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Adenovirus vector-mediated assay system for hepatitis C virus replication

Takeshi Yoshida[1], Masuo Kondoh[1,*], Manabu Ojima[1], Hiroyuki Mizuguchi[2], Yoshiaki Yamagishi[1], Naoya Sakamoto[3] and Kiyohito Yagi[1,*]

[1]Laboratory of Bio-Functional Molecular Chemistry, [2]Department of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka and [3]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan

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ABSTRACT

The efficient delivery of the hepatitis C virus (HCV) RNA subgenomic replicon into cells is useful for basic and pharmaceutical studies. The adenovirus (Ad) vector is a convenient and efficient tool for the transduction of foreign genes into cells *in vitro* and *in vivo*. However, an Ad vector expressing the HCV replicon has never been developed. In the present study, we developed Ad vector containing an RNA polymerase (pol) I-dependent expression cassette and a tetracycline-controllable RNA pol I-dependent expression system. We prepared a hybrid promoter from the tetracycline-responsive element and the RNA pol I promoter. Ad vector particles coding the hybrid promoter-driven HCV replicon could be amplified, and interferon, an inhibitor of HCV replication, reduced HCV replication in cells transduced with the Ad vector coding HCV replicon. This is the first report of the development of an Ad vector-mediated HCV replicon system.

INTRODUCTION

Hepatitis C virus (HCV) is a member of *Flaviviridae* that contains a 9.6-kb positive-sense RNA genome. A total of 170-million people worldwide are infected with HCV, leading to chronic hepatic inflammation, hepatic fibrosis, hepatic cirrhosis and hepatocellular carcinoma (1). Chronic infection with HCV is a major cause of hepatocellular carcinoma (1). Interferon (IFN) therapy is the gold standard method for HCV patients, but it is effective in only 50% of patients and its use has been limited because of severe side effects (2–4). Additional pharmaceutical therapies are needed to overcome HCV. However, the tropism of HCV is limited to chimpanzees and humans, and the mechanism of HCV infection and replication is not fully understood. The HCV genome encodes a polyprotein precursor of about 3000 amino acids that is cleaved into at least 10 proteins: core, envelope 1 (E1), E2, p7, non-structural protein 2 (NS2), NS3, NS4A, NS4B, NS5A and NS5B (5). An HCV subgenome replicon (called HCV replicon in the present study) consisting of a reporter gene and HCV NS genes has allowed various studies of HCV replication and the development of anti-HCV agents (6–8). The delivery of the HCV genome or HCV replicon is a powerful tool for basic and pharmaceutical research, and the transduction of *in vitro* translated HCV RNA genome is often performed by electroporation. However, a convenient and efficient method to transfer the 9.6-kb HCV RNA genome or the 8–9-kb HCV replicon has never been fully developed.

Transcribed RNAs are classified into rRNAs, mRNAs and short RNAs (tRNAs) in mammalian cells. RNA polymerases differ among the transcribed RNA species: RNA polymerase (pol) I for rRNAs, RNA pol II for mRNA and RNA pol III for short RNAs. RNA pol I transcribes RNA without a 5′-cap structure or a 3′-poly-A tail, and a plasmid vector encoding RNA pol I promoter and terminator has been applied to the development of RNA virus-expression system. For instance, influenza viruses, arenavirus and uukuniemi viruses are generated using RNA pol I-driven expression plasmid vectors coding each segment of negative-sense RNA (9–12). Recombinant adenovirus (Ad) vectors have been widely used to deliver foreign genes to a variety of cell types and tissues *in vitro* and *in vivo* in basic research and clinical therapy. Ad vector can be easily prepared, grown to a high titer, and used to efficiently transfer genes into dividing and non-dividing cells. Furthermore, several types of Ad vectors have been developed to expand their tropism and to increase the size of encoded genes (13,14). Ad vector encoding RNA pol I-driven expression of influenza virus

*To whom correspondence should be addressed. Tel: +81 6 6879 8196; Fax: +81 6 6879 8199; Email: masuo@phs.osaka-u.ac.jp
Correspondence may also be addressed to Kiyohito Yagi. Tel/Fax: +81 6 6879 8195; Email: yagi@phs.osaka-u.ac.jp

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

RNA has been developed for the generation of vaccine seed strains and for basic influenza virus studies (15). These findings indicate that the RNA pol I Ad vector system can be a promising tool for basic and pharmaceutical studies on HCV. However, the development of an RNA pol I-driven vector system expressing the HCV RNA genome has never been reported.

