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Original Article

Inhibition of human cytomegalovirus DNA replication by small interfering RNAs targeted to UL49

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Human cytomegalovirus (HCMV) is a ubiquitous virus. Although the infection in healthy children and adults is usually asymptomatic, in immunocompromised individuals and newborns it is a significant cause of morbidity and mortality. *UL49*, an essential gene of HCMV, is highly conserved among various HCMV strains. The expression of UL49 is correlated with the production of virions. When UL49 is inhibited in the HCMV, the production of virions is reduced severely. In this study, RNA interference was applied to further investigate the roles of UL49 in viral replication. Two effective small interfering RNAs against UL49 were selected. Silencing of UL49 in HCMV-infected human foreskin fibroblast cells reduced the transcription levels of early and late genes, but not immediate-early ones. In addition, the viral DNA content was significantly reduced. This is the first time to uncover the role of UL49 in viral DNA synthesis, which indicates that UL49 might play an important role in this period. So the down-regulation of *UL49* mRNA using RNAi might be a potential clinical therapy against the virus.

Keywords human cytomegalovirus; UL49; siRNA; DNA replication

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Introduction

As a member of the β-herpes virus subfamily, human cytomegalovirus (HCMV) is a ubiquitous pathogen of worldwide importance that can cause severe diseases in immunocompromised individuals and newborns. The virus is also a leading cause of retinitis-associated blindness and other debilitating conditions such as pneumonia and enteritis among AIDS patients [1,2]. Moreover, HCMV causes mental and behavioral dysfunctions in children who were infected *in utero*. Therefore, development of effective antiviral compounds and approaches is crucial for controlling HCMV infections and preventing HCMV-associated complications.

During lytic replication, the expression of HCMV genes follows a strictly coordinated pattern. Like other herpesviruses, the open reading frames (ORFs) are expressed in a temporally regulated cascade consisting of three sequential phases, termed immediate-early (IE), early (E), and late (L) [3–5]. Virion factors and IE genes pave the way for the following stages of the viral replicative cycle, whereas the onset of viral DNA replication takes place at the start of the E state [6], which is required for the entry into the L phase, the culmination in virion assembly and finally the release of infectious progeny virus from host cells [7].

HCMV contains a complex double-stranded DNA genome of about 240 kb, having the potential to encode >200 putative ORFs [8,9]. However, no more than 80 ORFs encode proteins that have been well characterized or are homologous to viral proteins of other herpesviruses with known functions [10]. The other proteins have not been identified experimentally, and their functions still need to be clarified. UL49 is highly conserved among various HCMV strains such as laboratory stains AD169, Towne, and Toledo, as well as the clinical isolates TR, Fix, and Merlin. Thus, UL49 might serve as a potential target for novel drug development to combat HCMV infection.

Nucleic acid-based gene interference technologies represent promising gene-targeting strategies for specific inhibition of mRNAs [11,12]. For example, ribozymes have been shown to cleave viral mRNA sequences and inhibit viral replication in human cells [13–15]. In the past decade, small interfering RNAs (siRNAs) are found to be effective to inhibit gene expression and growth of several human viruses [11,16,17]. Although HCMV replication was effectively inhibited while UL49 was knockdown [18], it still needs to be clarified at which period that UL49 was required.

In order to further explore the role of UL49 in viral replication, RNA interference (RNAi) was used in human foreskin fibroblast cells (HFFs). To further investigate the effect

of UL49 knockdown on viral genes expression and viral DNA replication, the other viral mRNAs and the content of viral DNA was tested in Towne-infected siRNA-transfected HFFs.

Materials and Methods

Cells and virus

All cells (COS-7 and HFFs) were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma, St Louis, USA) supplemented with 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, and 10% fetal bovine serum (Sijiqing, Shanghai, China). HFFs at passages 5–10 were used for infection and experiments were carried out with confluent cell monolayers. The HCMV Towne stocks used in this study were kindly provided by Dr Fenyong Liu (University of California, Berkeley, USA), and were prepared as described previously [19]. In order to initiate infection, sub-confluent HFFs were infected with Towne. The inoculum was replaced with fresh warm medium after 2 h.

