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Targeted delivery of hepatitis C virus-specific short hairpin RNA in mouse liver using Sendai virosomes

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Internal ribosome entry site (IRES)-mediated translation of input viral RNA is the initial required step for the replication of the positive-stranded genome of hepatitis C virus (HCV). We have shown previously the importance of the GCAC sequence near the initiator AUG within the stem and loop IV (SLIV) region in mediating ribosome assembly on HCV RNA. Here, we demonstrate selective inhibition of HCV-IRES-mediated translation using short hairpin (sh)RNA targeting the same site within the HCV IRES. sh-SLIV showed significant inhibition of viral RNA replication in a human hepatocellular carcinoma (Huh7) cell line harbouring a HCV monocistronic replicon. More importantly, co-transfection of infectious HCV–H77s RNA and sh-SLIV in Huh7.5 cells successfully demonstrated a significant decrease in viral RNA in HCV cell culture. Additionally, we report, for the first time, the targeted delivery of sh-SLIV RNA into mice liver using Sendai virosomes and demonstrate selective inhibition of HCV-IRES-mediated translation. Results provide the proof of concept that Sendai virosomes could be used for the efficient delivery of shRNAs into liver tissue to block HCV replication.

INTRODUCTION

Hepatitis C virus (HCV) has infected over 180 million people and the majority of them are at risk of developing liver cirrhosis and/or hepatocellular carcinoma (Saito *et al.*, 1990). The current treatment regimen available for HCV infection includes pegylated-interferon-α in combination with ribavirin, which has limitations in its efficacy, as many patients don't respond to this therapy (Iwasaki *et al.*, 2006), and also results in unwanted side effects. There is therefore an urgent need to develop an effective and specific antiviral for combating HCV disease progression. Several approaches targeting different regions of the viral genome are being investigated, such as serine–protease inhibitors, monoclonal antibodies, anti-fibrotics, immunosuppressants and immunomodulators (Pawlotsky *et al.*, 2007; Tan *et al.*, 2002). There are initiatives to develop nucleic acid-based antiviral agents, such as short hairpin (sh)RNA, short interfering (si)RNA, ribozymes and DNAzymes targeting non-coding regions, structural and non-structural proteins, which have shown moderate or better inhibition at the level of protein synthesis and replication (Trepanier *et al.*, 2006).

The synthesis of HCV proteins is mediated by the 5′ cap-independent internal initiation mechanism, where the loading of the ribosome onto HCV RNA is mediated through a highly structured RNA element, the internal ribosome entry site (IRES) (Wang *et al.*, 1993). Since the mechanism of ribosome assembly is unique and fundamentally different from the cap-dependent translation of the host cell mRNA, it can be targeted to selectively inhibit viral protein synthesis and consequently its replication (Dasgupta *et al.*, 2004; Hellen & Sarnow, 2001; Trowbridge & Gowans, 1998). Previously, we have demonstrated the importance of the GCAC sequence near the iAUG for ribosome assembly onto HCV IRES RNA (Pudi *et al.*, 2003, 2005). In fact, human La protein (an important host factor) has been shown to bind to the above sites and a peptide derived from the RNA-binding region of La protein has been shown to interfere with the ribosome assembly and inhibit viral RNA translation (Ali *et al.*, 2000; Mondal *et al.*, 2008; Pudi *et al.*, 2004, 2005). Here, we have investigated the ability of an shRNA targeting this region of HCV IRES to inhibit translation and replication of viral RNA.

Several approaches have been reported to inhibit HCV RNA translation or replication targeting different regions of the viral RNA, using either small RNA decoy, DNA or RNA aptamers, siRNAs or shRNAs, ribozymes, DNAzymes,

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Two supplementary figures are available with the online version of this paper.

peptides etc. (Dasgupta *et al.*, 2004; Pawlotsky *et al.*, 2007; Roy *et al.*, 2008). However, to date, a suitable system for selective and efficient delivery of small nucleic acid molecules into liver cells is lacking. F-virosomes derived from Sendai virus have been shown to be capable of liver-specific membrane-fusion-mediated delivery of drugs/genes to hepatocytes. It has been well established that these virosomes are selectively targeted to the hepatocytes of mice both *in vitro* and *in vivo* (Bagai *et al.*, 1993; Nijhara *et al.*, 2001; Ramani *et al.*, 1998). Thus, in this study we have exploited the F-virosomal delivery of shRNA for its targeted expression and report for the first time the targeted delivery of HCV-specific shRNA into mouse liver as a proof of concept to demonstrate significant inhibition of HCV IRES-mediated translation *in vivo* using mice.