In the present study, we developed an RNA pol I-driven vector system to monitor HCV replication using an HCV replicon in which structural genes were replaced by the luciferase gene. We prepared an Ad vector containing a tetracycline (tet)-regulated RNA pol I-expression cassette consisting of an RNA pol I-driven responsive vector and a *trans*-activator vector, and we successfully developed an Ad vector-mediated HCV replication system.

MATERIALS AND METHODS

Cell culture

Huh7.5.1 1bFeo [genotype 1b HCV replicon cell line, (8)] were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) and G418 (Nacalai Tesque, Kyoto, Japan) at 500 μg/ml. Huh7 and 293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS. The cells were maintained in a 5% CO_2 atmosphere at 37°C.

Preparation of RNA pol I-driven plasmid vectors

An RNA pol I expression-cassette was subcloned as follows: pHH21 (kindly provided by Dr Kawaoka, Tokyo, Japan) containing RNA pol I expression cassette was digested with *Afl*III, blunted by the Klenow fragment of DNA polymerase, ligated with *Eco*RI linker and digested with *Eco*RI/*Nhe*I, resulting in a fragment of the RNA pol I expression-cassette. The RNA pol I cassette was inserted into the *Eco*RI-*Xba*I site of pHM5 (16), generating pPol I. A fusion gene of enhanced green fluorescent protein and firefly luciferase (EGFPLuc, Clontech, Mountain View, CA, USA) was inserted into pPol I, resulting in pP_IWT-EL.

The subgenomic HCV sequence and the replication-incompetent subgenomic HCV sequence deleting GDD motif (MLVNGDDLVV) in NS5B were amplified by polymerase chain reaction (PCR) using pRepFeo as a template (8). The PCR fragments were inserted into pPol I, generating pPol I-1bFeo and pPol I-1bFeoΔGDD. The Feo fragment in pPol I-1bFeo or pPol I-1bFeoΔGDD was replaced with firefly luciferase, generating pPol I-HCV or pPol I-ΔGDD coding firefly luciferase reporter, HCV NS3, NS4A, NS5A and NS5B or mutated NS5B, respectively. A plasmid expressing β-galactosidase, pCMVβ, was purchased from Marker Gene Inc. (Eugene, OR, USA).

Preparation of tet-controllable RNA pol I-driven plasmid vectors

To develop the tet-controllable RNA pol I promoter expression system, the minimal cytomegalovirus promoter was replaced by fragments of RNA pol I promoters (from −235 to −1, from −311 to −1 or from −412 to −1) in pHM5-TREL2 (17), generating pP_I235, pP_I311 or pP_I412. These RNA pol I plasmid vectors were used for optimization of the tetracycline responsive element (TRE)/RNA pol I chimeric promoter. pHM5-tTA, pHM5-rtTA and pHM5-TREL2 were used in tet-regulated experiments (17).

Preparation of Ad vector expressing HCV replicon

The HCV replicon fragments cloned from pPol I-HCV or pPol I-ΔGDD were inserted into pP_I235, and then the firefly luciferase was replaced by the renilla luciferase to form pP_I235-HCV or pP_I235-ΔGDD. Ad vectors were constructed by an improved *in vitro* ligation method (18). Briefly, pP_I235-EL, pP_I235-HCV and pP_I235-ΔGDD were digested with I-*Ceu*I and PI-*Sce*I, and then ligated with I-*Ceu*I/PI-*Sce*I-digested pAdHM4 and pAdHM36, respectively. The resulting plasmids were digested with *Pac*I and transfected into 293 cells with SuperFect (Qiagen, Valencia, CA, USA). AdP_I235-EL, AdP_I235-HCV and AdP_I235-ΔGDD were purified by $CsCl_2$ gradient centrifugation and dialyzed with a solution containing 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$ and 10% glycerol. The multiplicity of infection (MOI) of Ad vectors was measured using an Adeno-X rapid titer kit (Clontech). Ad-tTA vectors were prepared as previously described (17).

Expression of plasmid-based HCV replicon

Huh7 cells were transfected with 0.8 μg of pPol I-HCV. After 24 h of incubation, the cells were lysed in LCβ (Toyo Ink, Tokyo, Japan). The cell lysates were frozen-thawed and centrifuged at 32 000 *g* for 5 min. The luciferase activity in the resulting supernatant was measured using a commercially available kit (PicaGene; Toyo Ink).

