Arch Virol (2007) 152: 1305–1314
DOI 10.1007/s00705-007-0951-z
Printed in The Netherlands

Archives of Virology

Silencing E1A mRNA by RNA interference inhibits adenovirus replication

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Received September 3, 2006; accepted January 31, 2007; published online March 20, 2007
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Summary

The adenovirus family contains 51 human serotypes, and most human adenoviruses cause widespread respiratory tract infections. Adenovirus infections can result in severe complications in some cases, such as in adenovirus type 11 infection in immunocompromised patients. However, effective treatment methods for adenovirus infections are currently unavailable. This prompted the search for antiviral agents effective against adenovirus infections. In the present study, adenovirus E1A was targeted by RNA interference (RNAi) using synthetic small interfering RNAs (siRNAs) in an attempt to inhibit viral replication, since adenovirus E1A proteins are known to be involved in the transcriptional activation of the viral and cellular genes necessary for controlling the cell cycle and viral replication. The results indicated that the siRNAs effectively reduced the amount of adenovirus E1A mRNA and the levels of replicative intermediates. Additionally, siRNA-mediated gene silencing inhibited adenovirus replication by suppressing the E1A mRNA. These results suggest that the RNAi-mediated targeting of adenovirus E1A may have a potentially therapeutic effect in controlling adenovirus infections.

Introduction

The adenovirus (Adv) family consists of 51 known serotypes, which fall into six subgenera, A through F [7]. Adv infects the respiratory tract, intestinal tract, conjunctiva, and other organs. The clinical manifestations caused by Adv infections vary considerably; most of them are mild and resolve without special chemotherapy. However, a severe Adv infection may occur in immunocompromised patients, such as those with leukemia, acquired immune deficiency syndrome (AIDS), and kidney or bone marrow allograft transplantation. Several reports of severe Adv type 11 (Adv11) urinary infections in immunocompromised patients prompted the search for antiviral agents effective against Adv replication [10, 12]. A number of investigators have reported several agents that exhibit antiviral activity against Adv *in vitro* [7, 10]. However, there is currently no effective and specific antiviral chemotherapy for Adv infections.

The Adv replication cycle is divided into two stages: the early stage and the late stage. The six distinct early regions are E1A, E1B, E2A, E2B, E3

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and E4. The E1 region is the only viral region that requires immediate transcription, and the first mRNA/protein produced is E1A. Three different types of open reading frames (ORFs), with varying splicing patterns, have been identified in the E1A region of Adv. The spliced E1A mRNAs encoding the E1A proteins are expressed during the early stage of infection. The E1A proteins activate transcription from early viral promoters via a mechanism that requires a forty-amino-acid zinc finger activation domain [12, 18]. Furthermore, the E1A proteins interact with the cellular regulators of transcription to reprogram gene expression in the infected cell [1, 21]. These observations suggest that the degradation of E1A mRNA may inhibit Adv11 replication.

RNA interference (RNAi) is a process by which double-stranded RNA (dsRNA) directs the sequence-specific degradation of mRNA [5]. The effectiveness of RNAi in down-regulating or knocking down gene expression has recently been demonstrated in a variety of mammalian systems [11]. The key players in RNAi are small interfering RNAs (siRNAs), which consist of 21–23 nucleotides. Once introduced into a cell, the siRNA is incorporated into a nuclease complex, called an RISC (RNA-induced silencing complex), that subsequently recognizes and cleaves the target mRNA [6, 8, 9, 15, 16]. RNAi-mediated gene silencing has the potential to be a powerful tool for inhibiting viral replication [6]. The present study was designed to examine whether siRNAs targeted at the E1A region of Adv11 are capable of inhibiting viral replication by inducing the degradation of viral mRNA.

Materials and methods

Virus

Adv 2K2/507/KNIH, serotype 11, was isolated in Busan, Korea, in 2002 from a patient suffering from a respiratory infection presenting with symptoms of a fever and cough. The human Adv11 prototype strain, Slobitski, was used as the control. The viruses were cultured in A549 cells, a human lung carcinoma cell line. The A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, JBI, Republic of Korea) supplemented with 5% fetal bovine serum (FBS, GIBCO, USA) at 37 °C in a 5% CO_2 incubator.

Sequence analysis

The E1A region of Adv 2K2/507/KNIH was amplified using forward (5′-GAGTGCCAGCGAGAAGAG-3′) and reverse (5′-GTAAACACGGATATGGAACACT-3′) primers. Direct sequencing of the PCR products was performed. The sequencing reaction was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). DNA sequencing was performed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, USA). The analysis of the E1A sequence of the Adv 2K2/507/KNIH genome was accomplished using DNAStar software from the Lasergene package (DNASTAR Inc., Madison).