Plasmids construction

For the plasmid pcDNA3.1-flag construction, *via* two endonuclease sites *Xho*I and *Xba*I (TaKaRa, Dalian, China), a pair of primers for 3 × flag sequence (Flag-F: 5′-TCG AGGACTACAAAGACCATGACGGTGATTATAAAGAT CATGACATCGATTACAAGGATGACGATGACAAGT-3′; Flag-R: 5′-CTAGACTTGTCATCGTCATCCTTGTAATCG ATGTCATGATCTTTATAATCACCGTCATGGTCTTTGT AGTCC-3′) was annealed into pcDNA3.1 (+). For pcDNA3.1-UL49flag (pc49f) construction, UL49 was constructed by polymerase chain reaction (PCR) using HCMV Towne genomic DNA as the template and oligonucleotides UL49F: 5′-CGGGATCCGTCATGGCCAGTCGTCGTCTC CG-3′ and UL49R: 5′-CCGCTCGAGGACATGGGGCAG GCCGT-3′ as 5′ and 3′ primers, respectively. The reactions were performed as follows: denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s for 36 cycles. Then, the PCR fragments were digested with *Bam*HI and *Xho*I (TaKaRa), and then cloned into pcDNA3.1-flag.

Western blot analysis

Extracts from cells were prepared by boiling in loading buffer, separated on 10% (*w/v*) polyacrylamide gel (Bio-Rad, Munich, Germany) and then transferred onto a poly(vinylidene difluoride) membrane (Amersham, Freiburg, Germany). The membrane was blocked for 1 h in PBST (phosphate-buffered saline plus 0.1% Tween-20) containing 5% skimmed milk powder and then washed with PBST. After incubation with appropriate monoclonal antibodies: anti-Flag (Cali-Bio, Coachella, USA); anti-β-actin (Clontech, Mountain View, USA); or anti-IE 72/86 (Santa Cruz, Santa Cruz, USA), the membrane was washed with PBST for 1 h. Then horseradish peroxidase-conjugated goat anti-mouse horseradish antibody (Clontech) was used to detect primary antibodies. Finally, the proteins were visualized by standard procedures using an enhanced chemiluminescence system (Roche, Basel, Switzerland) and exposed to Kodak X-ray film.

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

For quantification of *UL49* mRNA inhibited by siRNA, 1×10^6 HFFs were first transfected with or without siRNAs NC, 49-11, or 49-15 by Lipofectamine™ 2000 (Invitrogen, Carlsbad, USA), and then infected with Towne (MOI of 1) at 24 h after transfection. The inoculum was replaced with DMEM supplemented with 10% (*v/v*) fetal bovine serum at 2 h after incubation. Two days later, total RNA was extracted from cells using Trizol reagent (Invitrogen) according to the manufacturers' instruction. cDNAs were amplified and quantified by ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, USA) using the dye SYBR green PCR master mix (TaKaRa). The primers used were as follows: *UL49*, forward 5′-CGTTCTTGCGTCCTTCATCT-3′ and reverse 5′-CACAAAGTAGGGCTTGGTCAT-3′; *IE2*, forward 5′-T TTGAACGAGTGACCGAGGA-3′ and reverse 5′-CCCAA TACACTTCATCTCCT-3′; *UL44*, forward 5′-GCTGGTGA TCTTGTGCTGCTCGTAT-3′ and reverse 5′-GTCTCGGC GGTCTGGGAGGAGGTGG-3′; *UL83*, forward 5′-CCTG GATGCGATACTGGCTGGTGAA-3′ and reverse 5′-GC AGCAGATCTTCCTGGAGGTACAA-3′; and *β*-actin, forward 5′-TCGTCCACC GCAAATGCTTCTAG-3′ and reverse 5′-ACTGCTGTCACCTTCACCGTTCC-3′. *β*-Actin was used as the internal control to calculate $2^{-\Delta\Delta C_t}$. C_t represents the threshold cycle for each transcript. Real-time PCR was run using an ABI 7300HT device, and the data were analyzed using the SDS 2.2.1 software (Applied Biosystems). Each real-time PCR assay was performed in triplicate.