Inhibition assays of HCV replication in plasmid- or Ad-based RNA pol I HCV system

Huh7 cells were transfected with 0.8 μg of pPol I-HCV and 0.2 μg of pCMVβ or infected with AdP_I235-HCV (10 MOI) and Ad-tTA (50 MOI). After 2.5 or 1.5 h of transfection, the cells were treated with recombinant human interferon-α8 (IFN-α8) at the indicated concentration. After an additional 72 h of incubation, the cells were lysed in LCβ. Luciferase activity and β-galactosidase activity in the lysates was measured with PicaGene and a Luminescent β-gal Kit (Takara Bio Inc., Shiga, Japan), respectively. The cell viability was measured with a WST-8 kit according to the manufacturer's instruction (Nacalai Tesque).

Evaluation of tetracycline-controllable promoters in plasmid vector

Huh7 cells were co-transfected with 0.1 μg of reporter plasmid (pP_I235-EL, pP_I311-EL, pP_I412-EL or pP_IWT-EL), 0.8 μg of tet-responsive *trans*-activator plasmid (pHM5-rtTA in the tet-on system or pHM5-tTA in the tet-off system) and 0.1 μg of pCMVβ. After 2.5 h, the cells were treated with doxycycline (Dox) at the indicated

concentration for 48 h. Then, luciferase and β-galactosidase activities in the lysates were measured.

Expression of Ad vector containing tetracycline-controllable promoter system

Huh7 cells were transfected with a reporter Ad vector (AdP_I235-EL or AdP_I235-HCV at MOI of 5 or 10) and a *trans*-activator vector (Ad-tTA at MOI of 10 and 50). After an additional 48 h of incubation, luciferase activity in the cell lysates was measured.

Western blotting

Huh7 cells were co-infected with AdP_I235-HCV at 10 MOI and Ad-tTA at 50 MOI. The cells were lysed in RIPA buffer [50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA] containing a cocktail of protease inhibitors (Sigma, St Louis, MO, USA). The cell lysates (30 μg of protein) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by western blotting onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). After blocking in 5% skim milk, the filter was incubated with mouse anti-NS5A (Meridian Life Science, Sacao, ME, USA) or anti-β-actin Ab (Sigma). Then, the peroxidase-labeled secondary antibodies were added. The immunoreactive bands were visualized by chemiluminescence reagents (GE Healthcare, Buckinghamshire, UK).

Evaluation of NS5B-dependent replication

Huh7 cells were transfected with AdP_I235-HCV or AdP_I235-ΔGDD at 3 MOI and Ad-tTA at 15 MOI. After 24 h, the cells were treated with 10 μg/ml of Dox for 48 h. Then, luciferase activities in the lysates were measured.

Detection of a fragment of the HCV negative strand RNA

Huh7 cells were co-infected with AdP_I235-HCV or AdP_I235-ΔGDD at 3 MOI and Ad-tTA at 15 MOI. After 24 h, the cells were treated with 10 μg/ml of Dox for 48 h. The total RNAs were purified with High Pure RNA Isolation kit (Roche, Mannheim, Germany). The RNAs were reverse-transcribed to cDNA using a commercial available kit [TaKaRa RNA PCR Kit (AMV) Ver. 3.0] and a primer for the HCV negative strand RNA (5′-GCCAGCCCCCGATTGGGG-3′) or a primer for GAPDH (5′-TCTACATGGCAACTGTGA-3′), respectively. The transcription products of NS3 and GAPDH were amplified by PCR using paired primers (5′-ATGG CGCCTATTACGGCC-3′ and 5′-TGGTCTACATTAG TGTAC-3′) and (5′-GGTGGTCTCCTCTGACTTCAA CA-3′ and 5′-GTGGTCGTTGAGGGCAATG-3′), respectively. The putative sizes of the PCR products were 242-bp for NS3 and 89-bp for GAPDH. The PCR products were separated on 2% agarose gel.

RESULTS

RNA pol I-driven plasmid vector

First, we constructed an RNA pol I-driven plasmid coding an HCV replicon in which structural coding genes were replaced by the luciferase gene (Figure 1A). To investigate the expression of the HCV replicon from the RNA pol I plasmid vector, we transfected the plasmid vector into Huh7 cells. As shown in Figure 1B, the luciferase activity was observed in the RNA pol I vector-transfected cells. IFN is the most popular agent used to inhibit HCV replication. To examine whether the RNA pol I plasmid vector functions as an assay system for anti-HCV activity, we investigated the effect of IFN on the expression of the HCV replicon in the RNA pol I plasmid-transfected Huh7 cells. IFN dose-dependently reduced the replication of the HCV genome (Figure 1C), reaching 29.2% of the control at 5 pg/ml. IFN treatment did not cause any cytotoxicity (Figure 1D). These data suggest that the RNA pol I plasmid coding the HCV replicon works as an assay system for HCV replication.