Design and synthesis of siRNAs

Each designed siRNA molecule consisted of 19 nucleotides corresponding to the E1A region of Adv 2K2/507/KNIH and to two additional deoxythymidine nucleotides located at the 3′ end of each molecule. The GC content of each siRNA molecule was approximately 30%. Five different siRNA molecules were synthesized by Hokkaido System Science (HSS, Japan), and the siRNA sequences are listed in Table 1. Equal amounts of complementary oligonucleo-

Table 1. siRNA molecules targeted to the Adv 2K2/507/KNIH E1A region

Name	Polarity	Sequence	Position
siRNA 135	sense	UCA GGA ACU GUA UGA UUU ATT	135–153
	antisense	UAA AUC AUA CAG UUC CUG ATT	
siRNA 295	sense	GGU ACA GGU GUA AGA AAA UTT	295–313
	antisense	AUU UUC UUA CAC CUG UAC CTT	
siRNA 499	sense	GAA UUU CAC AGG AAA AAU ATT	499–517
	antisense	UAU UUU UCC UGU GAA AUU CTT	
siRNA 509	sense	GGA AAA AUA CUG GAG UAA ATT	509–527
	antisense	UUU ACU CCA GUA UUU UUC CTT	
siRNA 794	sense	UGG AAA AAC UUG AGG ACU UTT	794–812
	antisense	AAG UCC UCA AGU UUU UCC ATT	

tides were mixed, denatured in boiling water, and then allowed to cool to room temperature over a 60-min period to allow for annealing [2]. The annealed siRNAs were aliquoted and stored at −80 °C until use. The scrambled siRNA (Qiagen, Germany) was used as a negative control and was annealed according to the same procedure used with the designed siRNAs.

siRNA transfection

Approximately 24 h prior to transfection, DMEM containing 5% FBS lacking antibiotics was added to cells in 12-well plates. For each transfection mixture, 8 μL of siPORT Lipid (Ambion, Austin, TX, USA) was diluted with 22 μL of Opti-MEM I (GIBCO, USA) reduced serum medium. Ten microliters of siRNA was mixed with 360 μL of Opti-MEM I and then added to the pre-diluted siPORT Lipid. The mixture was incubated at room temperature for 20 min, and the prepared plate was washed twice with Opti-MEM I. The final mixture was then added to each plate along with Opti-MEM I. Each plate was incubated at 35 °C for 4 h, and then 1 mL of DMEM with 10% FBS was added to the plates.

In vitro transcription of Adv E1A RNA

The restriction enzyme *Hin*dIII was used to linearize the pcDNA E1A vector, which was constructed by cloning the E1A gene into the pcDNA 3.1 vector (Invitrogen, Karlsruhe, Germany). Adv E1A RNA was transcribed *in vitro* from the T7 promoter using a Megascript T7 transcription kit (Ambion, Austin, TX, USA) according to the instructions supplied by the manufacturer. The template plasmid DNA was removed by incubation with DNase I at 37 °C for 15 min. The template DNA was further removed by extraction with the Tri reagent (MRC, Cincinnati, USA). The resulting RNA pellet was resuspended in DEPC-treated water (GENO TECH, Washington, USA). Aliquots of RNA were stored at −80 °C.

Quantitative real-time PCR

A549 cells were infected with Adv following transfection with or without siRNA. One and two days after infection, the cells were harvested and the mRNA was isolated from the cell pellets using the automated nucleic acid extraction system of the MagNA Pure LC mRNA Isolation Kit I (Roche, Mannheim, Germany). Quantitative real-time PCR was carried out using the TaqMan One-step RT-PCR Master Mix Reagents (Roche, Branchburg, New Jersey, USA) and the ABI 7900 HT Sequence Detection System (Applied Biosystems, Forster City, USA). The PCR reaction volume was 20 μL, and included mRNA and specific primers. Two primers and an FAM-labeled TaqMan probe corresponding to the sequence in the E1A region of Adv 2K2/507/KNIH were used. The primer sequences were 5′-TGGTGCACG CCCTGATG-3′ (sense) and 5′-CTGAAGCGTAGGAG GCTCAAAA-3′ (antisense), and the probe sequence was 5′-FAM-ACGATCCGGAGCCACC-3′. Reverse transcription (RT) was performed for 30 min at 48 °C. After incubation for 10 min at 95 °C, amplification was performed and consisted of 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. The internal loading control reactions were carried out using the Human β-Actin-Certified LUX Primer Set purchased from Invitrogen (Karlsruhe, Germany) [3]. All reactions were done in duplicate.

