Silencing of African horse sickness virus VP7 protein expression in cultured cells by RNA interference

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Abstract

RNA interference (RNAi) is the process by which double-stranded RNA directs sequence-specific degradation of homologous mRNA. Short interfering RNAs (siRNAs) are the mediators of RNAi and represent powerful tools to silence gene expression in mammalian cells including genes of viral origin. In this study, we applied siRNAs targeting the VP7 gene of African horse sickness virus (AHSV) that encodes a structural protein required for stable capsid assembly. Using a VP7 expression reporter plasmid and an in vitro model of infection, we show that synthetic siRNA molecules corresponding to the AHSV VP7 gene silenced effectively VP7 protein and mRNA expression, and decreased production of infectious virus particles as evidenced by a reduction in the progeny virion titres when compared to control cells. This work establishes RNAi as a genetic tool for the study of AHSV and offers new possibilities for the analysis of viral genes important for AHSV physiology.

Introduction

RNA interference (RNAi) is a post-transcriptional gene silencing mechanism in which the expression of a gene is specifically inhibited by its cognate double-stranded (ds)RNA [*1*]. Mechanistically, RNAi is believed to proceed via a two-step mechanism [*2*]. In the

RNAi initiating step, long dsRNA is processed into discrete 21- to 23-nucleotide (nt) small interfering RNA (siRNA) molecules by a RNA nuclease termed DICER. The siRNAs are then incorporated into a RNA-induced silencing complex (RISC) that recognizes and degrades homologous mRNAs. In mammalian cells, dsRNA longer than 30 nt may induce the interferon response, leading to the inhibition of protein synthesis by the activation of the RNA-regulated protein kinase (PKR) pathway and activation of the OAS/RNase L system [*3*]. This nonspecific effect can be circumvented by use of 19–23 nt siRNA, which mediate strong and specific suppression of gene expression [*4*, *5*]. Consequently, siRNAs have rapidly developed into a powerful experimental tool for manipulating gene expression [*6*] and, at least at an experimental level, siRNA approaches is being utilized increasingly to inhibit the replication of viral pathogens [*7*–*10*].

African horse sickness virus (AHSV) belongs to the family Reoviridae under the genus *Orbivirus* [*11*]. The virus is the causative agent of African horse sickness (AHS), a highly infectious disease of equines and of which the mortality rate in horses may exceed 90% [*12*]. AHS is enzootic in sub-Saharan Africa, although outbreaks, resulting in considerable economic loss to the equestrian industry, have occurred in Morocco, the Middle East, and in Europe [*13*]. Like bluetongue virus (BTV), the prototype orbivirus, the AHSV virion is composed of seven structural proteins (VP1–VP7) organized into a two-layered protein capsid containing the 10 dsRNA segments of the viral genome [*14*, *15*]. The icosahedral core consists of two major proteins, VP3 and VP7, and three minor structural proteins (VP1, VP4 and VP6). The core is surrounded by the outer capsid, consisting of two proteins, VP2 and VP5, both of which are attached to the VP7 layer of the core [*15*, *16*]. Biochemical information has indicated that entry of BTV into mammalian cells involves virus attachment by VP2 and membrane penetration via VP5, followed by release of the core particle into the cytoplasm [*17*, *18*].

Since viral capsid assembly is a precise process that is fundamental to the success of viral infection, and keeping in view the significance of VP7 in the formation of a stable outer capsid, its down-regulation would also be expected to impact negatively on the proper assembly of infectious AHSV particles. We therefore, selected AHSV VP7 as candidate

target gene in order to determine the efficacy of RNAi for inhibition of AHSV gene expression. We here report the identification of siRNA target regions on the AHSV-9 VP7 gene transcript that are able to silence VP7 protein and mRNA expression, and impair the production of infectious viral progeny.

Materials and methods

Cell culture and viruses

Baby hamster kidney (BHK-21) cells (ATCC CCL-10) and Vero cells (ATCC CCL-81) were propagated in Minimal Essential Medium (MEM) supplemented with 2.5% fetal bovine serum (FBS) and antibiotics (Highveld Biological) at 37°C with 5% CO_2. AHSV serotype 9 was kindly provided by F. Wege (University of Pretoria, South Africa) and propagated in BHK-21 cells as described previously [*19*].