RNA pol I-driven Ad5 vector

The Ad vector is the most efficient gene transfer vector for a variety of mammalian cells *in vitro* and *in vivo* (13,14,19,20). There are more than 51 serotypes of Ad. The Ad type 5 (Ad5) vector has been frequently used in basic research and clinical studies (21). Ad5 vectors are 100- and 1000-fold more efficient at mediating gene transduction than cationic lipids, an effective non-viral vector (22). A reverse genetics system for the generation of influenza virus using RNA pol I-driven Ad5 vector produced 1000-fold the virus titer of the RNA pol I plasmid system (15). These findings indicate that the Ad5 vector may have advantages for the preparation of an HCV replicon system. We prepared RNA pol I-driven Ad5 vectors and confirmed the expression of a reporter gene from the Ad5 vectors coding luciferase (Supplementary Figure S1). However, we did not succeed in preparing Ad5 vector particles coding the HCV replicon. Indeed, there have been no previous reports of the preparation of Ad5 vector expressing the HCV RNA genome.

We think that two problems must be solved in order to develop Ad5 vectors coding the HCV RNA genome. These problems are the influence of the HCV replicon on the preparation of Ad5 particles and the packaging limit of Ad5 vectors.

Preparation of the TRE/RNA pol I chimeric promoter

The tet-regulated system comprises a regulator vector that expresses tet-controlled *trans*-activators and a response vector consisting of TRE within the promoter that controls expression of the gene of interest. The tet-controlled *trans*-activators are classified into tTA and rtTA that binds to the TRE promoter and activates expression from the TRE promoter in the absence and presence of Dox, respectively (23,24). We speculated that a tet-regulated vector system would minimize the influence of the HCV replicon on the preparation of Ad vector

