scavenger receptor class B type 1 (SR-B1), and Claudin1 (CLDN1). Considering the importance of HCV E2 gene and cellular receptors in virus infection and silencing effects of RNAi, the current study was designed to target the cellular and viral factors as new therapeutic options in limiting HCV infection.

Results: In this study the potential of siRNAs to inhibit HCV-3a replication in serum-infected Huh-7 cells was investigated by combined treatment of siRNAs against the HCV E2 gene and HCV cellular receptors (CD81 and LDLR), which resulted in a significant decrease in HCV viral copy number.

Conclusion: From the current study it is concluded that the combined RNAi-mediated silencing of HCV E2 and HCV receptors is important for the development of effective siRNA-based therapeutic option against HCV-3a.

Keywords: HCV, siRNA, HCV receptors, HCV envelope genes and viral titer

Introduction

HCV infection is a major health problem with nearly 10% chronically infected population in Pakistan and 350 million people worldwide [1,2]. About 75% of patients achieve no therapeutic benefit from the present combination therapy with pegylated interferon α (PEG-IFN-α) and ribavirin mainly depending upon HCV genotype, whereas in 40-60% patients chronic infection is mainly associated with liver cirrhosis and steatosis leading to hepatocellular carcinoma (HCC) [3-5]. In Pakistan the major HCV genotype is 3a followed by 3b and 1a, with a strong correlation between chronic HCV infection and HCC in Pakistan associated with genotype 3a [6,7]. There is a desperate need to develop more efficient and better therapeutic alternative for treatment of HCV infections.

Due to the absence of suitable animal model and competent in vitro cell culture system the mechanism of HCV cell entry was unrevealed after a long time. Recently, different groups have studied HCV replication in serum infected liver cell lines which mimics the naturally occurring HCV virions biology and kinetics of HCV infection in humans liver cells [8-11]. HCV envelop glycoproteins E1 and E2 are involved in HCV entry, fusion and defense against neutralization by envelop-specific host antibodies [12-18]. E2 glycoprotein works as a key component in interaction between the virus and its major cellular receptors i.e., CD81, SR-B1 and CLDN1 [15-17]. CD81 is a main HCV cell surface receptor, whereas additional role is played by the scavenger receptor class B type I (SRBI) and the low-density-lipoproteins receptor (LDLR) [19-22]. LDLR is potentially involved in the uptake of lipoprotein-associated HCV into hepatocytes as serum fraction...
composed of HCV with LDL, or very low-density lipoprotein (VLDL), which are involved in binding to the LDL receptor as a possible mechanism of HCV cell entry [23-25]. Hence, HCV envelope glycoprotein and cellular receptors is good target for the development of antiviral molecules that could block HCV entry.

Being a RNA virus HCV is highly susceptible to RNA interference (RNAi) induced by small interfering RNA (siRNA), which is a sequence specific gene silencing mechanism [26-28]. siRNAs can be used as a potential therapeutic agent against HCV because HCV replication takes place in the cytoplasm of liver cells without integration into the host genome. siRNA directed against HCV genotype 1a and 1b has been shown to effectively block the replication of viral replicons in Huh-7-derived cell lines [29-35]. In our previous study, the development of siRNA targeting envelope proteins of the local HCV-3a genotype showed that these genes are crucial for viral entry providing better choice for developing a rational antiviral strategy against HCV [36]. Several investigators have reported the inhibition of HCV RNA by targeting structural and non structural genes of HCV and cellular genes by using siRNAs in combination [33,36-38]. In this report, we investigated the effect of siRNA induced silencing of receptor genes and HCV E2 on viral load of HCV followed by a combined effect which showed a significantly decreased viral RNA.

Results
Cellular genes CD81 and LDLR are functionally involved in HCV entry. Our previous results also show that sequence specific siRNAs against each receptor significantly inhibit the expression of their respective genes of receptors CD81, LDLR, SRBI and CLDN1 (data submitted for publication). Keeping all these in view, we used in-vitro transcribed siRNA against HCV E2 gene and cellular receptors CD81 and LDLR and observed the effect of silencing of these receptors on viral titer. Previously, we have successfully inhibited HCV by E2 siRNAs [36], from that study we selected the best one for this study. The effect on viral titer was analyzed by silencing each receptor and E2 gene individually and then in combination of siRNAs against two receptors and E2 gene simultaneously. To evaluate the role of HCV E2 gene and HCV receptors in HCV infection and HCV pathogenesis, Huh-7 cells were infected with HCV-3a serum with or without siRNAs against HCV receptors CD81, LDLR and HCV E2-3a gene for 48hrs and viral loads were quantified by Real Time PCR. Results showed a decrease of 62%, 45%, with HCV receptor siRNA CD-81, LDL, 60%, and 72% with HCV E2 siRNA, (E2si873), respectively in viral load of HCV followed by a combined effect which showed a significantly decreased viral RNA.