Northern blot analysis

Total RNA isolated from Adv 2K2/507/KNIH-infected cells was separated on a 1% formaldehyde-agarose gel and transferred onto a nylon membrane. The probe for the E1A mRNA corresponding to 250–453 base pairs (bp) of the coding sequence for E1A was labeled with DIG via PCR amplification. Hybridization was performed overnight at 42 °C in ULTRAhyb (Ambion, Austin, TX, USA). The membrane was washed twice with High Stringency Wash Solution (Ambion, Austin, TX, USA) at 42 °C, and the hybridized probe was detected using a DIG DNA Detection Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol.

Plaque reduction assay

Adv particles were quantified using the plaque reduction assay in A549 cells transfected with or without siRNA. The cells were infected with Adv 4 h after transfection. The growth medium was then removed and overlaid with a 1:1 combination of 0.8% agarose dissolved in PBS and 2 × MEM (GIBCO, USA). The plate was incubated at 35 °C for five days. Following incubation, the plate was fixed with 10% PBS saturated with formaldehyde (SIGMA-ALDRICH, St. Louis, USA) and stained with 1% crystal violet (SIGMA-ALDRICH, St. Louis, USA) [14].

Fluorescence assay (FA)

The cells were infected with Adv 4 h after transfection. Cells transfected with or without siRNA were harvested two days after infection with the virus. The cell pellet was washed twice with PBS and suspended in 20 μL of PBS. The suspended cells were then plated on a slide glass in a 5-mm diameter spot, and the cells were fixed in the fixation solution (ethanol:acetone = 1:4) for 15 min. The fixed virus-infected cells were incubated with an adenovirus-specific monoclonal antibody against the hexon region of adenovirus type 11 (MAB805, CHEMICON, California, USA) at a 1:50 dilution. The secondary antibody, fluorescein isothiocyanate (FITC)-conjugated goat polyclonal anti-mouse IgG secondary antibody (Abcam, Cambridgeshire, UK), was used at a dilution of 1:250. Both the primary and secondary antibodies were diluted

in PBST (0.05% Tween20 in PBS). The immunofluorescence was observed under a Leica DC 300F microscope.

Results

Designing E1A-specific siRNAs on the basis of sequence analysis

The sequence of the E1A region of Adv 2K2/507/KNIH was compared to a previously reported sequence of an Adv11 prototype strain named Slobitski. The results showed that the amino acid homology in the E1A region between Adv 2K2/507/KNIH and the Adv11 prototype strain, Slobitski, was 96.6%. The E1A region of Adv11 produces three different types of ORFs with varying splicing patterns. ORFs encoding polypeptides of 262, 231 and 58 residues (R) have been identified in the E1A region of Adv11. The mRNA encoding 262R of Adv11 E1A, which contains a zinc-finger motif (i.e., $CX_2CX_{13}CX_2C$), is approximately 870 bp in length [12]. It was focused on designing siRNAs that remain effective despite the