Real-time quantitative PCR analysis of viral DNA synthesis

Viral DNA content was analyzed by real-time quantitative PCR (qPCR) as described previously [20,21]. First, HFFs were starved without serum for 72 h, and then transfected with each siRNA using Lipofectamine™ 2000 [16]. At 12 h post-transfection (hpi), siRNA-containing medium was removed and cells were washed once with pre-warmed medium. After another 12 h, cells were infected with Towne (MOI of 1). Then, cells were harvested at 72 hpi. DNA was isolated from the samples using a Viral DNA Kit (OMEGA, Norcross, USA) according to the manufacturers' instructions. Viral genomes were quantified with a primer

pair for *UL83*, and the number of viral genomes was normalized to the number of cellular copies of *β-actin* with primers described above. The primers used for *UL83* and *β-actin* were the same as above. PCR mixtures contained 1 μl extracted DNA, 50 nM primers, 10 μl 2 × SYBR green PCR master mix (TaKaRa), and nuclease-free water was added to a final volume of 20 μl. Each qPCR assay was performed in triplicate.

Results

UL49 was over-expressed in COS-7 cells

To construct pc49f recombinant plasmid, UL49 ORF was inserted into pcDNA3.1-flag. The pc49f plasmid was confirmed by PCR, digested with *Bam*HI and *Xho*I [**Fig. 1(A)**], and then sequenced. The plasmids pc49f and pcDNA3.1-flag were transfected into COS-7 cells, respectively. The cells were collected and lyzed after 48 h. Western blot analysis showed that UL49flag was expressed with the expected molecular size of 68 kDa [**Fig. 1(B)**].

Screening of the highly efficient siRNAs targeting to UL49

To examine the inhibition efficiency of three designed siRNAs [**Fig. 2(A)**] targeting UL49, COS-7 cells were simultaneously transfected with pc49f and siRNA. The expression of UL49 protein was tested by western blot analysis. As shown in **Fig. 2(B)**, UL49 expression was inhibited by specific siRNAs (49-10, 49-15 and 49-11) compared with the nonsense control siRNA (NC). The data also demonstrated that the expression of UL49 was significantly reduced by 49-15 siRNAs and 49-11 siRNA, and a less inhibited by 49-10 siRNA. Same results were also obtained in HEK293T cells and HeLa cells (data not shown).

Knocking-down UL49 at mRNA level by siRNAs in HFFs

To determine the influence of the effective siRNAs on the amount of UL49 mRNA, the siRNAs 49-15 and 49-11 were chosen. Total mRNAs were extracted from infected siRNA-transfected HFFs at 48 hpi, and used for quantification of *UL49* mRNA. As shown in **Fig. 3**, 49-15 siRNA and 49-11 siRNA inhibited the expression of *UL49* mRNA at 24, 48, and 72 hpi, respectively. Similar to the results in **Fig. 2(B)**, the 49-15 siRNA knocked down UL49 more dramatically.

Expression of other viral genes was affected by UL49 inhibition

It was previously reported that the HCMV replication was reduced by external guide sequences (EGS) and RNase P [18]. We next investigated whether knocking down UL49 reduced other viral genes expression. We detected the mRNA levels of three other viral genes, *IE2*, *UL44*, and *UL83*, standing for genes of different replication stages (IE, E, and L). As shown in **Fig. 4**, silencing UL49 had different effects on the viral transcription. The mRNA levels of *UL44* [**Fig. 4(B)**] and *UL83* [**Fig. 4(C)**] were obviously decreased in cells transfected with UL49 siRNA (49-15 or 49-11) compared with NC at 48 hpi. However, the mRNA level of *IE2* was less affected [**Fig. 4(A)**].

To test whether UL49 inhibition has similar minor effect on IE proteins, two IE proteins (IE72 and IE86) were detected in Towne-infected siRNA (NC and 49-15)-transfected HFFs. **Figure 4(D)** showed that silencing of UL49 had little effect on IE proteins at 24, 48, and 72 hpi. In conclusion, silencing of UL49 decreased the expression of the E and L genes, but not IE genes.

Viral DNA synthesis was affected during UL49 knockdown

The viral DNA synthesis starts as early as 24 hpi in cell culture [6], which is required for the expression of L genes [21,22]. We hypothesized that the obvious reduction of E and L mRNAs might be a result of reduced viral DNA synthesis. Viral DNA synthesis was determined in infected siRNA-transfected HFFs. The number of viral genomes present in each sample was determined by relative qRT-PCR, specifically. Then the copy number of a viral locus (*UL83*) was normalized to the copy number of a cellular locus (*β-actin*). Compared with infected-cells transfected with Lipo-only or NC, both UL49 siRNAs (49-15 and 49-11) did not change the numbers of the copies *UL83* at 6 hpi [**Fig. 5(A)**], indicating that UL49 did not affect the entry of viral DNA to its host cells. Nevertheless, 49-15 caused a reduction of 90% in the number of copies of *UL83*, and 49-11 caused a reduction of 65% at 72 hpi [**Fig. 5(B)**], which confirmed that UL49 plays an important role during HCMV DNA replication. It also indicated that the reduction of E and L mRNAs may be caused by the reduction of the viral genomes.