METHODS

Plasmid constructs. A relatively conserved and unique sequence within HCV IRES was selected to construct shRNA lacking homology with cellular genes. shRNA oligonucleotides were designed to contain a sense strand of 19 nt, followed by a short spacer (TTCAAGAGA), the reverse complement of the sense strand and five thymidines as a stop signal. sh-Stem and loop IV (SLIV) was designed by targeting the HCV IRES corresponding to nt 331–350. Additionally, to generate a non-specific control shRNA (Nsp-sh) a non-specific sequence was also cloned in the poly-linker region of the pSUPER vector. The target sequence of sh-SLIV is 5′-GACCGUGCACCAUGAGCAC-3′ and of Nsp-shRNA is 5′-CAGUCGCGUUUGCGACUGG-3′. To clone the shRNA-encoding DNA sequences, sense and antisense strand oligonucleotides corresponding to the target sequences were denatured at 95 °C and annealed by gradually cooling to 4 °C; these were then cloned into the *Bgl*II and *Hin*dIII sites of pSUPER vector (Brummelkamp *et al.*, 2002). The bicistronic plasmid construct (RLuc–HCV-IRES–FLuc) containing HCV IRES in between renilla luciferase (Rluc) and firefly luciferase (Fluc) genes was cloned into the pCDNA3.1 vector. In this bicistronic construct, the Rluc reporter gene represents the cap-dependent translation, whereas Fluc translation is mediated by the HCV IRES (Pudi *et al.*, 2003). Similarly, the Coxsackievirus B3 (CVB3) bicistronic construct, which contains the CVB3 IRES between Rluc and Fluc (Bhattacharyya *et al.*, 2008), was used as a non-specific IRES control. pSV-*β*-gal plasmid (Promega) was used as a control reporter plasmid for normalizing transfection efficiency. pSUPER vector alone (without shRNA) was used as a control.

Cell cultures. Human hepatocellular carcinoma (Huh) 7 and Huh7.5 cell monolayers were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum (FBS) at 37 °C in 5 % CO_2. To cells harbouring the HCV monocistronic replicon (a kind gift from Dr Ralf Bartenschlager, Heidelberg University, Germany), 25 μg hygromycin B ml^{-1} was added to the culture medium (Frese *et al.*, 2003).

Transient co-transfection of shRNA. Monolayers (70 % confluent) of Huh7 cells in 35 mm dishes were co-transfected with the shRNA plasmid construct and the HCV bicistronic plasmid DNAs or the CVB3 bicistronic construct along with a *β*-galactosidase (*β*-gal) construct using Tfx20 transfection reagent (Promega). Cells were harvested 24 h post-infection using passive lysis buffer (Promega) and Fluc and Rluc activities were measured using the Dual Luciferase assay system (Promega) in a TD 20/20 Luminometer (Turner Designs); the activities were normalized with respect to *β*-gal expression of the cells.

A cell culture-adapted infectious H77s–cDNA construct (a generous gift from Stanley Lemon, University of Texas, Galveston, USA) was used to generate H77s RNA after linearization with *Xba*I followed by run-off transcription. The respective shRNA constructs (0.5 μM) and the infectious H77s RNA (0.4 μM) were co-transfected into Huh7.5 cells using Lipofectamine 2000 (Invitrogen). Total cellular RNA was extracted 72 h post-transfection (p.t.) and quantitative RT-PCR (qRT-PCR) was performed.

shRNA transfection in Huh7 cells harbouring the HCV replicon. Transfection of shDNA constructs into cells harbouring HCV replicons was performed as described previously (Roy *et al.*, 2008). Briefly, Huh7 cells harbouring the monocistronic HCV (genotype 2a) replicon, were transfected with either pSUPER vector (a generous gift from Dr Rene Bernards, Netherlands) alone (as control) or with 0.5 μM of the plasmid constructs encoding sh-SLIV or Nsp-sh RNA. Total RNA was isolated 48 h p.t. using Tri Reagent (Sigma) and RT-PCR was carried out as stated below. For the colony formation assay, cells were trypsinized 24 h p.t. and 4×10^3 cells were seeded in 35 mm dishes, incubated for 10 days in the presence of 25 μg hygromycin B ml^{-1}. The cells were fixed with 90 % acetone, stained with Brilliant Blue G-250 in 40 % methanol and 10 % acetic acid; colonies were counted and the approximate percentage c.f.u. was calculated compared with the control.

Semiquantitative RT-PCR analysis. Semiquantitative RT-PCR was performed for HCV IRES positive and negative strand RNAs using standard protocols, as described previously (Roy *et al.*, 2008). The PCR products were analysed on 1.2 % agarose gels. cDNA synthesis from the positive and negative strands was performed using strand-specific primers. GAPDH was also amplified using the relevant primer as an internal control in the same reaction. Quantification of the replicon RNA was performed by real-time PCR analysis of the respective cDNAs synthesized using the qRT-PCR SYBR green kit (Finnzymes) following the manufacturer's protocol, using an Applied Biosystems 7900HT machine.