Design and synthesis of siRNAs

siRNAs that target the VP7 gene of AHSV-9 (GenBank accession no. U90337) were designed using the Qiagen siRNA design tool (available at http://www.qiagen.com) and according to published guidelines [*20*]. The selected sequences were subjected to BLAST analysis to ensure lack of homology to sequences other than the target gene. The following chemically synthesized siRNAs targeted to the VP7 gene were supplied as annealed duplexes by Qiagen: siVP7-336, corresponding to nt 336–356, had the sequence 5′-CGGGUCAGAUGCAAACAUUdTT-3′ (sense) and 5′-AAUGUUUGCAUCUGACCCGdTT-3′ (antisense); siVP7-441, corresponding to nt 441–461, had the sequence 5′-CGCGUGGUGGGUACAUCAAdTT-3′ (sense) and 5′-UUGAUGUACCCACCACGCGdTT-3′ (antisense). A double-stranded siRNA that does not share identity with any known sequence was also obtained from Qiagen and used as a nonsilencing control. The control siRNA had the sequence 5′-UUCUCCGAACGUGUCACGUdTT-3′ (sense), and 5′-ACGUGACACGUUCGGAGAAdTT-3′ (antisense).

Construction of reporter plasmid

A truncated AHSV-9 VP7, lacking a stop codon, was obtained by PCR-amplification using plasmid pBSS7PCR, containing a full-length cDNA copy of the VP7 gene [*21*], as template DNA together with oligonucleotides VP7-F (5′-CACAGATCTATGGACGCGATAGC-3′) and VP7-R (5′-CACAAGCTTGTGGTAGGCTGCTA-3′), which contain a *Bgl*II and *Hin*dIII site, respectively (underlined). The amplicon was cloned into pGEM®-T Easy (Promega) and sequenced to verify the absence of extraneous mutations. The insert DNA was subsequently recloned into the *Bam*HI and *Hin*dIII sites of the mammalian expression vector pCMV-Script® (Stratagene), under control of the CMV promoter, to generate pCMV-VP7trunc. Oligonucleotides eGFP-F (5′-CACAAGCTTATGGTGAGCAAGG-3′) and eGFP-R (5′-CACCTCGAGTTACTTGTACAGCTCGT-3′), which contain a *Hin*dIII and *Xho*I site, respectively (underlined), were used with plasmid pGEM-eGFP (kindly provided by J. Weyer, University of Pretoria, South Africa) as template DNA to PCR-amplify the sequence encoding the enhanced green fluorescent protein (EGFP). The amplicon was transferred into pGEM®-T Easy and then cloned into the *Hin*dIII–*Xho*I digested pCMV-VP7trunc vector to generate the reporter plasmid pCMV-VP7-EGFP, in which the EGFP gene was fused in-frame with the 3′ end of the VP7 coding sequence. For some experiments, cells were transfected with plasmid pCMV-EGFP (kindly provided by J. Roos, University of Pretoria, South Africa), which directs the expression of the EGFP protein.

Cell transfections and reporter plasmid analysis

BHK-21 cells were seeded 24 h prior to transfection in 24-well culture dishes and transfected at 80% confluence with 0.8 μg reporter plasmid DNA, in the presence and absence of 50 nM of VP7-specific or control siRNA, using Lipofectamine™ 2000 (Invitrogen) according to the protocol provided by the manufacturer. After 24 h of incubation, cells were observed for expression of EGFP on a Zeiss Axiovert 200 fluorescence microscope and photographed using a Nikon DXM1200 digital camera. For fluorometry analysis, the cells were trypsinized, washed with 1× PBS (137 mM NaCl; 2.7 mM KCl; 4.3 mM Na_2HPO_4 . $2H_2O$; 1.4 mM KH_2PO_4; pH 7.4) and resuspended in

1 ml of 1× PBS to analyze fluorescence, using a BioRad Versafluor™ fluorometer with filters (emission at 515–525 nm, and excitation at 485–495 nm) for EGFP expression. AlexaFluor488-labelled siRNA (Allstars Negative Alexafluor488-siRNA; Qiagen) was used to assay for transfection efficiency. The transfected cells were observed by fluorescence microscopy and the percentage of fluorescent cells in at least three randomly selected fields from three independent experiments was determined. About 80–90% of the BHK-21 cells were successfully transfected under the conditions described in this study.