Discussion

To analyze the function of viral proteins *in vivo*, the deletion mutants are often used. However, it can not be used on essential gene products, such as UL49 of HCMV, for the virus with the deletion would produce a non-replicating or even lethal phenotype [23]. In the case of HCMV, it has been shown by random mutagenesis of the genome that 41 open reading frames are essential for viral replication [24,25]. As one of them, UL49 was inhibited in AD169-infected human embryonic lung fibroblast cells previously treated with EGS [18].

A new promising approach is the use of siRNAs that allow the analysis of the gene function [26,27]. In this study,

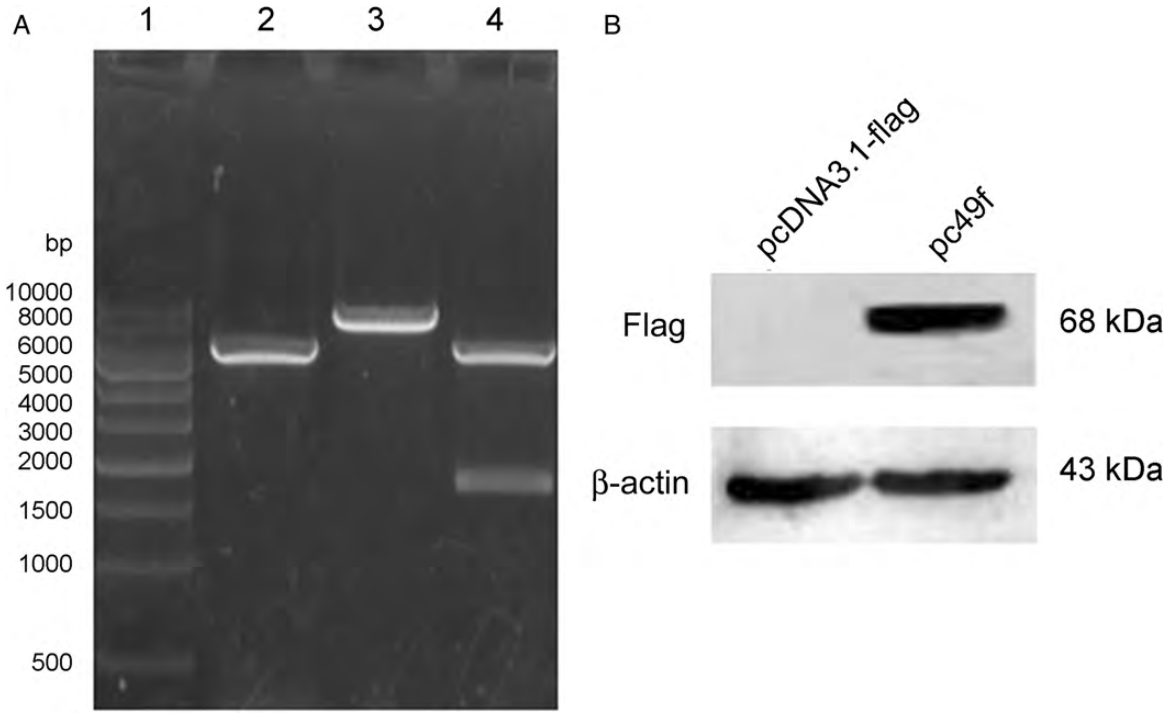

Figure 1 Efficient expression of the UL49 protein in COS-7 cells (A) Identification of the construction of pc49f. Lane 1, 1 kb DNA Ladder Marker; lane 2, pcDNA3.1-Flag digested by *Bam*HI and *Xho*I; lane 3, pc49f digested by *Xho*I; lane 4, pc49f digested by *Bam*HI and *Xho*I. (B) The over-expressed UL49flag protein was tested by western blot analysis.