Sendai virus culture and preparation of plasmid DNA-loaded F-virosomes. Sendai virus (Z strain) was grown in 10–11-day-old embryonated chicken eggs, harvested and purified by following published protocols (Bagai *et al.*, 1993). Viral yield was estimated in terms of protein and its fusogenic activity was checked. Reconstituted Sendai viral envelopes containing the F-protein (F-virosomes) were prepared as described by Bagai *et al.* (1993) and Ramani *et al.* (1998). Briefly, 100 mg Sendai virus envelope was reduced with 3 mM DTT at 37 °C followed by dialysis to remove haemagglutinin neuraminidase (HN) protein. The suspension was finally resuspended in non-ionic detergent for 1 h; this resulted in removal of HN as well as the viral genetic material containing the RNA genome. The supernatant from the detergent extract was mixed with the desired plasmid DNA (HCV bicistronic DNA construct with or without shRNA constructs) and reconstituted by stepwise removal of detergent using SM-2 Biobeads (Bio-Rad). The final virosome pellet was suspended in 10 mM PBS.

Fusion-mediated delivery of plasmids to cell lines. Monolayers of Huh7 cells (in 12-well culture plates; Falcon) were washed twice with 1 ml DMEM without serum. Cells were incubated with loaded F-virosomes (0.3 mg F-protein) containing 3–4 μg HCV bicistronic plasmid construct with or without sh-SLIV or Nsp-sh plasmid at 37 °C at 5 % CO_2 in 2 ml serum-free medium. Cells alone were used as controls. After 2 h of fusion, medium was replaced with DMEM containing 10 % FBS and cells were further incubated for 24 h at

37 °C at 5% CO_2. The luciferase reporter assay was performed as stated above.

Administration of loaded virosomes to BALB/c mice. Twelve-week-old male BALB/c mice were maintained under specific pathogen-free conditions in the animal facility. These BALB/c mice (~20 g) were given two injections (at an interval of 24 h) intravenously into the tail vein. The injection consisted of HCV bicistronic plasmid with or without sh-SLIV or Nsp-sh plasmid-loaded F-virosomes (containing 4 μg DNA) in a final volume of 0.2 ml Tris-buffered saline containing 2 mM Ca^{2+}. The equivalent volume of PBS was injected as a control (with or without the same amount of free DNA). Two days post-injection, animals were sacrificed for hepatocyte isolation.

Isolation and gene expression in isolated hepatocytes. Liver parenchymal cells were isolated by collagenase perfusion of the liver following a standard procedure (Nijhara *et al.*, 2001). Briefly, the perfused liver was excised from the animal, cut into small pieces and washed thoroughly with phosphate buffer, pH 7.4. The liver pieces were incubated at 37 °C for 30 min in a buffer containing collagenase A (hepatocyte qualified; Gibco). The resulting liver mass was filtered through a nylon mesh and the filtrate was centrifuged at 500 ***g*** for 10 min at 4 °C to obtain a pellet containing the hepatocytes. The non-hepatocyte pellet was obtained by centrifuging the supernatant at 1500 ***g*** for 10 min at 4 °C. Both of these cell types were washed three times with ice-cold PBS, pH 7.4, lysed with passive lysis buffer (Promega) and the cell extracts were then subjected to a dual luciferase assay.

Statistical analysis. All statistical analyses were performed using Student's *t*-test. All graphs represent mean ± SD. *P*-values were determined by using a paired *t*-test. *P*-values of less than 0.01 were considered to be statistically significant.

RESULTS

Inhibition of HCV RNA translation by shRNA targeting the IRES element

Specific shRNA targeting SLIV (encompassing the GCAC and the iAUG sequence) of the HCV IRES (Fig. 1) has been designed and cloned into pSUPER vector (Brummelkamp *et al.*, 2002) to generate sh-SLIV. A non-specific sequence was also cloned in the poly-linker region of the pSUPER vector to generate Nsp-sh.