Discussion
HCV entry into hepatocytes, a multistep process mediated by HCV envelope glycoprotein E1 and E2 and several cell surface receptors, is first step of virus life
...receptors activity to reduce the HCV infection in which showed potent RNAi against HCV genes and cellular serve as potential targets for RNAi. Several reports in HCV entry like CD81, LDLR, SR-BI and CLDN1 also been reported that cellular genes functionally involved into viral replication via negative strand intermediate. Previously it has been shown that the viral messenger RNA and template for RNA replication (+) sense single stranded RNA that functions as both an attractive target for RNAi therapy as its genome is a posed to be used in treatment of viral diseases. HCV is treated serum-infected Huh-7 cells.

Quantifying the viral titer in siRNA-treated and non-structural gene and receptor genes on viral entry by siRNA separately and in combination against HCV infection were investigated in serum infected Huh-7 cell culture model to evaluate the effect of our previous study, here to we utilized serum infected HCV 3a E2 gene for 48 hrs. Protein levels were quantified by western blot analysis using specific antibodies of CD81, LDLR, E2 and GAPDH. A) Silencing of CD81 gene and HCV 3a E2 gene alone and in combination using specific antibodies showing reduction at protein expression level. B) Silencing of LDLR gene and HCV 3a E2 gene alone and in combination, using specific antibodies showing reduction at protein expression level. Protein levels for GAPDH gene are also shown as internal control and scramble siRNA (Sc) as siRNA control.

RNAi is an exciting new therapeutic technology proposed to be used in treatment of viral diseases. HCV is an attractive target for RNAi therapy as its genome is a (+) sense single stranded RNA that functions as both the viral messenger RNA and template for RNA replication via negative strand intermediate. Previously it has been reported that cellular genes functionally involved in HCV entry like CD81, LDLR, SR-BI and CLDN1 also serve as potential targets for RNAi. Several reports showed potent RNAi against HCV genes and cellular receptors activity to reduce the HCV infection in which expression of HCV was distinctly inhibit HCV serum infection (30%-90%) [11,36,42-45]. In our current project, we utilized siRNAs to silence the expression of HCV cellular receptors and E2 gene to block the HCV entry in serum derived HCV infected Huh-7 cell culture model and analyze its effect on viral load. HCV infection pathway employs enhancement in expression of cell surface receptors that may facilitates to increase viral load. In account of these, we knock down the expression of host cell surface HCV receptors by using siRNA to block HCV entry, against each receptor gene separately and in combination of siRNA against two receptors gene in Huh-7 cells which were further infected with HCV serum of genotype 3a and observed the viral titer by detection of 5'UTR of viral copies by Real Time PCR in cells from 3rd day post infection. Our results indicate significant decrease in HCV viral load by 67% and 58% due to the silencing of HCV receptor CD81 (33 fold) and LDLR (42 fold) respectively when compared to control (S3a), (Figure 1). Since, LDLR is important for HCV-E1-pseudotype infectivity, whereas CD81 determines the infectivity of HCV-E2-pseudotype virus, the silencing effect of siRNAs against selected HCV infection host cellular proteins has been evaluated to reduce viral titer significantly. Thus use of combinations of siRNAs against both the virus and host genes in HCV infection are likely to be a potent approach in the treatment of chronic hepatitis C due to their additive HCV RNA inhibition effects. Moreover, different studies exhibit the feasibility of targeting host cellular factors involved in infection, as they are not prone to mutations, as potential targets for siRNA therapy. Henry and colleagues [46] targeted the IRES, NS5B, and host cell receptor CD81 by triple shRNA expression vector which concurrently reduced the HCV replication, CD81 expression, and E2 binding. Targeting multiple sites of the HCV genome and host factors involved in HCV infection are a realistic and valid approach aimed at preventing the virus from developing resistance.