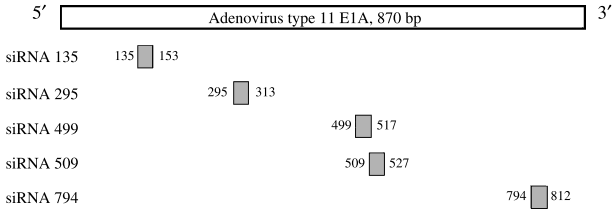

Fig. 1. Locations of E1A sequences targeted by siRNAs

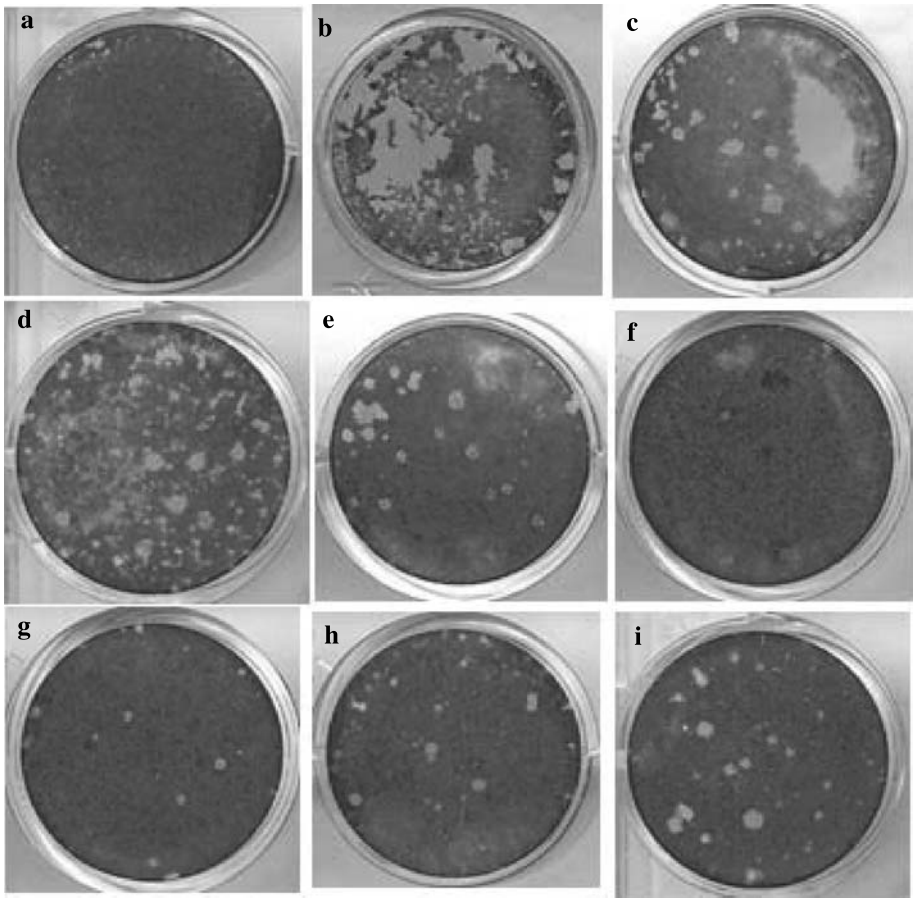

Fig. 2. Selection of effective siRNAs. The siRNA molecules were tested for their ability to inhibit viral replication in a virus plaque reduction assay. **a**, Mock infection (lipid only, no virus); **b**, siRNA negative (lipid, scrambled siRNA); **c**, lipid control (lipid, virus); **d**, virus control (virus only, no lipid); **e**–**i**, siRNA treatment (all siRNAs were treated at a concentration of 200 pmole); **e**, siRNA 135; **f**, siRNA 295; **g**, siRNA 499; **h**, siRNA 509; **i**, siRNA794

genetic variation and target sequences located at 262R of the E1A region. The designed siRNAs did not have more than 1 mismatch in the 21 nucleotides among the different types of Adv, and they did not share homology with any known human genes. Five siRNA molecules corresponding to the E1A region of Adv11 were designed and evaluated (Fig. 1, Table 1).

Selection of effective siRNAs

It was hypothesized that the siRNA-mediated silencing of E1A results in the inhibition of viral replication. A quantitative real-time PCR and a plaque reduction assay were used to evaluate the ability of siRNA molecules to induce mRNA silencing and to subsequently inhibit viral replication. A relatively low level of plaque formation was observed, especially with siRNAs 295 and 499, when the virus-infected cells were transfected with the siRNAs using the transfection agent, siPORT *Lipid* (Fig. 2). In contrast, a large number of plaques formed as expected when the virus-infected cells were treated with siPORT *Lipid* containing scrambled siRNA, which demonstrated that the scrambled siRNA alone did not affect viral replication in the cells.