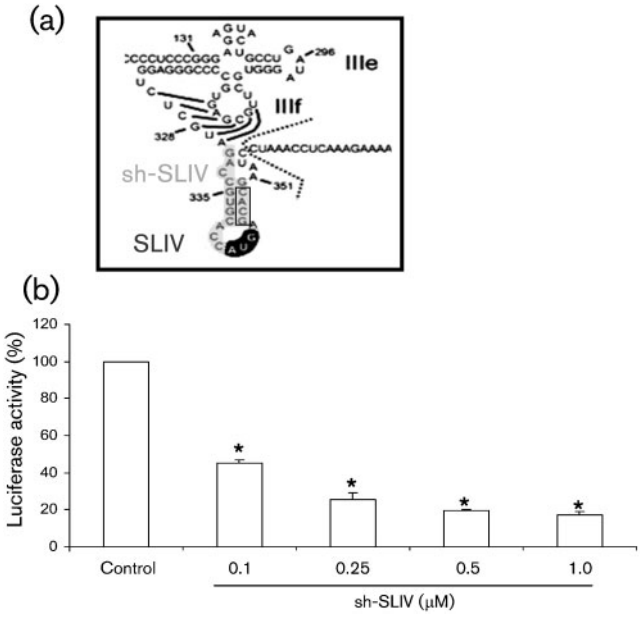

Fig. 1. Targeting the SLIV region of HCV IRES using shRNA. (a) Schematic representation of stem–loop III and SLIV of the HCV IRES (adapted from Brown *et al.*, 1992). The target site within SLIV is indicated by grey shading and the GCAC sequence is boxed. (b) A range of concentrations of sh-SLIV construct were co-transfected with 0.25 μM HCV bicistronic contruct and pSV40 β-gal plasmid DNA to normalize transfection efficiency. Cells were harvested 24 h p.t. and luciferase activities were measured. The luciferase activities were normalized with protein concentration of the lysates and the β-gal activity for the transfection efficiency. *Values that significantly differ from controls ($P<0.001$).

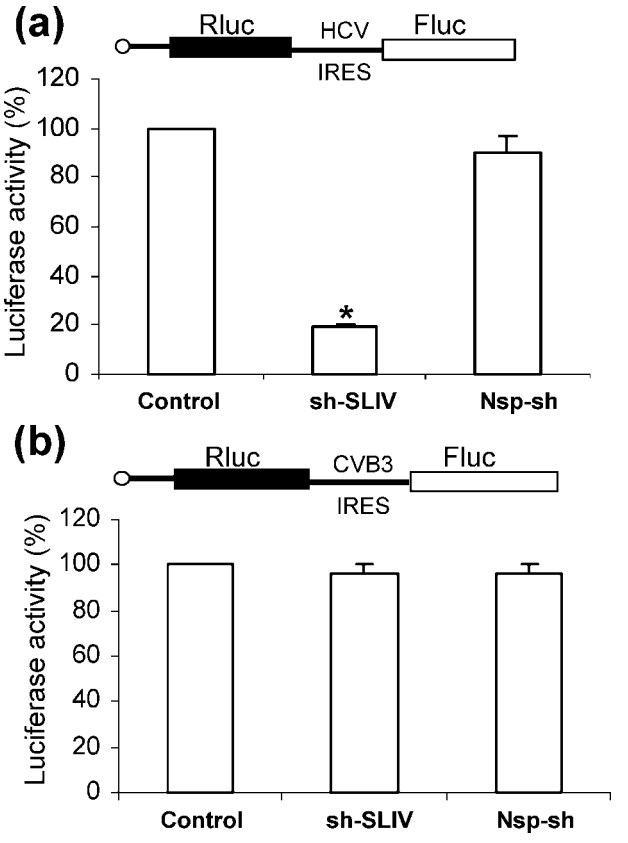

Fig. 2. sh-SLIV RNA specifically inhibits HCV-IRES-mediated translation in Huh7 cells. Bicistronic DNA constructs containing HCV (a) or CVB3 (b) IRESs were co-transfeted with shRNA constructs and β-gal. Luciferase activity was measured and normalized with β-gal (Control, taken as 100%). Data shown are the mean ± SD of three independent experiments carried out in duplicate. *Values which significantly differ from controls ($P<0.001$).

A range of concentrations of the sh-SLIV construct were co-transfected with the HCV bicistronic contruct (RLuc–HCV-IRES–FLuc) and pSV40 β-gal plasmid DNA and the luciferase activity was measured in cells harvested 24 h p.t. The luciferase activities were normalized to the protein concentration of the lysates and the β-gal activity for the transfection efficiency (Fig. 1b).

Our results showed that there was significant inhibition of HCV-IRES-mediated translation upon transfection of Huh7 cells with sh-SLIV (Fig. 2a). However, Nsp-sh did not significantly inhibit HCV-IRES-mediated translation. To further demonstrate the specificity of sh-SLIV function, a similar experiment was performed using the bicistronic construct containing the IRES element of CVB3. These data showed that there was no significant inhibition of CVB3 translation by HCV-specific shRNA (Fig. 2b), reconfirming the target specificity of the sh-SLIV for the HCV IRES RNA.

Effect of sh-SLIV RNA on HCV replication in replicon cell line and in HCV cell culture

To investigate the effect of the sh-SLIV on HCV RNA replication, sh-SLIV, Nsp-sh or pSuper vector control DNA was transfected into Huh7 cells harbouring the HCV-monocistronic replicon, maintained with hygromycin B (Frese *et al.*, 2003) (Fig. 3a). Total cellular RNA was isolated 48 h p.t. Semiquantitative RT-PCR analysis of RNA iolated 48 h p.t. indicated that there was considerable inhibition of both positive- and negative-strand synthesis (Fig. 3b). To reconfirm these data, the replicon RNA was quantified by real-time PCR analysis. The results showed