In correspondence to the latest reports, we also investigated the down regulation of viral titer by silencing the expressions of HCV envelope gene alone using HCV E2 specific siRNA (E2si873) and in combination with the silencing of CD81 or LDLR gene expression using siCD81-B, siLDLR in Huh-7 cells which were further infected HCV 3a serum. Our findings showed a significant decrease in HCV viral titer up to 67% and 58%, with siCD81-B, siLDLR and 72% with HCV E2 siRNA (E2si873), respectively in the Huh-7 cells. A significant suppression of HCV RNA (84% and 78%) was observed with the combination of both siRNAs against HCV E2 gene, E2 siRNA (E2si873), and CD81, LDLR siRNAs (Figure 1). Likewise, cell lysates from HCV serum infected Huh-7 cells were examined by western blot...
analysis using CD81, LDLR and HCV-E2 specific antibodies. Our results showed considerable decrease in the protein levels of CD81, LDLR and HCV-E2 in transiently transfected siCD81-B, siLDLR and E2si873 siRNAs, whereas combination of siCD81-B + E2si873 and E2si873 + siLDLR resulted in a more significant decrease of CD81, LDLR protein expression that ultimately reduced the protein level of HCV-E2 which depicts the reduced entry of HCV, hence lessen the HCV infection (Figure 2).

In summary, our data showed that CD81 and LDL specific siRNAs not only reduced their gene expression respectively but also reduced viral titer in siRNA treated cells confirming their role in HCV infection; combination of these siRNA (siCD81-B, siLDLR) with E2 effective siRNA (E2si873) showed dramatic reduction of HCV entry. Use of siRNA to inhibit the HCV E2 protein or HCV receptor protein expression alone or in combination could be helpful in reduction of HCV entry. In addition, we propose the use of combination siRNAs against HCV gene with host genes which could inhibit HCV entry better than separately used siRNA.

Materials and Methods
Source of samples
The local HCV-3a patient’s serum samples used in this investigation were obtained from the CAMB (Center for Applied Molecular Biology) diagnostic laboratory, Lahore, Pakistan after quantification and genotype determination. Serum samples were stored at -80°C prior to RNA extraction for cloning and viral inoculation experiments. Patient’s written consent and approval for this study was obtained from institutional ethics committee.

Designing and synthesis of siRNA
Designing and synthesis of siRNA were done as we have described earlier [36]. siRNA oligonucleotides were designed to express RNAi mechanism against host HCV receptors (LDLR and CD81) and E2 region of HCV-3a genome using the Ambion’s siRNA design tool http://www.ambion.com/techlib/misc/siRNA_finder.html after sequencing of local HCV-3a patient’s serum samples (Table 1). The designed siRNAs (cellular genes HCV receptors, HCV-3a E2 and control Scrambled) were synthesized using Silencer siRNA construction kit according to the manufacturer’s instruction (Ambion, USA).

Viral inoculation and co-transfection with siRNA
Huh-7 cell line was kindly provided by Dr. Zafar Nawaz (University of Miami, USA) and maintained in Dulbecco’s modified eagle medium (DMEM) supplemented with 100 μg/ml penicillin; streptomycin and 10% fetal bovine serum referred as complete medium (Sigma Aldrich, USA) at 37°C with 5% CO₂. The medium was renewed every 3 day and passaged every 4-5 days. Huh-7 cell line was used to establish the in vitro replication of HCV genotype 3a. A similar protocol was used for viral inoculation as described by earlier [36,45]. For these experiments high viral titer > 1 × 10⁸ IU/ml containing serum from HCV-3a patient was used as principle inoculum. Huh-7 cells were maintained in 6-well culture plates to semi-confluence, washed twice with serum-free medium then inoculated with 500 μl (5 × 10⁸IU/well) viral load of HCV-3a sera and 500 μl serum free media. Cells were maintained overnight at 37°C in 5% CO₂. Next day, the adherent cells were washed three times with 1 x PBS, complete medium was added and incubation was continued for 48hrs. Cells were harvested and assessed for the presence of viral RNA quantitatively by Real Time PCR. To analyze the effect of siRNA on HCV infection, serum infected Huh-7 cells were seeded after three days of infection in 24-well plates and grown to 80% confluence with 2ml medium. The cells were transfected with or without 40 μM/well cellular receptors CD81, LDL-R and E2 siRNAs alone or in combination using Lipofectamine™ 2000 (Invitrogen Life Technologies, CA) according to the manufacturer’s protocol.