Cell transfection and viral challenge assays

The viral challenge assays were performed according to the procedures described by Bitko and Barik [*4*]. Briefly, BHK-21 cells were seeded 24 h prior to transfection in 6-well culture dishes and transfected at 80% confluence with control or VP7-specific siRNA (50 nM or 100 nM), either individually or with a mixture of the siRNAs, using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's specifications. Cells were incubated for 8 h after transfection, washed twice with MEM (lacking serum and antibiotics) and subsequently infected with AHSV-9 at a multiplicity of infection of 1 plaque forming unit (pfu)/cell. After 1 h of adsorption, a second transfection was performed under identical conditions and the cells were incubated in MEM containing FBS. As a control, untreated AHSV-9 infected cells were included in the analyses. At 24 h post-infection, the virus-containing supernatants were collected and virus titres were determined by plaque assays on Vero cells, while the cells were processed for RNA isolation.

RNA extraction and semiquantitative real-time reverse transcription (RT)-PCR

Total RNA was extracted from siRNA-treated AHSV-9 infected cells, using the Aurum™ Total RNA mini kit (BioRad) according to the manufacturer's protocol. RT was carried out by using a QuantiTect® Reverse Transcription kit (Qiagen) in a 20-µl reaction mixture, containing total RNA and random primers, at 42°C for 30 min. One microlitre of RT reaction mixture was used for the real-time PCR, performed in the LightCycler 1 system (Roche Diagnostics), using the QuantiTect™ SYBR® Green PCR kit (Qiagen). Following a denaturation step at 95°C for 15 min, 55 cycles of amplification was

performed at 94°C for 15 s, 60°C for 20 s, and 72°C for 10 s. The sequences of the AHSV-9 VP7 gene-specific oligonucleotides used in the real-time PCR were 5′-CTGGAGATGTCGTCGCATGGAATAC-3′ (sense) and 5′-GAGCCAATTCCGGAACCGTG-3′ (antisense). Real-time RT-PCR was also performed for β2-microglobulin as endogenous reference and used for data normalization and calculation of fold changes in VP7 transcripts using the Relative Expression Software Tool (REST©) [*22*]. The sequences of the β2-microglobulin (GenBank accession no. X17002) gene-specific oligonucleotides were 5′-AGTGGAGCTGTCAGATCTGTCCTTC-3′ (sense) and 5′-TGACCACCTTGGGCTCCTTC-3′ (antisense). To confirm specific amplification, melt-curve analysis of the RT-PCR products were performed according to the manufacturer's protocol, using the LightCycler version 3.5.3 software program (Roche Diagnostics), and the RT-PCR products were analyzed on a 2% agarose gel stained with ethidium bromide.

Immunoblot analysis

Twenty-four hours post-infection, cell lysates were prepared from control nontransfected BHK-21 cells and AHSV-9 infected BHK-21 cells, as well as from siRNA-treated AHSV-9 infected BHK-21 cells. The proteins were separated by 10% SDS-PAGE and electroblotted onto a nitrocellulose membrane (Hybond™-C; Amersham Biosciences). The membrane was incubated overnight at 4°C in 1× PBS (pH 7.4) supplemented with 1% fat-free milk powder, followed by incubation at room temperature with anti-AHSV-9 antibody (diluted 1:100; Onderstepoort Veterinary Research Institute) for 2 h with gentle shaking. The blots were washed three consecutive times with wash buffer (1× PBS containing 0.05% Tween-20), and incubated for 1 h with the secondary antibody, Protein-A conjugated to horseradish peroxidise (diluted 1:1000; Sigma-Aldrich). After three successive washes with wash buffer, the blots were rinsed with 1× PBS (pH 7.4) and immunoreactive proteins were detected by an enzyme-substrate solution (4-chloro-1-naphtol; BioRad).

Plaque assays

Virus titres were determined in two independent experiments by plaque assays on Vero cells according to the method described by Oellerman [*23*], with the following

modifications. Vero cells in 6-well culture dishes were inoculated with 100 µl of serially diluted virus in 2 mM Tris and allowed to adsorb for 1 h. The inoculums were removed and the cells overlaid with 2 ml agarose (1:1 ratio of 0.7% agarose and MEM containing 10% [v/v] FBS). After 5 days, cells were stained with 0.1% neutral red and the plaque counts were determined.

