Down-regulation of human cytomegalovirus UL138, a novel latency-associated determinant, by hcmv-miR-UL36

Yujing Huang[2], Ying Qi[1], Yanping Ma[1], Rong He[2], Yaohua Ji[1], Zhengrong Sun[1] and Qiang Ruan[1,*]

[1]*Virus Laboratory, The Affiliated Shengjing Hospital,* [2]*Clinical Genetics Department, the Affiliated Shengjing Hospital, China Medical University, 110004 Shenyang, Liaoning, P.R. China*

**Corresponding author (Fax, +0086-24-23892617; Email, ruanq@sj-hospital.org)*

MicroRNAs (miRNAs) are small RNAs, 19–23 nucleotides in length, which regulate a variety of cellular processes. Human cytomegalovirus (HCMV) encodes only one intronic miRNA: human cytomegalovirus microRNA UL36 (hcmv-miR-UL36). In this study, we found that over-expression of hcmv-miR-UL36 resulted in a more than threefold increase in HCMV DNA synthesis at 24 h post infection. Fifteen putative targets of hcmv-miR-UL36 were identified using hybrid PCR, one being the HCMV UL138 gene that has previously been identified as a novel latency-associated determinant of HCMV infection. Down-regulation of UL138 expression by hcmv-miR-UL36 was validated using luciferase reporter assays and Western blot analysis in HEK293 cells. In the presence of hcmv-miR-UL36, we observed a 74.6% decrease in luciferase activity and a 46.2% decrease in HCMV UL138 protein expression. Our results indicate that hcmv-miR-UL36 may be a viral miRNA contributing to HCMV replication.

[Huang Y, Qi Y, Ma Y, He R, Ji Y, Sun Z and Ruan Q 2013 Down-regulation of human cytomegalovirus UL138, a novel latency-associated determinant, by hcmv-miR-UL36. *J. Biosci.* **38** 479–485] **DOI** 10.1007/s12038-013-9353-4

1. Introduction

Human cytomegalovirus (HCMV) is a ubiquitous beta-herpes virus known to infect a broad range of cell types within its human host, which contributes to its complex and varied pathogenesis. HCMV infection rate varies from 50% to 70% of the general population, dependent upon the socioeconomic status of the country, population age and geographic location (Ho 1990; Staras *et al.* 2006). Although a typical HCMV infection is asymptomatic, reactivation of the virus in immunosuppressed/immunocompromised individuals can result in serious disease (Khoshnevis 2002; Whitman *et al.* 2005; Crough and Khanna 2009). Identification of specific viral molecules that are required for the establishment and maintenance of HCMV latency is still under investigation.

MicroRNAs (miRNAs) are an abundant class of small non-coding RNA molecules that target messenger RNAs (mRNAs), generally within their 3′ untranslated region (3′UTR). They suppress target gene expression mainly through inhibition of translation or mRNA degradation (Ambros 2004; Filipowicz 2005). miRNAs act as key regulators in diverse biological pathways in cells, including early development, differentiation, proliferation, metabolism and apoptosis (Reinhart *et al.* 2000; Brennecke *et al.* 2003; Dostie *et al.* 2003; Xu *et al.* 2003). HCMV clinical isolates encode at least 22 miRNAs, present across the viral genome (Dunn *et al.* 2005; Pfeffer *et al.* 2005; Stark *et al.* 2012). Several HCMV-encoded miRNAs have been identified as playing an important role in the establishment and maintenance of viral latency and persistence. It has been reported that hcmv-miR-UL112-1 targets the immediate early transactivator IE72, which is essential for driving HCMV replication (Grey *et al.* 2007). Hcmv-miR-UL112-1 can also reduce the expression of UL114, which affects HCMV replication (Stern-Ginossar *et al.* 2009). Furthermore, both hcmv-miR-US25-1 and hcmv-miR-US25-2 inhibit HCMV DNA replication through the reduction of IE72 and pp65 expression (Grey *et al.* 2010).