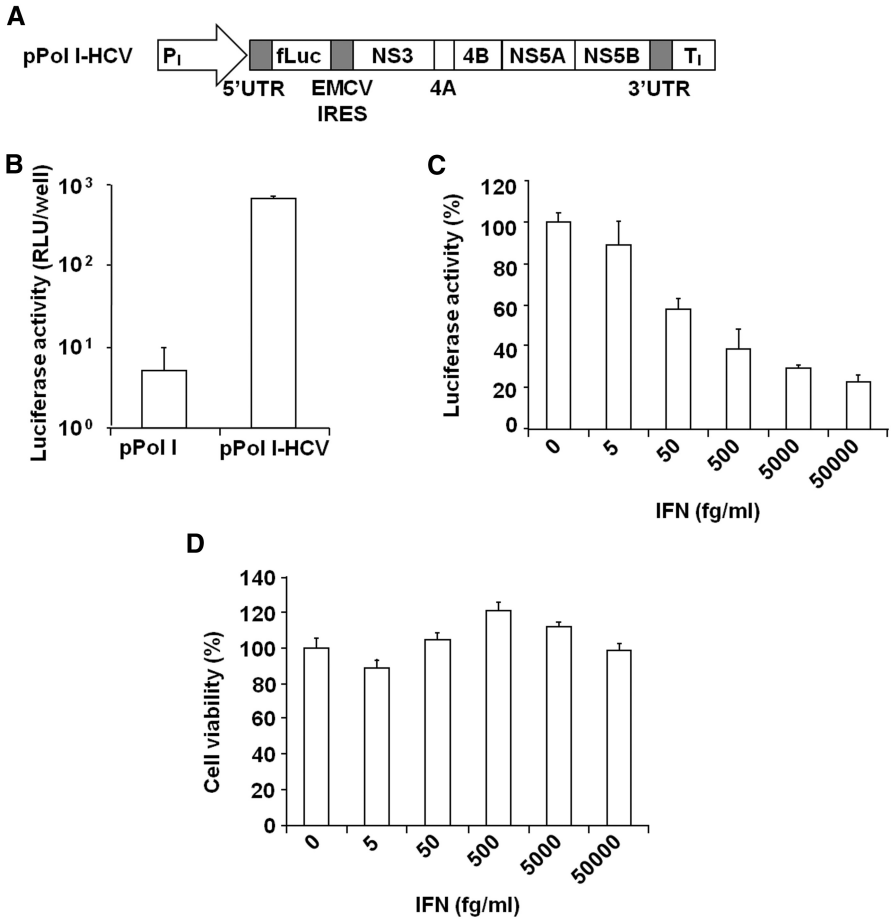

Figure 1. Preparation of plasmid expressing HCV replicon driven by RNA pol I promoter. (**A**) Schematic construct of HCV replicon-expression cassette. The HCV replicon gene was driven by the RNA pol I promoter (P_I) and terminator (T_I). (**B**) Transgene expression in Huh7 cells. Cells were transfected with pPol I-HCV. After 24 h of transfection, the luciferase activities were measured. Data are mean ± SD ($n = 3$). (**C** and **D**) Effect of IFN on HCV replication in RNA pol I vector-transfected cells. Huh7 cells were transfected with pPol I-HCV. After 2.5 h of transfection, the cells were treated with IFN at the indicated concentration. After an additional 72 h of incubation, the luciferase activity (C) and the cell viability (D) were measured. The luciferase activity (%) was calculated as a percentage of that in the vehicle-treated cells. Data are mean ± SD ($n = 3$).

particles. First, we optimized the chimeric promoter of TRE and the RNA pol I promoter. As shown in Figure 2A, the RNA pol I promoter is a 412-bp fragment containing an upstream control element (UCE) and the binding site of a transcription factor (Core). We constructed three chimeric promoter-driven plasmid vectors and checked the expression profiles using luciferase as a reporter gene. The chimeric vector was co-transfected into Huh7 cells with response vectors coding tTA or rtTA (23,24). As shown in Figure 2B and C, co-transfection with tTA exhibited a higher expression level than that of rtTA. The P_I235 promoter had the lowest luciferase expression in the absence of response vectors (Supplementary Figure S2). We used tTA and the P_I235 promoter in further studies. To investigate whether the chimeric RNA pol I promoter works in the Ad vector, we prepared Ad5 vector coding the chimeric RNA pol I-driven luciferase gene. AdP_I235-EL (MOI of 5) was co-transduced with Ad-tTA at MOI of 10 and 50. As shown in Figure 2D, the luciferase expression was increased in an Ad-tTA concentration-dependent manner.

Expression of the HCV replicon from Ad vector

The packaging limit of a foreign gene in the conventional Ad5 vector has been estimated to be 8.1–8.2-kb (25). The HCV replicon is ~8.9-kb and contains a 1.7-kb firefly luciferase gene and sequence derived from the HCV genome. Thus, another reason for no previous reports regarding the preparation of Ad5 vector coding the HCV replicon appears to be the packaging limit of the Ad5 vector. Mizuguchi and Hayakawa found that Ad5/35 vector containing chimeric fibers of Ad5 and Ad35 increased the size limit of foreign genes to 8.8-kb (26). We were successful in preparing Ad5/35 vector particles (9.53×10^8 IFU/ml) coding the TRE/RNA pol I chimeric promoter-driven HCV replicon containing the 1.0-kb renilla luciferase gene and sequence derived from the HCV genome (Figure 3A). To investigate the expression of the HCV replicon, Huh7 cells were transfected with the Ad vector coding the HCV replicon and Ad-tTA at MOI of 10 and 50, respectively. As shown in Figure 3B, western blot analysis showed that NS5A was expressed in Huh7 cells transfected with the vectors in the absence of Dox.