Results

Inhibitory effects of siRNA on the expression of VP7-EGFP fusion proteins

To determine whether the siRNAs we designed could efficiently reduce expression of VP7 in cultured cells, we cotransfected BHK-21 cells with pCMV-VP7-EGFP in the absence or presence of the different siRNAs, and assessed VP7-EGFP expression at 24 h post-transfection by using fluorescent microscopy. The results showed that expression of the VP7-EGFP fusion protein was markedly reduced in cells cotransfected with siVP7-336 and siVP7-441, while the control nonsilencing siRNA had no apparent effect (Fig. 1a, panel 1). Notably, siVP7-336 appeared to silence VP7-EGFP gene expression more efficiently than siVP7-441, since in most transfected cells VP7-EGFP expression was reduced to near background levels. To show specificity of the siRNA targeting, we also transfected BHK-21 cells with the reporter plasmid pCMV-EGFP in a parallel series of experiments. Results showed that the EGFP protein was highly expressed in cells transfected with the reporter plasmid in the presence of cotransfected siVP7-336, siVP7-441, and control siRNA (Fig. 1a, panel 2). The expression of the VP7-EGFP and EGFP proteins was analyzed quantitatively by fluorometry in the siRNA-cotransfected cells, as shown in Fig. 1b. The data showed that compared to cells cotransfected with the control siRNA, siVP7-336 and siVP7-441 reduced expression of the VP7-EGFP fusion protein by 91% and 67%, respectively. In contrast, no significant inhibition of EGFP protein expression was found in cells cotransfected with the VP7-specific siRNAs. Together, these data showed that the siRNAs targeting the AHSV-9 VP7 gene could induce a significant and specific reduction in the level of VP7 protein.

Fig. 1 Effect of siRNAs on the expression of AHSV-9 VP7-EGFP in BHK-21 cells. (**a**) Fluorescence micrographs of cells transfected with reporter plasmids pCMV-VP7-EGFP and pCMV-EGFP, and cotransfected with the VP7-specific siRNAs (siVP7-336, siVP7-441), as well as a control nonsilencing siRNA. At 24 h post-transfection, representative fields were photographed. Magnification, 5×. (**b**) Cells were analyzed for VP7-EGFP and EGFP expression by fluorometry and the level of fluorescence relative to cells cotransfected with control nonsilencing siRNA was quantitated. Data are the means ± standard deviation (S.D.) from three independent experiments

siRNA treatment of BHK-21 cells reduced AHSV-9 VP7 transcripts

Since siRNA functions by identifying and degrading mRNA that shares sequence complementarity with the siRNA, we next examined the effect of siVP7-336 and siVP7-441 on the abundance of VP7 mRNA in AHSV-9 infected cells by making use of semi-quantitative real-time RT-PCR. Moreover, we also explored the extent of inhibition using an increased dose of siVP7-336 (100 nM) and by using both VP7-specific siRNAs (50 nM each) for cell transfections. In contrast to BHK-21 cells transfected with the control nonsilencing siRNA, VP7 mRNA expression was reduced in BHK-21 cells transfected with the VP7-specific siRNAs (Fig. 2). The results indicated that in BHK-21 cells treated with siVP7-336 and siVP7-441, the siRNAs induced a 7.9- and 3.9-fold reduction in VP7 mRNA transcripts, respectively, as compared with control siRNA. Compared to the use of 50 nM siVP7-336, the inhibitory effect on VP7 mRNA observed for siVP7-336 at a higher dosage or by using both siVP7-336 and siVP7-441 was weakened in both instances, and resulted in 5.9- and 5.7-fold reduction in VP7 mRNA, respectively (Fig. 2). These results therefore suggested that the suppression of VP7 protein expression observed earlier (Fig. 1) was indeed due to degradation of VP7 mRNA.

Fig. 2 Real-time RT-PCR analysis for relative quantification of VP7 mRNA expression in AHSV-9 infected BHK-21 cells. Fold changes in VP7 mRNA expression levels were calculated by relative quantification of VP7 mRNA in VP7-specific (siVP7-336, siVP7-441) siRNA-treated cells as compared with control nonsilencing siRNA-treated cells.

Real-time RT-PCR for β2-microglobulin gene transcripts was included in the assays as endogenous reference and used for data normalization of VP7 mRNA fold changes. Data are shown as the means ± S.D. of three samples

siRNA treatment of BHK-21 cells reduced AHSV-9 VP7 protein expression

To investigate whether the reduction in VP7 mRNA correlated with a down-regulation in VP7 protein in siRNA-treated AHSV-9 infected cells, immunoblotting using anti-AHSV-9 antibody was carried out. It is evident from the results that the VP7-specific siRNAs reduced the amount of detectable VP7 protein (Fig. 3). A marked reduction in the expression of VP7 was noticed in AHSV-9 infected cells treated with siVP7-336, and to a lesser extent in cells treated with siVP7-441 (50 nM). Virus-infected cells treated with siVP7-336 at a higher dose (100 nM) or with both siVP7-336 and siVP7-441 also induced inhibition of VP7. In contrast, transfection of BHK-21 cells with control nonsilencing siRNA failed to yield any reduction in the synthesis of VP7. These results were consistent with the data obtained at the mRNA level by semi-quantitative real-time RT-PCR assays. It is also noteworthy that expression of a non-targeted host protein (ca. 40 kDa), which verified equal sample loading, remained unaffected by all siRNAs (Fig. 3).