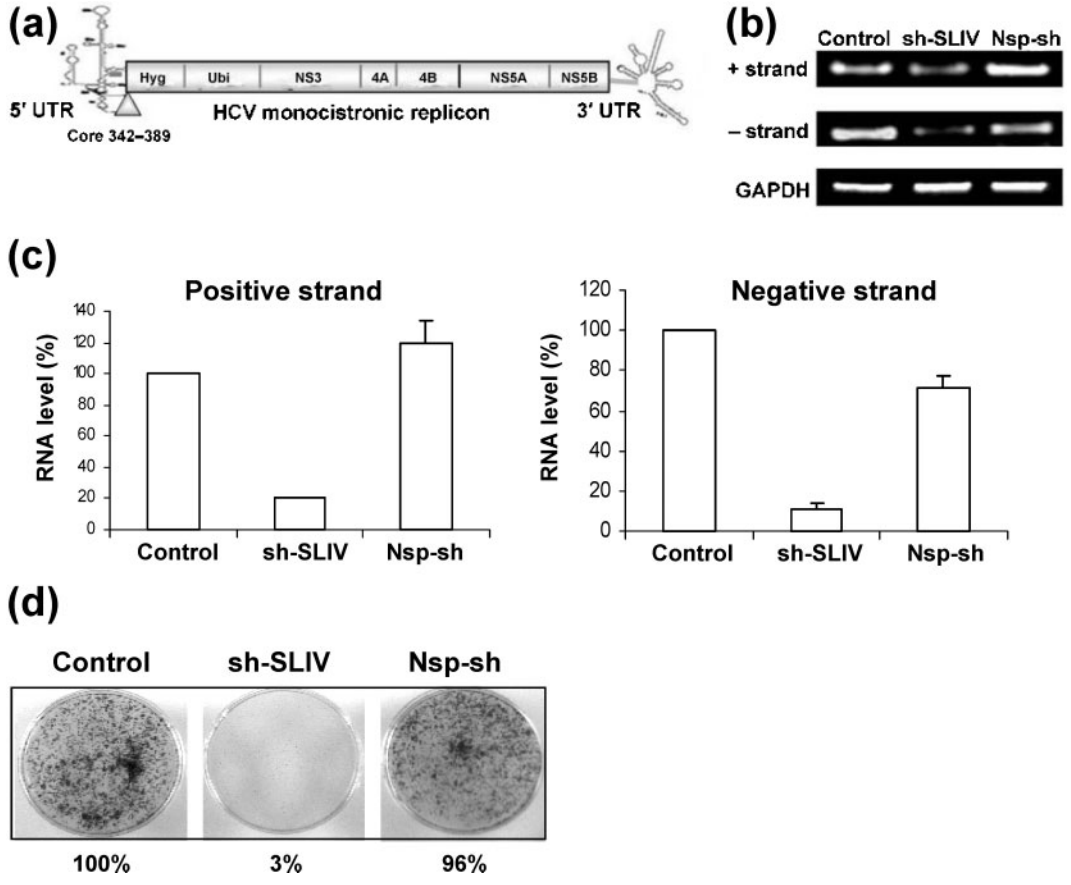

Fig. 3. Effect of shRNA *ex vivo* in Huh7 cells harbouring the HCV monocistronic replicon. (a) Schematic representation of the HCV monocistronic replicon (adapted from Frese *et al.*, 2003). (b) Either pSUPER vector alone (Control) or plasmid constructs encoding sh-SLIV or Nsp-sh were transfected in Huh7 cells harbouring the HCV monocistronic replicon. RNA [positive (+) and negative (–)] isolated 48 h p.t. was analysed by semiquantitative RT-PCR. (c) The RNA was also analysed by qRT-PCR. The HCV RNA levels for positive and negative strand RNA were calculated after normalizing with GAPDH. (d) Cells infected as in (a) were trypsinized, seeded into 35 mm dishes and incubated with hygromycin B for 10 days. Cells were fixed with 90 % acetone and stained with Brilliant Blue G-250 to calculate the c.f.u. Percentage values represent the curing of Huh7 cells from HCV replicon RNA in the presence of sh-SLIV (97 %) and Nsp-sh (3 %).

significant inhibition of both positive (2.25-fold decrease, 80 % inhibition) and negative (3.1-fold decrease, 90 % inhibition) strands when transfected with the sh-SLIV construct. In contrast, the Nsp-shRNA showed only marginal inhibition (Fig. 3c).

As the inhibition was between 80 and 90 %, we intended to determine whether the shRNAs completely eliminate viral RNA in a given cell. The efficacy of shRNA for curing of replicating HCV RNA in Huh7 cells at the single cell level was assessed by colony formation assay. This showed that around 97 % of cells were virus free or survived in the presence of sh-SLIV compared with the control or Nsp-shRNA (~3 %) (Fig. 3d).

To evaluate the efficacy of sh-SLIV RNA to inhibit HCV replication in cell culture, HCV-H77s RNA was generated from a cell culture-adapted infectious H77s–cDNA construct (Fig. 4a) (Yi *et al.*, 2006). Co-transfection of shRNA constructs and infectious H77s RNA resulted in a considerable inhibition of both the strands of HCV RNA. The positive strand was inhibited by 3.4-fold (93 %) and the negative strand was inhibited by 2.7-fold (80 %). However, the Nsp-sh or pSUPER vector did not show notable inhibition of either strand (Fig. 4b).