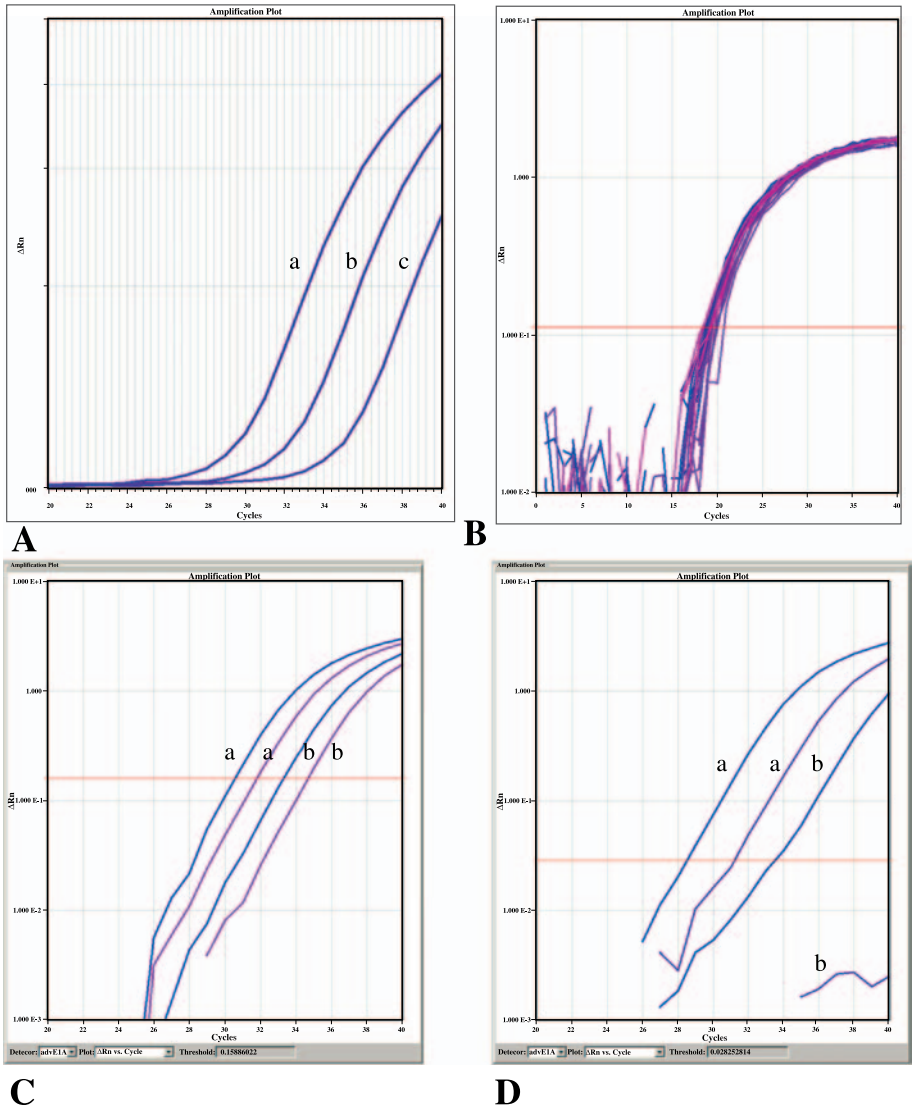

Fig. 3. Analysis of E1A mRNA levels by quantitative real-time PCR. **A** Standard curve for quantitative real-time PCR. **a**, 7.137×10^4; **b**, 7.137×10^3; **c**, 7.137×10^2 copies. **B** Quantitative real-time PCR for the internal control, β-actin mRNA, was performed for normalization. **C** RNA interference with siRNA 295. **D** RNA interference with siRNA 499. **C**, **D** *a*, post-infection day one; *b*, post-infection day two; blue line, virus control; pink line, siRNA treatment

The quantitative real-time PCR also confirmed the observation that two siRNA molecules (siRNA 295, siRNA 499) induced the silencing of E1A mRNA in the cell, which resulted in a significant inhibition of viral replication (data not shown). Therefore, siRNA 295 and siRNA 499 were chosen for further characterization in subsequent experiments.

Inhibition of Adv 2K2/507/KNIH replication by siRNAs 295 and 499

Quantitative real-time PCR and Northern blot analysis

The ability of siRNAs 295 and 499, which are specific for E1A of the Adv 2K2/507/KNIH, to knock down the level of E1A mRNA was evaluated by quantitative real-time PCR and by Northern blot analysis in order to determine whether siRNAs 295 and 499 are able to induce E1A silencing. Quantitative real-time PCR was performed on each mRNA sample to measure changes in the level of Adv E1A mRNA following siRNA transfection. In-vitro-transcribed E1A transcripts were used as standards for the quantification of E1A mRNA by real-time PCR (Fig. 3A). The β-actin mRNA levels of each sample were analyzed using quantitative real-time PCR to normalize the data, and all mRNA samples were found to be equal in Ct value (Fig. 3B) with respect to β-actin mRNA. The quantitative real-time PCR revealed that siRNAs 295 and 499, which proved to be effective at inhibiting Adv replication, were able to significantly reduce the level of Adv E1A mRNA on post-infection days one and two. The reductions in the E1A mRNA levels were approximately 60 and 70% upon treatment with siRNA 295 and siRNA 499, respectively (Fig. 3C, D). In addition, the silencing of Adv 2K2/507/KNIH E1A was confirmed by Northern blot analysis. The level of E1A mRNA was dramatically reduced in cells transfected with siRNA 295 or 499 (data not shown), whereas it was clearly detected in virus-infection-only control and negative control (transfected with a scrambled siRNA) samples.

Adv 2K2/507/KNIH-infected cells were harvested at 24-h intervals for five days following transfection with siRNA 295, and the extracted mRNAs were analyzed using quantitative real-time PCR in order to evaluate the duration of the siRNA effect. An efficient reduction in the number of Adv E1A transcripts by the siRNA was observed until day four after siRNA transfection. However, the silencing effect of the siRNA began to diminish starting at day five (Fig. 4).