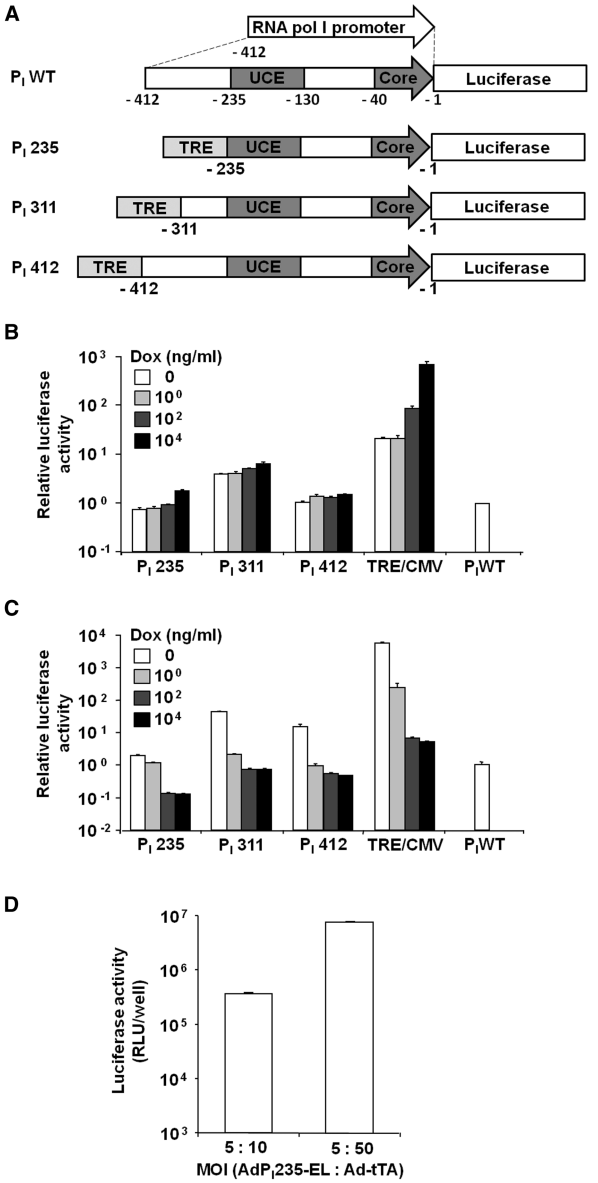

Figure 2. Development of tet-controllable RNA pol I promoter. (**A**) Construct of the chimeric RNA pol I promoter. The 412-bp human RNA pol I promoter contains Core (from −40 to −1), the binding site of the transcription factor, and UCE (from −235 to −130). TRE is connected to the full or partial RNA pol I promoter at the indicated sites, resulting in P_I235, P_I311 and P_I412. (**B** and **C**) Promoter activities of the chimeric promoter in Huh7 cells. Huh7 cells were co-transfected with the chimeric RNA pol I plasmid coding EGFPLuc, pCMVβ and *trans*-activator plasmid [rtTA (B) or tTA (C)]. After 2.5 h of transfection, the cells were treated with Dox at the indicated dose. After an additional 48 h of incubation, the luciferase and β-galactosidase activities were measured. The luciferase activity was normalized by the β-galactosidase activity and expressed relative to that of pP_IWT-EL-transfected cells. Data are mean ± SD (n = 3). (**D**) Transgene activity of Ad vector coding the chimeric promoter construct. Huh7 cells were co-infected with AdP_I235-EL and Ad-tTA. The MOI ratio of AdP_I235-EL to Ad-tTA was 5:10 or 5:50. After an additional 48 h of incubation, the luciferase activity was measured. Data are the mean ± SD (n = 3).

Luciferase was also expressed (Figure 3C). Dox dose-dependently attenuated expression of luciferase (Supplementary Figure S3). To discriminate between translation of the RNA pol I-transribed HCV RNA derived from the vector DNA and translation of HCV RNA derived from autonomous HCV replication in the transcribed cells, we prepared replication-incompetent HCV replicon deleting GDD motif in NS5B. Luciferase expression was attenuated in the cells transfected with the GDD-deleted Ad vector (AdP_I235-ΔGDD) (Figure 3D). A fragment of the HCV negative strand RNA, an essential replication intermediate, amplified by RT-PCR has been detected in the cells transfected with AdP_I235-HCV but not AdP_I235-ΔGDD (Figure 3E). Autonomous replication of the HCV RNA may occur in this system. To evaluate whether the Ad vector systems could be used to evaluate inhibitors of HCV replication, we investigated the effect of IFN on luciferase expression from HCV replicon in the Ad vector. As shown in Figure 3F, treatment of cells with 5 pg/ml of IFN reduced luciferase expression (33.3% of vehicle-treated cells). Cell viability was not affected by IFN treatment (Figure 3G). These findings indicate that the tet-controllable RNA pol I Ad vector may be useful for evaluation of anti-HCV activity.

DISCUSSION

HCV is an RNA virus containing the positive strand of a 9.6-kb RNA genome. A technique to transfer all or part of the HCV RNA genome to cells could be widely applicable for basic studies on HCV and pharmaceutical therapy against HCV. However, efficient and convenient methods to transduce the HCV RNA genome have never been fully developed. Electroporation of *in vitro* translated HCV RNA genome into cells is the most popular method. In the present study, we used a tet-controllable expression system to successfully develop an Ad vector system expressing the HCV RNA genome.