Effect of virosome-loaded sh-SLIV RNA *ex vivo* in Huh7 cells and *in vivo* in BALB/c mice

To explore a suitable delivery system for the shRNA constructs, we opted to use Sendai virosomes, which are popular for selective and effective delivery into liver cells (Ramani *et al.*, 1998). Fig. 5(a) represents the process used for preparation of virosomes loaded with shRNA construct. The engineered virosome and the delivery strategy in BALB/c mice are shown in Supplementary Fig. S1 (available in JGV Online). Essentially, the engineered Sendai virosomes (suitable for the delivery of the shRNAs) were packed with HCV bicistronic DNA construct with or without shRNA construct and used for transfection into Huh7 cells and BALB/c mice. In order to achieve a systematic assessment of the ability of F-virosome-mediated delivery of this shRNA and its ability to inhibit HCV IRES in a liver-derived cell line, monolayers of Huh7 cells were transfected with virosomes loaded with HCV bicistronic construct and sh-SLIV or Nsp-sh construct. Luciferase activity was measured 24 h p.t. Significant inhibition (~80 %) of HCV-IRES-mediated translation was observed in the presence of the sh-SLIV compared with the control. However, Nsp-sh showed very little or negligible inhibition of HCV-IRES-mediated translation (Fig. 5b). The results suggest that there is efficient delivery of the virosome-entrapped shRNA constructs inside liver-derived Huh7 cells and selective inhibition of HCV-IRES-mediated translation. We have also tried the experiment of separate trapping of HCV-bicistronic DNA and the sh-SLIV construct in the virosomes; subsequent delivery to Huh7 cells showed only 16–20 % inhibition (Supplementary Fig. S2, available in JGV Online).

To investigate whether the Sendai virosomes packed with reporter gene constructs could deliver the DNAs selectively into liver cells in the context of the whole animal (BALB/c mouse), we first tested the preferential expression of

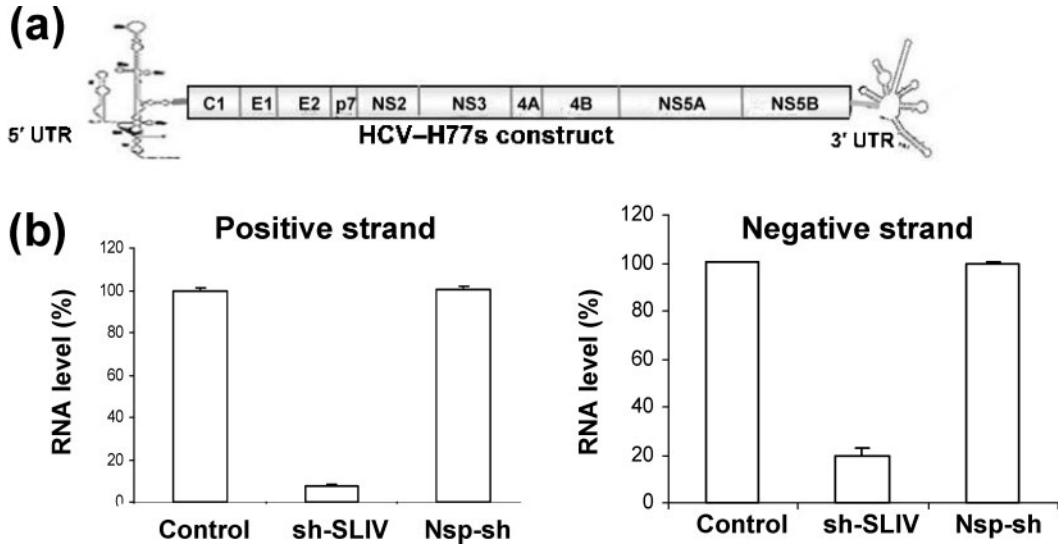

Fig. 4. Effect of shRNA *ex vivo* on the replication of HCV–H77s RNA in cell culture. (a) Schematic representation of the HCV–H77s construct (adapted from Yi *et al.*, 2006). (b) *In vitro*-transcribed HCV RNA from the HCV–H77s construct was transiently co-transfected with either pSUPER vector alone (Control) or sh-SLIV or Nsp-sh plasmid constructs in Huh7.5 cells. RNA was extracted 72 h p.t. and qRT-PCR analysis was performed. The percentage HCV RNA levels (mean ± SD) were calculated for the positive and negative strand after normalizing with GAPDH (Control).