Plaque reduction assay and fluorescence assay

A plaque reduction assay was performed with cultures infected with Adv 2K2/507/KNIH at 10^{-4} dilution in the presence or absence of siRNA 295 or 499 in order to estimate viral titers more pre-

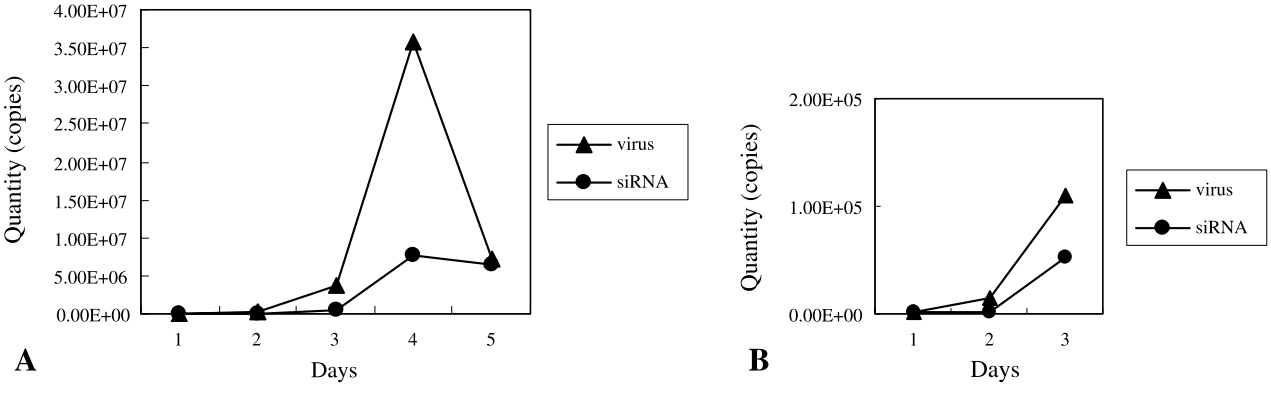

Fig. 4. Duration of siRNA-mediated E1A silencing. The graph shows the changes in the copy number of E1A mRNA of Adv 2K2/507/KNIH for 5 days with or without siRNA treatment. **B** shows an enlarged view of the data corresponding to days one to three. Closed triangles indicate virus, virus-only infection without siRNA and the circles indicate siRNA, siRNA treatment after virus infection

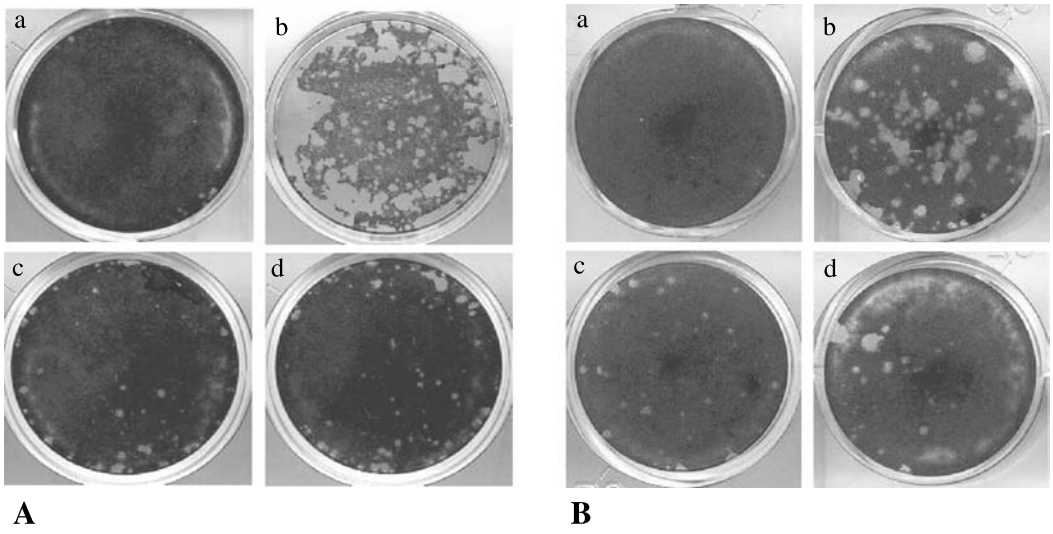

Fig. 5. Plaque reduction assay for siRNAs 295 and 499 against Adv 2K2/507/KNIH. A plaque reduction assay was used to test the concentration-dependent inhibition of viral replication by siRNA 295 **A** or siRNA 499 **B**. *a*, Uninfected cell control; *b*, virus control; *c*, siRNA treatment (200 pmole); *d*, siRNA treatment (20 pmole)

cisely. A viral titer of 1.2×10^3 pfu/mL was detected in the mock culture at a dilution of 10^{-4}, and all subsequent experiments were performed with 10^{-4}-diluted viruses. Four hours after siRNA transfection, Adv 2K2/507/KNIH was inoculated onto the transfected A549 cells. A viral titer of 1.2×10^3 pfu/mL was detected at a 10^{-4} dilution in the virus control without siRNA transfection, whereas no or few plaques were detected in the A549 cells transfected with 200 pmole of siRNA 295 or 499. The plaque formation decreased by at least 3000-fold (Fig. 5). Even at a concentration of 20 pmole, Adv replication was inhibited by approximately 1000-fold (Fig. 5). In contrast, the scrambled siRNA alone did not induce any significant inhibition of viral replication under conditions identical to those described above (data not shown).