Keywords. Down-regulation; HCMV; hcmv-miR-UL36; UL138

Published online: 12 July 2013

In our present study, ectopic expression of hcmv-miR-UL36 resulted in an increased level of HCMV DNA synthesis at 24 h post infection (HPI). Hybrid PCR (Huang *et al.* 2011) is a simple and effective method to screen putative target mRNAs of a known miRNA. It is carried out using a miRNA-specific primer containing the reverse and complementary sequence of the seed region of a given miRNA at the 3′ terminal. Putative target sequences can be acquired by the reliance on imperfect base pairing through a low annealing temperature (37°C) in an initial PCR. Fifteen putative targets of hcmv-miR-UL36 were obtained using this method. The HCMV UL138 gene, which has been demonstrated to be a novel latency-associated determinant of HCMV infection (Goodrum *et al.* 2007; Petrucelli *et al.* 2009), was identified as a putative target of hcmv-miR-UL36. Down-regulation of UL138 expression by hcmv-miR-UL36 was validated using luciferase reporter assays and Western blot analysis.

2. Materials and methods

2.1 *Cell culture*

We received the HEK293 cells from Dr. Fangjie Chen of the Department of Medical Genetics, China Medical University. HEK293 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 μg/mL penicillin, 100 μg/mL streptomycin sulfate and 2 mM L-glutamine. Human embryonic lung fibroblast cells, MRC-5 cells, were acquired from the Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. MRC-5 cells were maintained in Modified Eagle's Medium (MEM) supplemented with 10% FBS, 100 μg/mL penicillin and 100 μg/mL streptomycin sulfates. All cell cultures were maintained at 37°C in a 5% CO_2 incubator.

2.2 *Virus preparation*

HCMV clinical strain H was isolated from a urine sample of a 5-month-old infant hospitalized in Shengjing Hospital, China Medical University. Stock virus was propagated in MRC-5 cells maintained in MEM supplemented with 2% FBS, 100 μg/mL penicillin and 100 μg/mL streptomycin. The supernatant was harvested, with aliquots stored at −80°C prior to use.

2.3 *Plasmid construction*

Plasmid pSilencer 4.1 (Ambion, Beijing, China) was used to construct the hcmv-miR-UL36 expression vector. A fragment predicted to express hcmv-miR-UL36 was amplified directly from the genome of H strain using both sense (5′-CGCGGATCCGCTCCGTTCGCGCAGCGCCCT-3′) and anti-sense (5′-CCCAAGCTTGGACCCTCGACGAAGC CAAAGG-3′) primers. It was then cloned into pSilencer 4.1 at the *BamHI- HindIII* sites to construct pSilencer-miR-UL36. The 3′UTR sequence of HCMV UL138 was amplified from mRNA-derived cDNA using both sense (5′-GGACT AGTCGAGCCCAAGCGTATCTGCAGCA-3′) and anti-sense (5′-CCCAAGCTTGCCACCGTTTTCTAACACC GATCAC-3′) primers. The product was digested by *SpeI* and *HindIII* and inserted into the multiple cloning regions of a luciferase reporter vector pMIR (Ambion, Beijing, China) to construct pMIR-UL138UTR, The sequence containing the UL138 open reading frame (ORF) and 3′UTR was also amplified from mRNA-derived cDNA using both sense (5′-CGCGGATCCATGGCATCAATGCAGAAGCTGATCTC AGAGGAGGACCTGGACGATCTGCCGCTGAACGTC G-3′) and anti-sense (5′-CCCAAGCTTGCCACCGTTTT CTAACACCGATCAC-3′) primers. An in-frame, N-terminal Myc epitope tag sequence was introduced into the PCR product. The PCR product was inserted into the pBI-CMV2 vector (clontech, Beijing, China) to construct pBI-MycUL138WS, which constitutively expressed the protein of interest as well as a green fluorescent protein AcGFP. Negative controls (pMIR-UL138MUT and pBI-MycUL138MUT) were constructed by deleting a 31 base pair (bp) fragment containing the hcmv-miR-UL36 putative binding site from the 3′UTR of UL138. All constructs were confirmed by DNA sequencing.

2.4 *Quantification of viral DNA*

MRC-5 cells were plated in 60 mm plates and transfected with pSilencer-miR-UL36 or pSilencer-neg (Ambion, Beijing, China) which expressed a random small RNA, using Lipofectamine 2000 (Lipo2000) (Invitrogen, Shanghai, China). The transfected MRC-5 cells were inoculated with H strain at 0.5 multiplicity of infection (MOI) 24 h later. Total DNA was extracted at 24, 48, 72 and 96 HPI, respectively. HCMV DNA copies were then determined by a real-time PCR assay using a commercial kit (Daan gene, Guangzhou, China). All measurements were done in triplicate and results were presented as mean±SD.