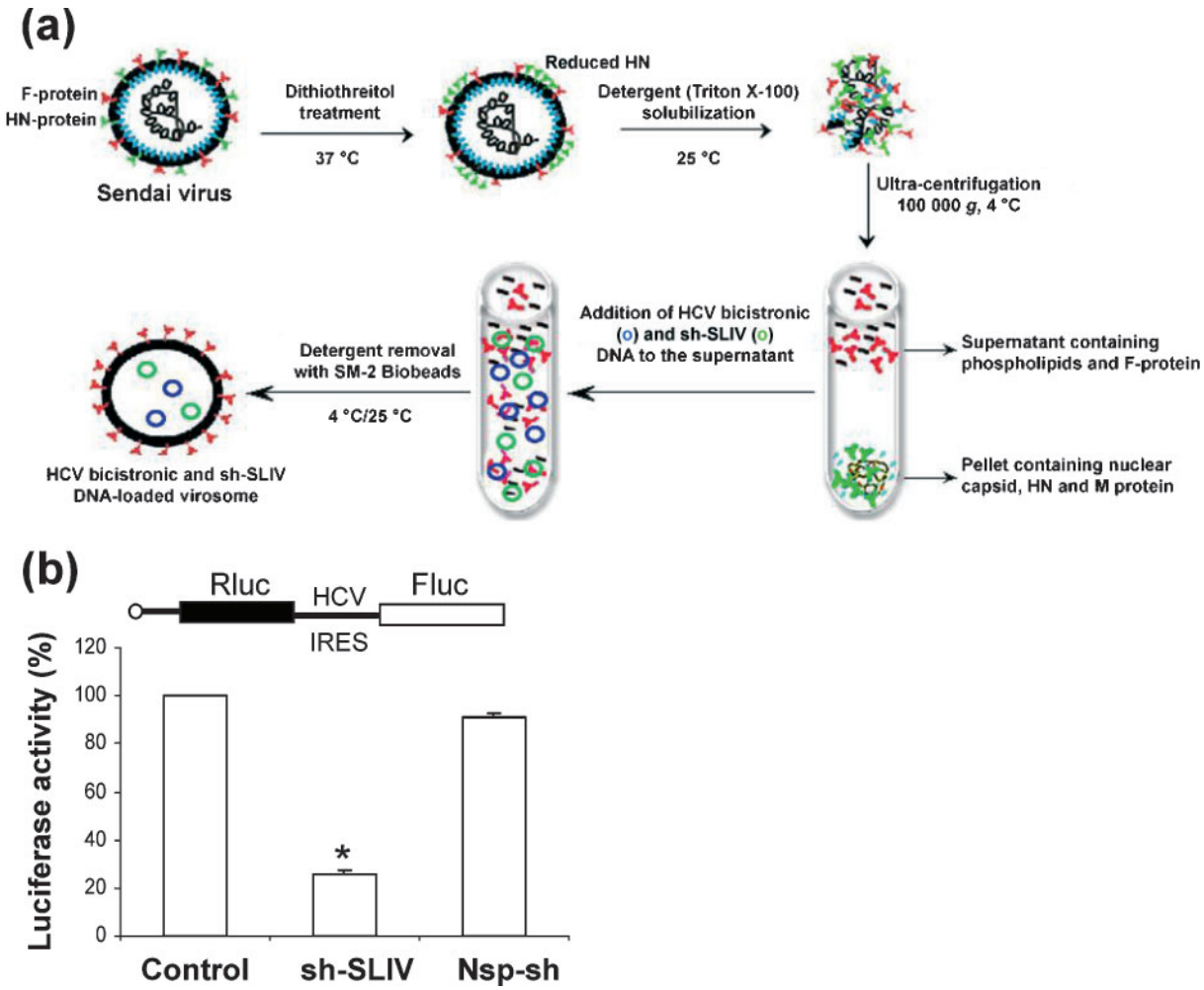

Fig. 5. Entrapment of shRNA-encoding plasmid constructs in the Sendai virosome. (a) Schematic representation of Sendai F-virosome preparation (following the protocol of Ramani *et al.*, 1998). (b) Dual luciferase activity of Huh7 cells harvested 24 h post-fusion of F-virosome with either sh-SLIV or Nsp-sh constructs. Data shown are mean ± SD, taking the control as 100 %. *Values which significantly differ from controls ($P<0.001$).

luciferase gene loaded into Sendai F-virosome by intravenous (i.v.) injection through the tail vein. It was observed that the expression of the luciferase reporter gene occurred preferentially in the parenchymal cells (hepatocytes) of the mouse liver, compared with Kupffer cells (non-hepatocyte cells) (Fig. 6a), which is consistent with an earlier publication (Ramani *et al.*, 1998). To assess the target specificity of F-virosomes to deliver sh-SLIV and inhibit HCV-IRES-mediated translation *in vivo*, F-virosomes containing HCV bicistronic construct and sh-SLIV or Nsp-sh encoding plasmids were delivered to BALB/c mice by using multiple i.v. injections through the tail vein. Livers from injected animals were analysed for luciferase activity. Mice treated with the sh-SLIV construct showed a significant reduction (75 %) in HCV-IRES-mediated translation of Fluc, compared with that in the control animal that received virosomes containing HCV bicistronic construct alone (Fig. 6b). Free plasmid/buffer-injected animals showed no inhibition and served as an additional negative control (data not shown).