Viral load quantification
Cells were harvested for viral load determination using Gentra RNA isolation kit (Gentra System Pennsylvania, USA) according to the manufacturer’s instructions. For viral quantification Sacace HCV quantitative analysis kit (Sacace Biotechnologies Caserta, Italy) was used. Briefly, 10 μl of extracted viral RNA was mixed with an internal control provided by Sacace HCV Real TM Quant kit and subjected to viral quantification using Real Time PCR SmartCycler II system (Cepheid Sunnyvale, USA).

Table 1

| Name          | Sequences                                                                 |
|---------------|---------------------------------------------------------------------------|
| Scramble-antisense | AAACCTGATACGGGACTCGACCCCTGTCTC                                               |
| Scramble-sense  | AAGTCGAGTCGCTATGAGAGGCGCTGTCTC                                              |
| CD81-B antisense | AAAGTATGCTACTGAGGCTGTCTC                                                   |
| CD81-B sense   | AACACCTTGTGATGAGATGCTACCTGCTCTC                                             |
| LDL antisense  | AAATGCATTCATCACAATGTCGGCGCTGTCTC                                             |
| LDL sense      | AACCCTGTAGGGACATGCTACCTGCTCTC                                               |
| E2si873-antisense | AACAAGGTGACCTGTCACCAATCCTGCTCTC                                              |
| E2si873-sense  | AAAGTATGCTACCTGGGACGTCTGCTCTC                                               |

Total RNA isolation and gene expression analysis
Total RNA from HCV serum infected and non-infected cells was isolated using TRizol reagent (Invitrogen life technologies, CA), 24 hrs and 48hrs post-transfection. To analyze the effect of siRNA on envelope gene
expression, cDNA was synthesized with 1 μg of total RNA using Superscript III cDNA synthesis kit (Invitrogen life technologies, CA) and semi-quantitative RT-PCR was done using primers of HCV receptors, E2 genes and GAPDH as control. Quantitative Real Time PCR was carried out using Real Time ABI 7500 system (Applied Biosystems Inc, USA) with SYBR Green mix (Fermentas International Inc, Canada) as we have described earlier [36]. The relative gene expression analysis was carried out by the SDS 3.1 software (Applied Biosystems Inc, USA). Each individual experiment was performed in triplicate.

Western blotting
To determine the effect of siRNAs on protein expression levels HCV receptors CD81 and LDL-R and E2, in HCV serum infected cells, cells were lysed using ProteoJet mammalian cell lysis reagent (Fermentas, Canada). Equal amounts of total proteins were subjected to electrophoresis on 12% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane according to the manufacturer’s protocol (Bio-Rad, CA). After blotting non-specific binding sites with 5% skimmed milk, blots were incubated with primary monoclonal antibodies specific to HCV cellular receptors like CD81 and LDL-R, HCV E2 and GAPDH genes (Santa Cruz Biotechnology Inc, USA) and secondary Horseradish peroxidase-conjugated anti-goat anti-mouse antibody (Sigma Aldrich, USA). The protein expressions were evaluated using chemiluminescence’s detection kit (Sigma Aldrich, USA).

Statistical analysis
All statistical analysis was done using SPSS software (version 16.0, SPSS Inc). Data are presented as mean ± SD. Numerical data were analyzed using student’s t-test and ANOVA. P value < 0.05 was considered statistically significant.

List of abbreviations
E1, E2: Envelop proteins 1, 2. HCC: Hepatocellular carcinoma, HCV: Hepatitis C virus, CD81: Cluster of differentiation B1, SR-BI: Scavenger Receptor Class B Type 1, LDL-R: Low-Density Lipoprotein Receptor, CLDN 1: Claudin 1, PEG-IFN-α: pegylated interferon alpha, RNAi: RNA interference, siRNAs: small interfering RNAs.