To our knowledge, development of Ad vector expressing HCV subgenome or genome has never been succeeded. The NS3 protease is essential for processing most of the NS proteins from the HCV polyprotein (27–30). The cleavage site of the NS3 protease is estimated to be between the P1 and P1′ position of an acidic amino acid at the P6 position, a Cys or Thr residue at the P1 position, and a Ser or Ala residue at the P1′ position (31). E1A, pIIIa, pol and V proteins of Ad have the cleavage site of the NS3 protease. The lack of previous success in generating Ad vectors coding the HCV genome and subgenome might be partly due to the degradation of Ad components by the NS3 protease during the preparation of Ad particles. In the tet-regulated system, when Ad vectors coding foreign genes driven by the TRE hybrid promoter are co-transfected with tTA or rtTA vector, the foreign gene can be expressed. Expression of the foreign gene could be suppressed during amplification of Ad vector particles in 293 cells, resulting in the preparation of Ad vector particles. The critical factor in the HCV replicon must be determined in a future study.

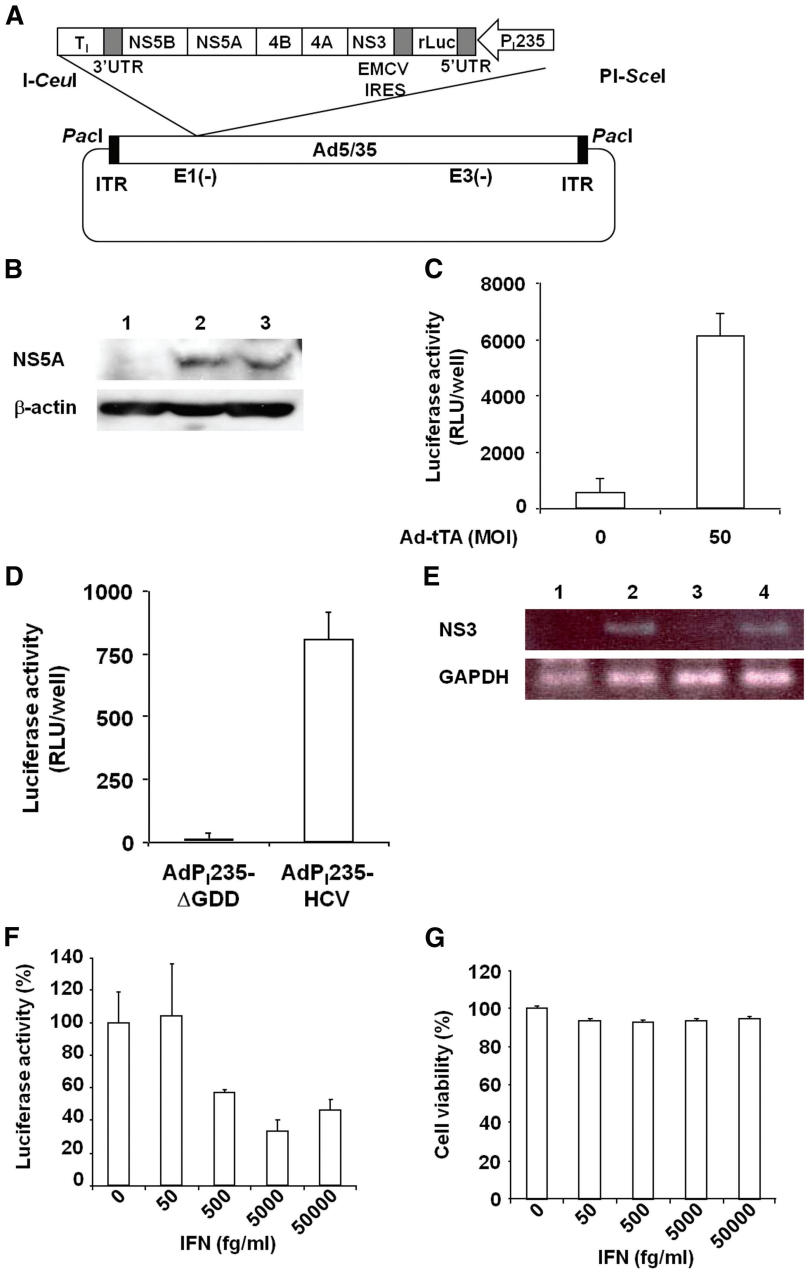

Figure 3. Preparation of Ad vector to monitor HCV replication. (**A**) Construct of Ad vector. The Ad vector contained the chimeric RNA pol I promoter (P_I235) and the HCV replicon to monitor HCV replication as the luciferase expression. (**B**) Expression of HCV NS5A protein in Huh7 cells transfected with AdP_I235-HCV. The cells were transfected with AdP_I235-HCV (10 MOI) and Ad-tTA (50 MOI). After 72 h of incubation, the cells were harvested, and the lysates (30 μg) were subjected to SDS–PAGE, followed by immunoblotting with antibody against NS5A. Huh7 cells and Huh7.5.1 1bFeo cells were used as the negative and positive controls, respectively. Lane 1, Huh7 cells; lane 2, Huh7 cells infected with AdP_I235-HCV; lane 3, Huh7.5.1 1bFeo cells. (**C**) Expression of luciferase in the Ad vector-transfected cells. Huh7 cells were co-infected with AdP_I235-HCV (10 MOI) and 0 or 50 MOI of Ad-tTA. After an additional 48 h of incubation, the luciferase activity was measured. Data represent the mean ± SD ($n = 3$). (**D**) Involvement of NS5B in expression of luciferase in the Ad vector-transfected cells. Huh7 cells were infected with AdP_I235-HCV or AdP_I235-ΔGDD (3 MOI) and Ad-tTA (15 MOI). After 24 h, the cells were treated with 10 μg/ml of Dox for 48 h. Then, the luciferase activity was measured. Data represent the mean ± SD ($n = 3$). (**E**) Expression of minus-stranded HCV RNA in the Ad vector-transfected cells. Huh7 cells were co-infected with AdP_I235-HCV or AdP_I235-ΔGDD at 3 MOI and Ad-tTA at 15 MOI. After 24 