These results were further confirmed by immunostaining the cells with a monoclonal antibody against an Adv antigen. In comparison to the virus control cells, the reduction in the number of fluorescence-positive cells was approximately 95%, further indicating the inhibitory effect of siRNA treatment on viral replication (Fig. 6).

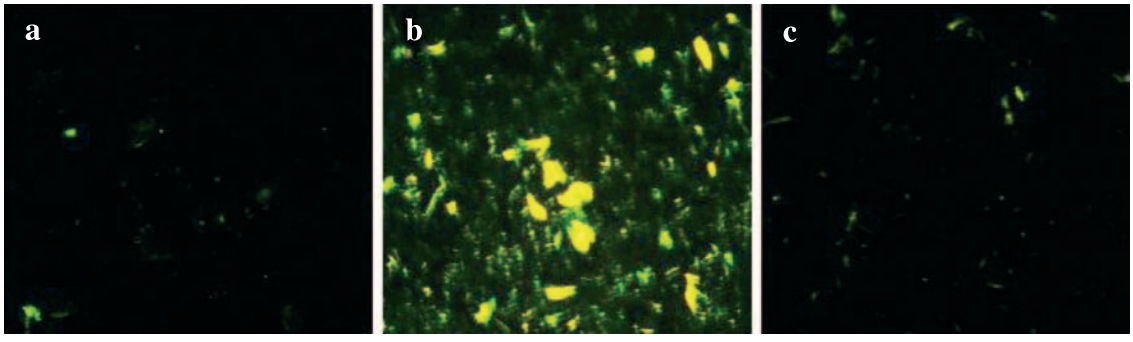

Fig. 6. FA (fluorescence assay) for the inhibition of Adv 2K2/507/KNIH replication by siRNA 295. FA was performed with an Adv-specific monoclonal antibody to analyze viral replication. **a**, Uninfected cell control; **b**, virus control; **c**, siRNA 295 (200 pmole) treatment

Cytopathic effect

The cells infected with Adv 2K2/507/KNIH exhibited marked morphological changes during the culture when compared with the uninfected cells. The uninfected cells were flattened, whereas the Adv 2K2/507/KNIH-infected cells became retractile and round. Based on the absence of morphological changes, siRNA 295 markedly inhibited the cytopathic effect of viral infection and replication (data not shown).

Inhibition of replication of Adv11 prototype strain by siRNAs 295 and 499

The effectiveness of siRNAs 295 and 499, which are specific for the E1A region of Adv 2K2/507/KNIH, was confirmed as shown above. Due to the fact that the Adv 2K2/507/KNIH showed no variation in the functional E1A region when compared to the Adv11 prototype, a supplementary test was performed to test whether these siRNAs were also effective against other Adv11 strains. The inhibitory effect of the siRNAs on the replication of the Adv11 prototype strain was assessed using quantitative real-time PCR and a plaque reduction assay as described above. Quantitative real-time PCR analysis of an internal control, β-actin mRNA, was performed to normalize the data.

Both siRNAs significantly reduced the levels of Adv11 prototype E1A mRNA and viral replication in comparison to the virus-infection-only control. Quantitative real-time PCR revealed that the Adv11 prototype E1A mRNA levels were reduced by approximately 60 and 70% upon siRNA 295 and 499 treatments, respectively, at days one and two of viral infection (data not shown). In the plaque reduction assay, a 1.3×10^4 pfu/mL viral titer was detected in the virus control without siRNA transfection, whereas few or no plaques were detected in the A549 cells transfected with 200 pmole of siRNAs (data not shown).