Fig. 3 Immunoblot depicting the expression of AHSV-9 VP7 in BHK-21 cells subjected to transfection with indicated siRNAs and concentrations. Immunoblot analysis was performed with total cell lysates prepared at 24 h post-infection, and the membrane probed with anti-AHSV-9 antibody. Molecular weight markers (kDa) are indicated to the left of the blot. BHK-21 cells and untreated AHSV-9 infected cells were included as controls

siRNA-treated BHK-21 cells reduced plaque formation

To evaluate the effect of VP7-silencing siRNAs on reducing the infectious virus count, we collected the supernatants from siRNA-treated AHSV-9 infected BHK-21 cells at 24 h post-infection, and determined the titre of virus produced by plaque assays. As a control, untreated AHSV-9 infected cells were also included. Since AHSV can replicate

rapidly in infected cells [*19*], we considered that the virus in the supernatants after 24 h of culture to represent newly replicated virus. The results indicated that the cells transfected with the control siRNA at different concentrations did not exhibit a reduction in virus titre (1.57×10^6 pfu/ml [50 nM] and 1.26×10^6 pfu/ml [100 nM]) compared to untreated AHSV-9 infected BHK-21 cells (1.17×10^6 pfu/ml). These results thus indicated that virus replication occurred in the cells during the 24-h culture period and that transfection of the cells with the control siRNA did not nonspecifically interfere with virus replication. However, a reduction in virus titre was observed in cells treated with the siRNAs corresponding to VP7 mRNA (Fig. 4). Whereas siVP7-336 reduced the virus titre by 84%, siVP7-441 induced a 63% reduction in virus titre. In BHK-21 cells treated with siVP7-336 at a higher dose or in cells treated with both siVP7-336 and siVP7-441, the virus titres were reduced by 71% and 73%, respectively.

Fig. 4 Effect of siRNAs on AHSV-9 progeny virus production expressed by plaque reduction assays. The virus-containing supernatants were collected 24 h after virus infection of BHK-21 cells treated with siRNAs, as indicated. Progeny virions in the culture supernatants were titrated by plaque assay. The effect of VP7-specific siRNAs (siVP-336, siVP7-441) on progeny virus titres is depicted as percent reduction in plaque forming units (pfu) as compared to control nonsilencing siRNA (with 0% reduction). Virus titres were determined from two independent experiments and values shown are the means ± S.D

Discussion

Despite its impact in probing gene function and in the development of novel therapeutics and antivirals, there have been only a few studies exploring the potential for RNAi approaches to viruses with a segmented dsRNA genome and these have focused on rotaviruses [*24–26*]. In the present report we show that AHSV, like rotavirus, is susceptible to the RNAi pathway. Both the siRNAs tested resulted in AHSV-9 VP7 gene silencing and also resulted in a reduction in progeny virion titres.

Since assays with infectious viruses are time-consuming, labor-intensive and have to be performed under elevated safety conditions, a simple method for the preselection of active siRNA species is desirable. In the present study, we demonstrate that fusion of the virus gene upstream of the EGFP reporter gene is an appropriate method to screen for active siRNAs. We designed two 21-nt siRNAs to target different regions on the AHSV-9 VP7 mRNA, termed siVP7-336 and siVP7-441, which were synthesized chemically. To facilitate screening of active siRNA we constructed the reporter plasmid pCMV-VP7-EGFP in which the VP7 coding region was fused in-frame with that of the EGFP gene, thereby expressing VP7-EGFP fusion mRNAs. Since siRNA that induce cleavage of the viral mRNA will lead to reduced expression of the reporter, the EGFP reporter gene therefore provided a simple and impartial model for assessing the efficacy of gene silencing by analysis at the protein level visually by fluorescent microscopy and quantitatively by fluorometry.