2.5 *RNA isolation*

MRC-5 cells were inoculated with H strain at 3–5 MOI. For preparation of RNA in the early stage of HCMV infection, 100 μL/mL of DNA synthesis inhibitor phosphonoacetic acid (PAA) (Sigma, Beijing, China) was immediately added to the medium post infection. Total RNA was isolated from approximately 10^7 infected cells harvested at 48 HPI using Trizol agent (Takara, Dalian, China), according to the manufacturer's protocol. Total RNA was dissolved in 200 μL RNase-free H_2O and treated by TURBO DNA-free™ kit (Ambion,

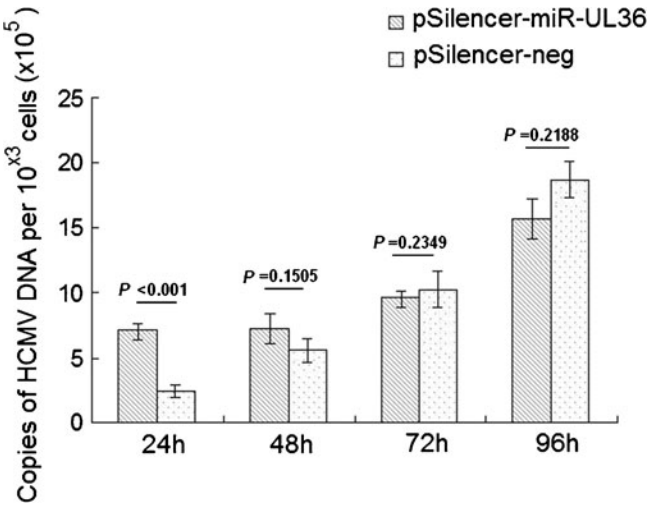


Figure 1. Alternative levels of HCMV DNA synthesis in MRC-5 with ectopic expression of hcmv-miR-UL36.

Beijing, China). RNA integrity was analyzed by gel electrophoresis using a 1% agarose gel alongside an RNA marker.

2.6 *Hybrid PCR*

Hybrid PCR was performed as previously described (Huang *et al.* 2011) using 3′-Full RACE Core Set (Takara, Dalian, China). The hybrid primer for hcmv-miR-UL36 (5′-TCTTTCCRGGT GTCTTCRRCG-3′) was designed according to its sequence. The presence of an 'R' in the hcmv-miR-UL36 hybrid primer indicates random insertions of adenines (A) or guanines (G). All PCR products were harvested using QIAEX ® II Gel Extraction kit (Qiagen, Beijing, China) and cloned into pMD-19T vectors (Takara, Dalian, China). The recombinant products were then transformed into *E. coli* to produce a pool which should contain specific sequences of putative target mRNAs that hcmv-miR-UL36 can bind to. Fifty-seven clones were randomly selected. Corresponding products inserted into the plasmids were sequenced on an ABI 3730 automated sequencer.

Table 1. Putative target mRNAs of hcmv-miR-UL36 identified by hybrid-PCR

Putative target mRNA	Accession number
Homo sapiens solute carrier family 25, member 6 (SLC25A6)	NM_001636.3
Homo sapiens guanine nucleotide binding protein (GNAI2)	NM_002070.2
Homo sapiens G protein-coupled receptor 37 (GPR37)	NM_005302.2
Homo sapiens tubulin folding cofactor A (TBCA)	NM_004607.2
Homo sapiens nucleolin (NCL)	NM_005381.2
Homo sapiens LSM12 homolog (LSM12)	NM_152344.3
Homo sapiens ribosomal protein L27 (RPL27)	NM_000988.3
Homo sapiens ribosomal protein S15	BC141832.1
Homo sapiens translation initiation factor 4e	AF038957.1
Homo sapiens cofactor A protein	AF038952.1
Homo sapiens putative splice factor transformer2-beta	U61267.1
HCMV US34A	GQ466044.1
HCMV UL84	GQ466044.1
HCMV UL138	GQ466044.1
HCMV UL145	GQ466044.1

2.7 *Sequence BLAST and downstream analysis*

Complete extremity of mRNA was confirmed when the hybrid primer and polyA structure were found in the sequence. To identify the original genes of hcmv-miR-UL36 putative targets, mRNA-specific sequences located between the corresponding sequence of the hcmv-miR-UL36 hybrid primer and polyA structure were intercepted and run through the online Basic Local Alignment Search Tool (BLAST) (*http://www.ncbi.nlm.nih.gov/blast*).