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Authors’ contributions
SJ, SK and BS contributed equally to this work, conceive the idea and performed all the lab work. MH and UAA helped SJ and SK in lab work and literature review. BI and WA helped SJ and SK in data analysis. SK and SJ critically reviewed and finalized the manuscript. SH provided all facilities to complete this work. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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References
1. Giannini C, Brechot C: Hepatitis C virus biology. Cell Death Differ 2003, 10(Suppl 1):S27-S38.
2. Raja NS, Janjua KA: Epidemiology of hepatitis C virus infection in Pakistan. J. Microbial Immunol Infect 2008, 41:4-8.
3. Athnal NR: The natural history of hepatitis C. Semin Liver Dis 2004, 24(Suppl 2):9-8.
4. Manns MP, McHutchinson JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Lint M, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001, 358:958-965.
5. Mengshol JA, Golden-Mason L, Roven HR: Mechanisms of Disease: HCV-induced liver injury. Nat Clin Pract Gastroenterol Hepatol 2007, 4:622-634.
6. Idrees M, Riazuddin S: Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infect Dis 2008, 8:69.
7. Idrees M, Rafique S, Rehman I, Akbar H, Youssuf MZ, Butt S, Awan Z, Manzoor S, Akram M, Alfabb M, Khubail M, Riazuddin S: Hepatitis C virus genotype 3a infection and hepatocellular carcinoma: Pakistan experience. World J Gastroenterol 2009, 15:5080-5085.
8. Buck M: Direct infection and replication of naturally occurring hepatitis C virus genotypes 1, 2, 3 and 4 in normal human hepatocyte cultures. PLoS One 2008, 3:e2660.
9. el-Awady MK, Tabil AA, Bahgat MW, Shoeb HA, Youssef SS, Bader el-Din NG, Redwan e, el-Demellawy M, Omran MH, el-Garf WT, Goueli SA: HepG2 cells support viral replication and gene expression of hepatitis C virus genotype 4 in vitro. World J Gastroenterol 2006, 12:4836-4842.
10. Lazzaro CA, Chang N, Tang W, Campbell J, Sullivan DG, Gretch DR, Corey L, Coombs RW, Fausto N: Hepatitis C virus replication in transfected and serum-infected cultured human fetal hepatocytes. Am J Pathol 2007, 170:478-489.
11. Molina S, Castet V, Pichard-Garcia L, Wychowski C, Meurs E, Brechot C, Sureau C, Fabre JM, Saccuhia A, Larrey D, Dubuisson J, Coste J, McKeaning J, Maurel P, Fournier-Wirth C: Functional hepatitis C virus E1 and E2 glycoproteins. J Virol 2003, 77:18-28.
12. Cortese R, Nicosia A, Cosset FL, Benzoni M, Scaglione R, Denslow ND, Cooms RW, Fausto N: Hepatitis C virus infection of primary human hepatocytes is tetraspanin CD81 dependent. J Virol 2008, 82:569-574.
13. Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL: Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. J Biol Chem 2003, 278:41624-41630.
14. Bartosch B, Dubuisson J, Cosset FL: The role of N-linked glycans in the functions of hepatitis C virus pseudoparticles containing functional E1-E2 envelope protein complexes. J Exp Med 2003, 197:633-642.
15. Drummer HE, Maerz A, Poumbourois P: Cell surface expression of functional hepatitis C virus E1 and E2 glycoproteins. FEBS Lett 2003, 546:385-392.
16. Gottard F, Callens N, Bartosch B, Wychowski C, Cosset FL, Montpellier C, Dubuisson J: Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins. J Virol 2005, 79:8400-8409.
C virus antibodies is modulated by specific glycans on the E2 envelope protein. J Virol 2007, 81:8101-8111.

17. Helle F, Dubuisson J. Hepatitis C virus entry into host cells. Cell Mol Life Sci 2008, 65:100-112.

18. Nielsen SJ, Bassendine MF, Burt AD, Bevitt DJ, Tomis GL. Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver. J Gen Virol 2004, 85:1497-1507.

19. Flint M, Maidaens C, Loomis-Price LD, Shotton C, Dubuisson J, Monk P, Higginbottom A, Levy S, McKeating JA. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J Virol 1999, 73:6235-6244.

20. Monazahian M, Bohme I, Bonk S, Koch A, Scholz C, Grethe S, Thomsen R. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999, 57:223-229.

21. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abbrignam S. Binding of hepatitis C virus to CD81. Science 1999, 282:938-941.