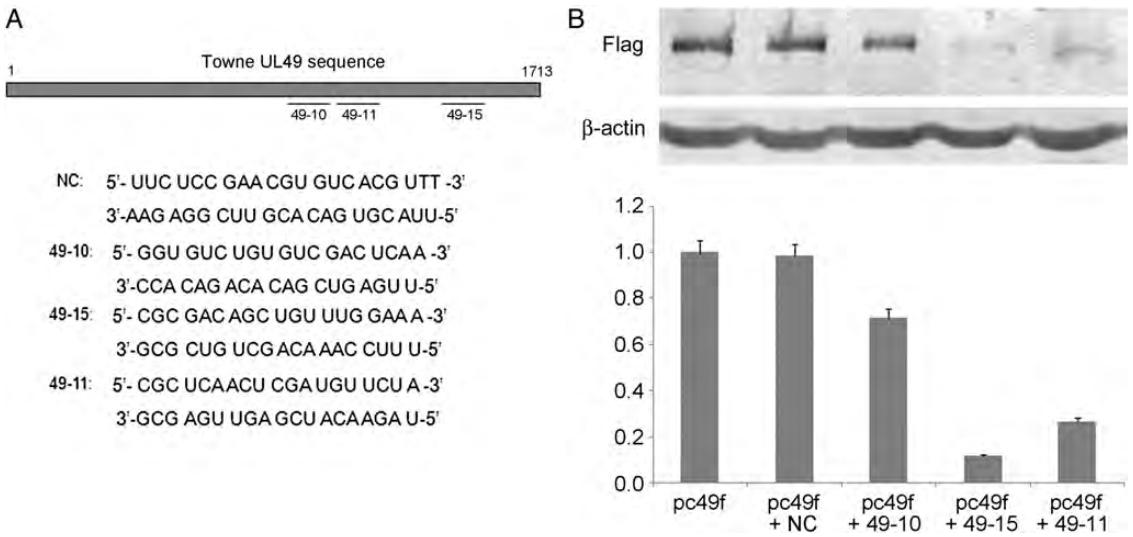

Figure 2 Screening of the highly efficient siRNAs targeting UL49 (A) Schematic diagram indicating the sequence of the siRNA constructs. The gray box on the top indicates the coding region for HCMV UL49 protein. The numbers at the end represent the full length of UL49 and regions from where siRNA sequences were selected. NC was used as a control. (B) UL49 protein was inhibited by siRNAs. Both 49-15 and 49-11 effectively inhibited the UL49 protein.

we used UL49-specific siRNAs as specific inducers of RNA silencing. As the optimum host cells for HCMV replication, including viral DNA replication, HFFs were widely used in most published articles, which were also chosen to perform the current experiments. Compared with Lipo-only and NC, both 49-15 and 49-11 inhibited *UL49* mRNA of Towne by nearly 60% (**Fig. 3**) at different time points post-infection, suggesting that the interference persisted over a long time in HFFs. The inhibition efficiency of UL49 may be higher by improving siRNAs transfection efficiency.

UL49 is necessary for the efficient replication of HCMV AD169 [18]. But it remains unclear at which stage UL49 is