h, the cells were treated with 10 μg/ml of Dox for 48 h. Then RT-PCR analysis was performed for detection of minus-stranded HCV NS3 and GAPDH. The PCR products were separated on 2% agarose gel. Huh7 cells and Huh7.5.1 1bFeo cells were used as the negative and positive controls, respectively. Lane 1, Huh7 cells; lane 2, Huh7.5.1 1bFeo cells; lane 3, Huh7 cells infected with AdP_I235-ΔGDD; lane 4, Huh7 cells infected with AdP_I235-HCV. (**F** and **G**) Effect of IFN on the replication of HCV replicon. Huh7 cells were infected with AdP_I235-HCV (10 MOI) and Ad-rtTA (50 MOI). After 1.5 h of infection, the cells were treated with IFN at the indicated concentration for 72 h. Then, the luciferase activity (F) and the cell viability (G) were measured. Data represent the percentage of vehicle-treated cells. Data are the mean ± SD ($n = 3$).

Transgenes delivered by a conventional Ad5 vector are limited to a size of 8.1–8.2-kb (32), and the size of HCV replicon is ~8.2-kb (containing a 1.0-kb luciferase gene and a 7.2-kb fragment of HCV genome) (8). The lack of a successful preparation of Ad5 vector may be partly due to limitation of packaging transgene. Mizuguchi and Hayakawa prepared a chimeric Ad vector containing type 5 and type 35 fiber proteins, which is a package 8.8-kb of foreign gene (26). CD46 is a receptor for Ad type 35 (Ad35), and CD46 is ubiquitously expressed in human cells (33,34). The Ad5/35 chimera vector can transduce various human cells more effectively than Ad5 vectors, indicating that the Ad5/35 vector may be a better system than Ad5 (26,35). In this study, we successfully prepared an Ad5/35 vector coding a tet-regulated RNA pol I-driven HCV replicon, and we found that the Ad5/35 vectors could be applied to evaluation of anti-HCV activity.

In conclusion, to the best of our knowledge, this is the first report to establish a novel strategy for the preparation of Ad vector expressing the HCV genome by using a tet-controllable expression system. Replication-incompetent HCV particles will be a promising candidate for vaccine therapy for HCV. As mentioned above, the packaging size (8.8-kb) of Ad5/35 vector used in the present study is smaller than that of the HCV RNA genome (9.6-kb), and, therefore, the preparation of inactive HCV particles using Ad5/35 vector is impossible. Helper-dependent Ad vector (HDAd), in which all viral coding sequences are deleted, can deliver a large capacity of ~37-kb to cells (36). Tet-controllable RNA pol I HDAd vector might contribute to the development of vaccine therapy for HCV.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Conflict of interest statement. None declared.

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