22. Scarselli E, Ansuini H, Cerino R, Roccasecca RM, Acili S, Filocamo G, Traboni C, Nicolaia A, Cortese R, Vittel A. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. EMBO J 2002, 21:5017-5025.

23. Agnello V, Abel G, Eftharas M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci USA 1999, 96:12766-12771.

24. Lavillette D, Tarr AW, Vioisset C, Donot P, Bartosch B, Bain C, Patel AH, Dubuisson J, Ball JK, Cosset FL. Characterization of host-range and cell entry properties of the major genotypes and subtypes of hepatitis C virus. Hepatology 2005, 41:265-274.

25. Wurschmann S, Muller HM, Stepp CS, Hemler ME, Stapleton JT. In vitro interaction between hepatitis C virus (HCV) envelope glycoprotein E2 and serum lipoproteins (LPS) results in enhanced cellular binding of both E2 E2 and LPS. J Infect Dis 2006, 194:1058-1067.

26. Hannon GJ. RNA interference. Nature 2002, 418:244-251.

27. Sharp PA. RNA interference–2001. Genes Dev 2001, 15:485-490.

28. Khaliq S, Khaliq SA, Zahur M, Ijaz B, Jahan S, Ansar M, Riazuddin S, Hassan S. Inhibition of core gene of HCV 3a genotype using synthetic expression cassettes. J Med Virol 2007, 81:350-356.

29. Khaliq S, Jahan S, Ijaz B, Ahmad W, Hassan S. Inhibition of hepatitis C virus glycoprotein-mediated viral infection. J Virol 2007, 81:1448-1455.

30. Kapadia SB, Bredaus-Andersen A, Chiariu PV. Interference of hepatitis C virus RNA replication by short interfering RNAs. Proc Natl Acad Sci USA 2003, 100:2014-2018.

31. Liu M, Ding H, Zhao P, Qin ZL, Gao J, Cao MM, Luan J, Wu WB, Qi ZT. RNA interference effectively inhibits mRNA accumulation and protein expression of hepatitis C virus core and E2 genes in human cells. Biosci Biotechnol Biochem 2006, 70:2049-2055.

32. Prabhu R, Vittal P, Yin Q, Flemington E, Garry R, Robichaux WH, Dash S. Inhibition of hepatitis C virus translation and subgenomic expression by siRNAs directed against highly conserved HCV sequence and cellular HCV cofactors. J Hepatol 2003, 43:225-234.

33. Liu S, Yang W, Shen L, Turner JR, Coyne CB, Wang T. Tight junction proteins Claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection. J Virol 2009, 83:2011-2014.

34. Timpe JM, McKeating JA. Hepatitis C virus entry: possible targets for therapy. Gut 2008, 57:1728-1737.

35. Ziesel MB, Koutoudiakis G, Schnober EK, Haberstroh A, Blum HE, Cosset FL, Wakita T, Jaek D, Doffoel M, Rooy C, Soulier E, Schroerer E, Schuster C, Stol-Keller F, Bartschlagler R, Pieschmann T, Barth H, Baumert TF. Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. Hepatology 2007, 46:1722-1731.

36. Evans MJ, Von HT, Tschemke DM, Syder AJ, Panis M, Wolk B, Hatziioannou T, McKeating JA, Bienzard PD, Rice CM. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. Nature 2007, 447:801-805.

37. Zhang J, Randall G, Higginbottom A, Monk P, Rice CM, McKeating JA. CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. J Virol 2004, 78:1448-1455.

38. Jahan S, Khaliq S, Ijaz B, Ahmad W, Hassan S. Role of HCV Core gene of genotype 1a and 3a and host gene Cox-2 in HCV-induced pathogenesis. Virology J 2011, 8:1-15.

39. Khaliq S, Jahan S, Ijaz B, Ahmad W, Asad S, Pervaz A, Samreen B, Khan M, Hassan S. Inhibition of core gene of HCV 3a genotype using synthetic and vector derived siRNAs. J Virol 2010, 74:7318.

40. Henry SD, van der WP, Metersalea HJ, Talinus HW, Scholte BJ, van der Laan LJ. Simultaneous targeting of HCV replication and viral binding with a single lentiviral vector containing multiple RNA interference expression cassettes. Mol Ther 2006, 14:485-493.

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