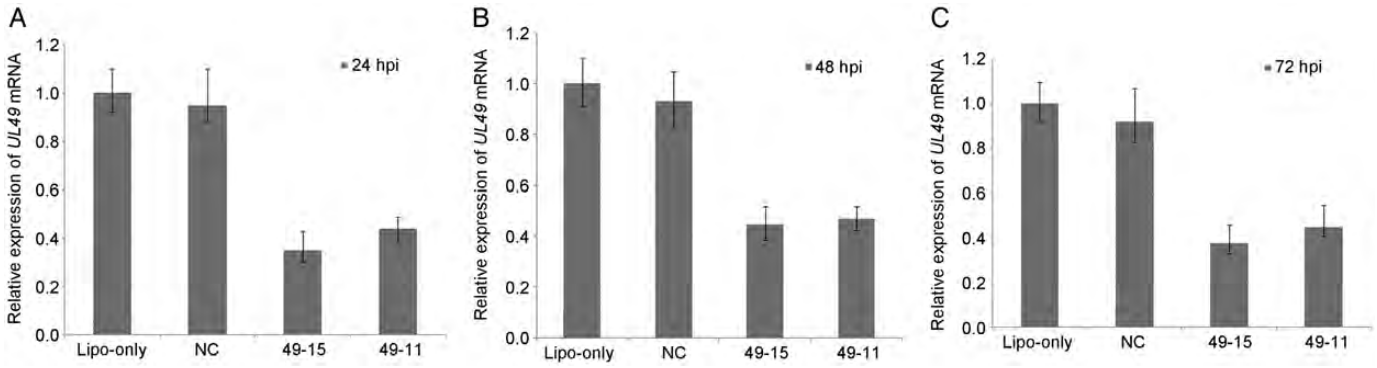

Figure 3 ***UL49*** **mRNA was inhibited in Towne-infected HFFs by siRNAs** Real-time PCR analysis of *UL49* mRNA extracted from Towne (MOI of 1) infected HFFs at 24 (A), 48 (B), and 72 (C) hpi, which were first transfected with siRNA NC, 49-15, 49-11, or Lipo-only. The data are expressed as the mean ± SD from three independent experiments.

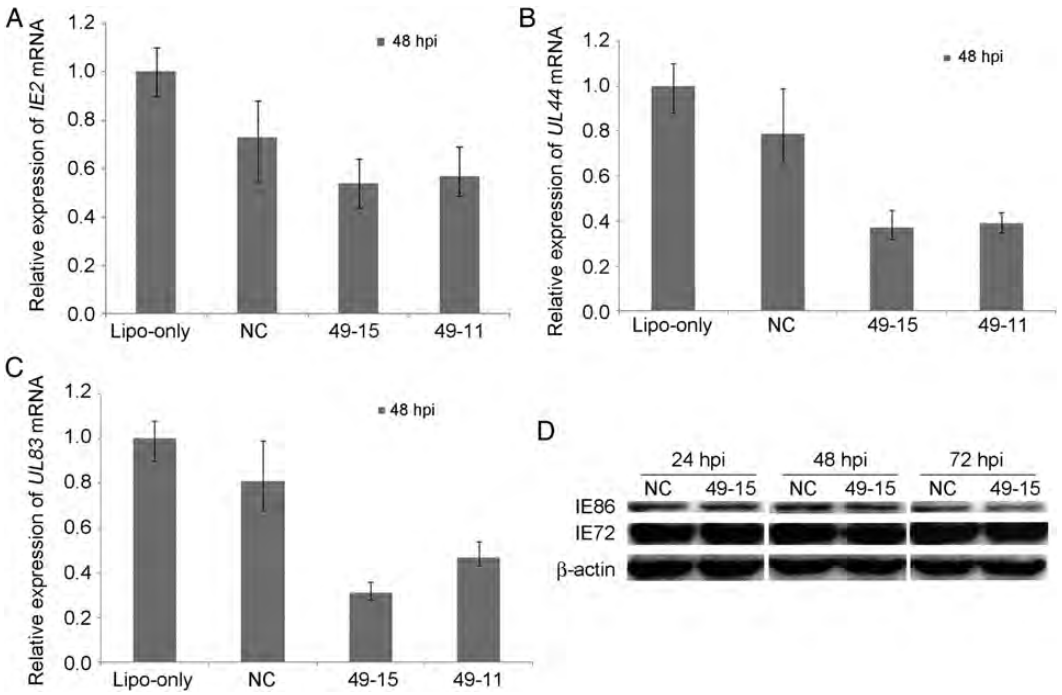

Figure 4 Expression of viral genes from infected siRNA-transfected HFFs Samples were treated as in **Fig. 3**. The cDNAs of *IE2* (A), *UL44* (B), and *UL83* (C) were screened by RT-PCR, respectively. The data are expressed as the mean ± SD from three independent experiments. (D) Lysates from NC- or 49-15-transfected HFFs infected with Towne (MOI of 1) were prepared at the indicated time points. Two IE proteins (IE72 and IE86) were detected by western blot analysis using antibodies recognizing IE72/86 or β-actin.

required in the HCMV replication cycle. Since the transcripts of all viral genes appeared at 48 hpi, the viral mRNAs were detected at this time point. With the knockdown of *UL49* mRNA, the content of E and L mRNAs was reduced, but the level of IE mRNAs changed only a little. It might due to the reduction of viral DNA content, as the viral DNA synthesis begins at 24 hpi.