DISCUSSION

So far, several shRNAs targeting different regions of the HCV genome have been reported to achieve significant inhibition of translation and replication of viral RNA (Kanda *et al.*, 2007; Kapadia *et al.*, 2003; Randall *et al.*, 2003; Wilson *et al.*, 2003). Some of the target sequences used were shown to be conserved across the genotypes. However, the scope of an siRNA-/shRNA-based antiviral strategy in general is limited because of the evolution of escape mutants. In this study, we purposely targeted the site (nt 331–350) within the SLIV region which encompasses *cis*-acting elements critical for ribosome assembly and which is likely to be more conserved in the HCV RNA

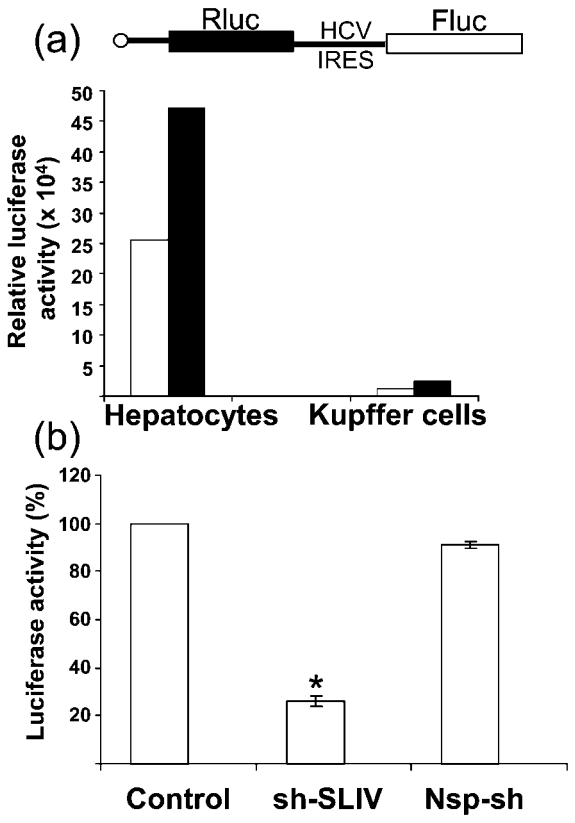

Fig. 6. Inhibition of HCV-IRES-mediated translation *in vivo* in BALB/c mice by shRNA delivered through F-Virosomes. (a) Preferential expression of HCV bicistronic construct in the liver cells following intravenous injection by F-virosomes. (b) Relative luciferase activities (mean ± SD) of liver parenchymal cells from BALB/c mice injected i.v. with F-virosomes loaded with the HCV bicistronic constructs and either sh-SLIV or Nsp-sh. *Values which significantly differ from controls ($P<0.001$).

and is protected from the generation of escape variants (Pudi *et al.*, 2004). Thus, the sh-SLIV could be used to achieve broad spectrum inhibition of HCV translation and replication.

Presumably, the sh-SLIV RNA inhibits HCV-IRES-mediated translation by cleaving the bicistronic RNA, as its sequence is exactly complementary to the target sequence. However, we do not rule out the possibility of any off-target activity by which shRNA might interfere with cellular factors critical for IRES function or induce double-stranded RNA-activated protein kinase, which in turn would contribute to inhibition of translation. sh-SLIV showed almost 80 % translation inhibition and up to 90 % replication inhibition of the replicon system and H77S RNA [full-length HCV genome that replicates and produces virus particles that are infectious in cell culture (HCVcc)] in an HCV cell culture assay, suggesting that sh-SLIV could be effective in inhibiting viral proliferation. Additionally, it would be interesting to use a cocktail of shRNAs (packed in a Sendai virosome) targeting different regions of the HCV genome to further potentiate the inhibitory activity.

Furthermore, in this study we have demonstrated target-specific silencing of the HCV RNA using Sendai virus F-virosomes, which are widely used for their liver specificity and efficient targeted delivery compared with cationic liposomes (Ramani *et al.*, 1998). Although several methods have been tried for the delivery of siRNA/shRNA molecules, which includes adenoviral and retroviral delivery systems (Kronke *et al.*, 2004; Zhang *et al.*, 2004), none of these are selective for hepatocytes. Studies using mice as the animal model utilize tail vein injection or hydrodynamic injection for delivery of shRNAs, which requires a high dose as it is distributed systemically (Wang *et al.*, 2005). Therefore, the present delivery mode using Sendai virosomes can ensure targeted delivery into the mouse liver and could minimize the effective dose.

Finally, this study provides, for the first time, proof of concept for the targeted delivery of anti-HCV molecules into mouse liver cells using Sendai virosome, which could be a novel and effective delivery strategy to block HCV proliferation in liver tissue.

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