Although both siRNAs had sequences homologous to the AHSV VP7 mRNA, they differed in their efficacy to establish VP7 protein silencing. In several independent experiments, siVP7-336 consistently inhibited VP7-EGFP expression more efficiently than did siVP7-441, indicating that the difference in efficacy cannot be ascribed solely to differences in transfection efficiency. Variation in the silencing activity of several siRNAs targeting the same mRNA has also been reported previously [*27–29*]. The reasons for the differences in RNAi efficacy are not well understood, but has been postulated to be due differences in the thermodynamic properties of the siRNAs [*30*], as well as secondary and tertiary RNA structure of the target site that disables recognition by the siRNA [*28*, *31*, *32*].

Moreover, results obtained during the course of this study also indicated that VP7 gene silencing was less efficacious when either a combination of the VP7-specific siRNAs or an increased dose of siVP7-336 was used. To date, the advantages of using a combination of siRNAs to improve inhibition remain controversial. In some cases the inhibition has been enhanced [*33*], whereas other reports have indicated a counter-effect by increasing the dosage [*34–36*]. The decrease in inhibition observed in the present study may have been due to the presence of siVP7-441 that inhibited the ability of the more efficacious

siVP7-336 molecule to bind to the RISC complex, perhaps through competition for RISC binding. Notably, it has recently been proposed that at high siRNA concentrations the RNAi effect may be down-regulated through the induction of a series of as yet unknown genes, coding for destabilizing enzymes that either limit or completely degrade the exogenous siRNAs [*35*].

RNAi is promising to provide antiviral therapies directed against viral diseases, including the many viral diseases of animals. Over the past few years, several laboratories have used RNAi to attenuate animal virus infection in cell culture, including foot-and-mouth disease virus (FMDV) [*37*], Anatid herpes virus-1 (AHV-1) [*38*], porcine reproductive and respiratory syndrome virus (PRRSV) [*39*], bovine viral diarrhea virus (BVDV) [*40*], and avian metapneumovirus (aMPV) [*41*]. In contrast to viruses with a positive strand RNA genome (e.g., FMDV, BVDV, and PRSVV), in which the genome is targeted by the RNAi machinery thereby inhibiting replication of the virus, in DNA viruses (e.g., AHV-1) and viruses with a negative strand RNA genome (e.g., aMPV) the inhibition is due to silencing of viral functions through viral mRNA cleavage. Our results showed that the AHSV-9 titres were reduced in cells treated with the VP7-specific siRNAs (Fig. 4). The reduction in the production of infectious virus progeny thus poses the question whether RNAi may be used as an effective antiviral strategy for AHSV. In this regard, it is important to note that in the case of AHSV, each of the 10 genome segments of dsRNA is transcribed into a single mRNA, each encoding a single protein (with the exception of one bicistronic segment) [*15*]. Moreover, results obtained with rotavirus, a dsRNA virus with 11 genome segments, showed that positive-strand RNAs that are susceptible to siRNA-induced degradation are located outside of the viroplasms and undergo translation, whereas those positive-strand RNAs which are located inside viroplasms and undergo replication are not [*26*]. These results therefore imply that siRNAs directed to a given transcript may impair the synthesis of the corresponding protein, but not that of the corresponding dsRNA genome segment. Similarly, AHSV positive-strand mRNAs that are located in the virus inclusion bodies (VIBs), the proposed sites of orbivirus replication and assembly [*15*, *42*], may thus also be inaccessible to the RNAi machinery. It therefore follows that it may not be possible to block AHSV genome replication. The

reduced viral titres observed in this study may thus be attributed to the RNAi-induced reduction in intracellular amount of VP7 protein and the subsequent lack of VP7 protein to facilitate stable outer capsid assembly, which is required for productive infection of susceptible host cells.

In conclusion, this study provides evidence that siRNA technology can be used as a tool to inhibit gene expression of the VP7 protein of AHSV in BHK-21 cells. We used various experimental proofs (fusion constructs, semi-quantitative real-time RT-PCR, immunoblotting, and virus titres) in the present study that are concordant with each other and thus enhance the reliability of the findings. Furthermore, the siRNA approach and methodology described here are likely to be generally applicable to the silencing of all AHSV genes. Although the use of RNAi as an antiviral therapeutic tool remains to be demonstrated, the segmented nature of the dsRNA genome of AHSV makes it particularly amenable to analysis by RNAi. Therefore, new experimental approaches in analyzing viral gene functions in the context of AHSV infection can now be designed, a task that has been elusive for a long time.

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