The replication cycle of HCMV can start immediately at the G_0 phase of cells [28], and virus replication performance is highest when cells are infected in the state of quiescence [29]. To generate infecting cells in G_0, HFFs were first serum-starved for 72 h. To detect the content of viral DNA, cells were harvested at 72 hpi, because at this time the virus replication machinery has reached maximal activity [30]. Knockdown of UL49 with its special siRNAs caused a reduction of viral DNA content, indicating that UL49 plays a very important role during HCMV DNA replication. It also indicated that UL49 might interact with some viral DNA synthesis factors or host proteins to accomplish viral genome synthesis. Our observations also lead to the hypothesis that RNAi can be used as a potential therapy against HCMV.

Recently, we used yeast two-hybrid to screen out some viral proteins interacting with UL49 protein, two of which

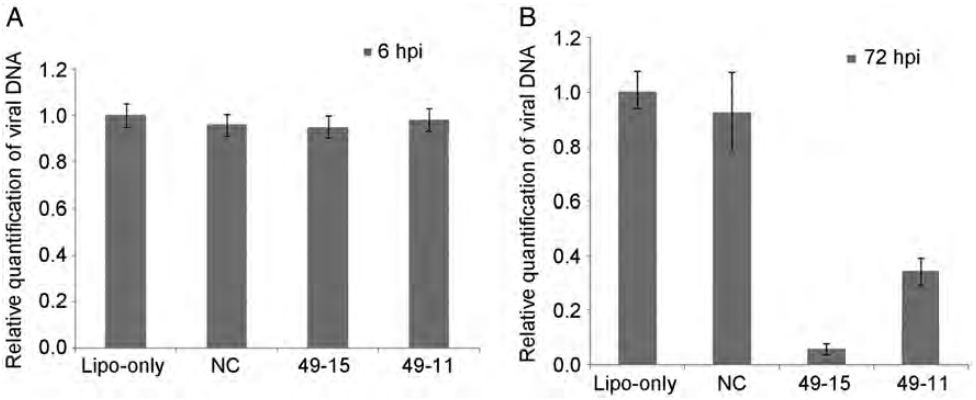

Figure 5 Viral DNA synthesis in HFFs cells transfected with siRNA Serum-starved HFFs were transfected with or without siRNA and then infected with Towne (MOI of 1). The cells were harvested at 6 hpi (A) and 72 hpi (B), respectively. Viral DNA synthesis was determined by qRT-PCR. Data are expressed as the mean ± SD.

are necessary for viral DNA replication (data not shown), suggesting that UL49 protein is required for DNA replication. Further investigations will be continued to elucidate the role of UL49 in viral DNA replication.

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References

1 Palella FJ, Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA and Aschman DJ, *et al*. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998, 338: 853–860.

2 Gallant JE, Moore RD, Richman DD, Keruly J and Chaisson RE. Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus disease treated with zidovudine. The Zidovudine Epidemiology Study Group. J Infect Dis 1992, 166: 1223–1227.

3 Demarchi JM. Human cytomegalovirus DNA: restriction enzyme cleavage maps and map locations for immediate-early, early, and late RNAs. Virology 1981, 114: 23–38.

4 McDonough SH and Spector DH. Transcription in human fibroblasts permissively infected by human cytomegalovirus strain AD169. Virology 1983, 125: 31–46.

5 Wathen MW, Thomsen DR and Stinski MF. Temporal regulation of human cytomegalovirus transcription at immediate early and early times after infection. J Virol 1981, 38: 446–459.

6 McVoy MA and Adler SP. Human cytomegalovirus DNA replicates after early circularization by concatemer formation, and inversion occurs within the concatemer. J Virol 1994, 68: 1040–1051.

7 Mocarski ES, Shenk T and Pass RT. Cytomegaloviruses. In: Knipe DM, Griffin DE, Lamb RA, Martin MA, Roizman B and Straus SE eds. Fields Virology. Vol. 2, 5th edn. Philadelphia: Lippincott Williams and Wilkins, 2007, 2701–2772.

8 Davison AJ, Dolan A, Akter P, Addison C, Dargan DJ, Alcendor DJ and McGeoch DJ, *et al*. The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. J Gen Virol 2003, 84: 17–28.

9 Murphy E, Yu D, Grimwood J, Schmutz J, Dickson M, Jarvis MA and Hahn G, *et al*. Coding potential of laboratory and clinical strains of human cytomegalovirus. Proc Natl Acad Sci USA 2003, 100: 14976–14981.