Discussion

Adv infections cause considerable clinical manifestations in the general population as well as in immunocompromised patients. However, no effective antiviral chemotherapy has been developed to reduce the symptoms or duration of the Adv infection. Adv11, particularly, is a causative agent of severe respiratory and urinary infections in immunocompromised patients, such as in acute hemorrhagic cystitis patients, bone marrow recipients, and AIDS patients. Therefore, an effective chemotherapy is urgently needed to treat severe cases. A number of investigators have reported several agents with antiviral activities against Adv *in vitro*. There are several reports that (s)-1-(3-hydroxy-2-phosphonylmethoxy-propyl) cytosine (HPMPC) and 2′, 3′-Dideoxycytidine (zalcitabine, ddC) inhibit Adv replication [10]. It is also known that ddC acts as an inhibitor of HIV reverse transcriptase, and it is also known to exhibit antiviral activity against Adv both *in vitro* and *in vivo* [7]. However, neither HPMPC nor ddC has been used clinically as an anti-Adv drug [7].

In the present study, RNAi-mediated gene silencing was evaluated as a possible method of inhibiting Adv replication. The technique, called RNAi, uses very small pieces of artificially made genetic material known as siRNA. The RNAi technique is based on a natural strategy, discovered just a few years ago [4, 19], that is used by plants, worms, and other lower species to ward off viruses. This technique was initially used to study the functions of individual genes. This evolutionarily conserved mechanism, triggered by dsRNA, specifically suppresses gene expression by selectively degrading mRNAs matching the sequence of the dsRNA responsible for triggering the response without affecting the expression of other genes. The recent demonstration that synthetic 21-nucleotide siRNA duplexes effectively inhibit mammalian endogenous gene expression in a sequence-specific manner without activating nonspecific dsRNA responses shows that this gene silencing pathway may have great potential for use in the study of the function of mammalian virus genes [20]. Ui-Tei et al. [17] reported that highly effective RNAi was found to occur in mammalian cells if the siRNA satisfied the following sequence criteria: (i) A/U at the 5′ end of the antisense strand; (ii) G/C at the 5′ end of the sense strand; (iii) AU-richness in the 5′ end, a 7-bp

long region of the antisense; and (iv) the absence of any long GC stretch greater than 9 bp in length [17]. Although five siRNAs were designed for use in the present study, not all siRNAs proved to be effective at silencing E1A. Only two siRNAs, siRNAs 295 and 499, were found to be effective. These two siRNAs well satisfied the above criteria for designing a highly effective siRNA, whereas siRNAs 135, 509 and 794 did not.

Advs have a double-stranded DNA genome, which is replicated in the cell nucleus. Replication is divided into the early and late phases; the latter is defined by the onset of DNA replication [12]. Prior to and independently of genome replication, the immediate early and early mRNAs are transcribed from the input DNA. The transcription of the Adv genome is regulated by virus-encoded trans-acting regulatory factors. The products of the immediate early genes regulate the expression of the early genes. The early genes are encoded at various sites on both strands of the DNA. E1A, an immediate early gene, is a very significant region at the acute phase of adenoviral infection. In addition, the virus replication cycle is highly lytic and rapid and is completed in approximately 12 h.

The features of Adv replication made it challenging for the RNAi machinery to silence the virus gene expression and to block the replication of the virus. The results of the present study showed that siRNAs with a sequence homologous to the gene encoding Adv E1A efficiently inhibited the synthesis of the E1A protein by degrading the E1A mRNA. Figure 3 shows a dramatic decrease in the level of E1A mRNA after transfection with siRNAs 295 and 499. Subsequently, as shown by the plaque reduction assay, the yield of progeny virus that resulted from an infection was reduced by 1000–3000-fold upon treatment with siRNAs 295 and 499, in comparison to the virus-infection-only control. As a result of the inhibition of plaque formation, the replication of Adv 2K2/507/KNIH in A549 cells was significantly hindered. These results verified the potential for the treatment of acute respiratory tract infection by adenovirus type 11.

The siRNA 295 and 499 treatments were designed to target the E1A region of Adv 2K2/507/KNIH. Since the Adv 2K2/507/KNIH showed low variation in the E1A region, the susceptibility of the Adv11 prototype virus to the siRNA-mediated inhibition of viral replication was tested. Treatment with siRNA also reduced the plaque formation of the Adv11 prototype by approximately 10^4-fold compared to that of the control infection performed in the absence of the siRNA (data not shown).

Since Adv11 is a causative agent of severe respiratory and urinary infection in immunocompromised patients, Adv11 infection in these patients is often fatal. Therefore, an effective chemotherapy for Adv11 infections is very much in need. In the present study, siRNAs 295 and 499 were shown to be effective in reducing the amount of Adv E1A mRNA, levels of replicative intermediates, and the viral protein. These results indicate that siRNA-mediated gene silencing inhibits Adv replication through the suppression of viral RNA. Such an siRNA-based method clearly shows potential as a novel therapeutic treatment for Adv11 infections.

Acknowledgments

This study was supported by then intramural grant of the National Institute of Health, Korea Centers for Disease Control and Prevention.

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