10 Qian Z, Xuan B, Hong TT and Yu D. The full-length protein encoded by human cytomegalovirus gene UL117 is required for the proper maturation of viral replication compartments. J Virol 2008, 82: 3452–3465.

11 Scherer LJ and Rossi JJ. Approaches for the sequence-specific knockdown of mRNA. Nature Biotechnol 2003, 21: 1457–1465.

12 Stein CA and Cheng YC. Antisense oligonucleotides as therapeutic agents—is the bullet really magical? Science 1993, 261: 1004–1012.

13 zu Putlitz J, Yu Q, Burke JM and Wands JR. Combinatorial screening and intracellular antiviral activity of hairpin ribozymes directed against hepatitis B virus. J Virol 1999, 73: 5381–5387.

14 Yu M, Ojwang J, Yamada O, Hampel A, Rapapport J, Looney D and Wong-Staal F. A hairpin ribozyme inhibits expression of diverse strains of human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 1993, 90: 6340–6344.

15 Sarver N, Cantin EM, Chang PS, Zaia JA, Ladne PA, Stephens DA and Rossi JJ. Ribozymes as potential anti-HIV-1 therapeutic agents. Science 1990, 247: 1222–1225.

16 Wiebusch L, Truss M and Hagemeier C. Inhibition of human cytomegalovirus replication by small interfering RNAs. J Gene Virol 2004, 85: 179–184.

17 Jacque JM, Triques K and Stevenson M. Modulation of HIV-1 replication by RNA interference. Nature 2002, 418: 435–438.

18 Zhang W, Li H, Li Y, Zeng Z, Li S, Zhang X, Zou Y and Zhou T. Effective inhibition of HCMV UL49 gene expression and viral replication by oligonucleotide external guide sequences and RNase P. Virol J 2010, 7: 100.

19 Lee HR, Kim DJ, Lee JM, Choi CY, Ahn BY, Hayward GS and Ahn JH. Ability of the human cytomegalovirus IE1 protein to modulate sumoylation of PML correlates with its functional activities in transcriptional regulation and infectivity in cultured fibroblast cells. Jo Virol 2004, 78: 6527–6542.

20 Saffert RT and Kalejta RF. Human cytomegalovirus gene expression is silenced by Daxx-mediated intrinsic immune defense in model latent infections established *in vitro*. J Virol 2007, 81: 9109–9120.

21 Strang BL, Boulant S and Coen DM. Nucleolin associates with the human cytomegalovirus DNA polymerase accessory subunit UL44 and is necessary for efficient viral replication. J Virol 2010, 84: 1771–1784.

22 Mercer J, Snijder B, Sacher R, Burkard C, Bleck CK, Stahlberg H and Pelkmans L, *et al*. RNAi screening reveals proteasome- and cullin3-dependent stages in vaccinia virus infection. Cell Rep 2012, 2: 1036–1047.

23 Dittmer A and Bogner E. Specific short hairpin RNA-mediated inhibition of viral DNA packaging of human cytomegalovirus. FEBS lett 2006, 580: 6132–6138.

24 Yu D, Silva MC and Shenk T. Functional map of human cytomegalovirus AD169 defined by global mutational analysis. Proc Natl Acad Sci USA 2003, 100: 12396–12401.
25 Dunn W, Chou C, Li H, Hai R, Patterson D, Stolc V and Zhu H, *et al*. Functional profiling of a human cytomegalovirus genome. Proc Natl Acad Sci USA 2003, 100: 14223–14228.
26 Hannon GJ. RNA interference. Nature 2002, 418: 244–251.
27 Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T and Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001, 293: 834–838.
28 Salvant BS, Fortunato EA and Spector DH. Cell cycle dysregulation by human cytomegalovirus: influence of the cell cycle phase at the time of infection and effects on cyclin transcription. J Virol 1998, 72: 3729–3741.
29 Noris E, Zannetti C, Demurtas A, Sinclair J, De Andrea M, Gariglio M and Landolfo S. Cell cycle arrest by human cytomegalovirus 86-kDa IE2 protein resembles premature senescence. J Virol 2002, 76: 12135–12148.
30 Wiebusch L and Hagemeier C. Human cytomegalovirus 86-kilodalton IE2 protein blocks cell cycle progression in G(1). J Virol 1999, 73: 9274–9283.