2.8 *Luciferase reporter assays*

Assays were conducted in 24-well plates, with HEK293 cells plated at a density of 4.0×10^5 cells per well and grown to 85% confluence at the time of transfection. 0.2 μg pMIR-UL138UTR, pMIR-UL138MUT or empty vector pMIR was co-transfected along with 0.4 μg pSilencer-miR-UL36 and 0.2 μg control renilla plasmid pRL-TK (Promega, Beijing, China) using Lipo2000 (Invitrogen, Beijing, China), according to the manufacturer's recommendations. Plasmid pSilencer-neg (Ambion, Beijing, China) was transfected as a miRNA negative control. The cells were collected at 48 HPI, with luciferase activity measured using the dual luciferase reporter assay system (Promega, Beijing, China), according to the manufacturer's guidelines. All measurements were done in triplicate and signals were normalized for transfection efficiency against the internal renilla control. The results were presented as the mean±SD.

2.9 *Western blot analysis*

For Western blot analysis, HEK293 cells were plated in 60 mm plates. Cells were co-transfected using Lipo2000, with 5 μg pSilencer-miR-UL36 or pSilencer-neg control vector, and 3 μg pBI-MycUL138WS or pBI-MycUL138MUT, respectively. The cells were collected at 48 HPI. In order to obtain protein extracts, cells were prepared by suspension in lysis buffer, followed by centrifugation. Protein concentration in the supernatant was

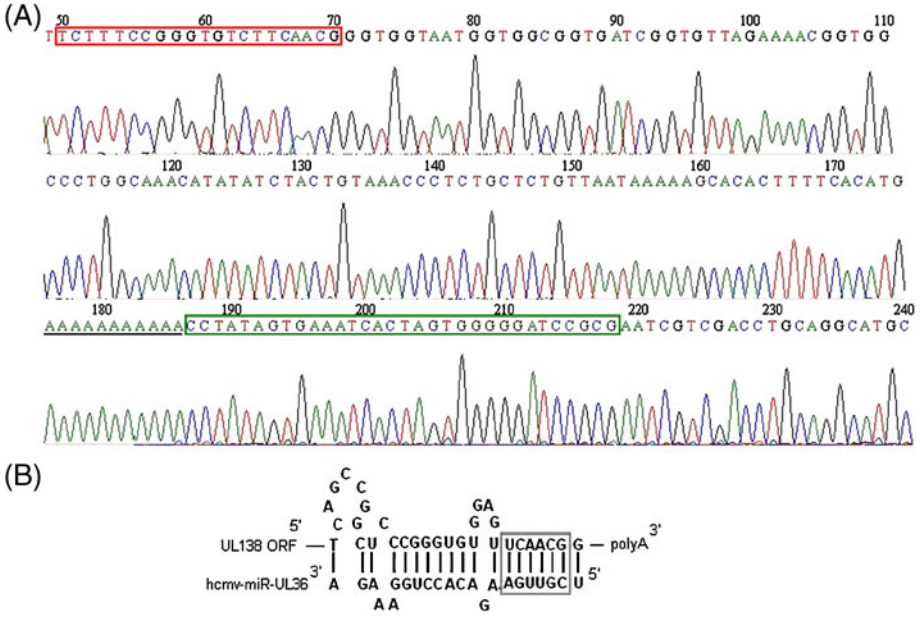


Figure 2. Identification of HCMV UL138 as a putative target of hcmv-miR-UL36. (**A**) Partial chromatogram of the clone, which was identified as containing a HCMV UL138-specific sequence. The sequence of the hcmv-miR-UL36 hybrid primer is indicated in the red box, and the binding site of the adaptor primer is indicated in the green box. The polyA sequence is underlined in black. (**B**) Diagram shows the predicted sequences of hcmv-miR-UL36 binding to HCMV-UL138 mRNA. The seed region of hcmv-miR-UL36 is indicated by a grey box surrounding nucleotides 2–7 of the miRNA target.

quantified using a protein assay kit (Beyotime, Jiangsu, China). The extracted proteins were then separated in a 10% acrylamide gel and transferred onto a nitrocellulose membrane. Western blot analysis was performed using Myc-, β-actin- and AcGFP-specific antibodies (Clontech, Beijing, China). Immunoblots were visualized via an electrochemiluminescence (ECL) detection system. Sample loading was normalized using β-actin and AcGFP strength was used to monitor the transfection efficiency.

2.10 *Statistical analysis*

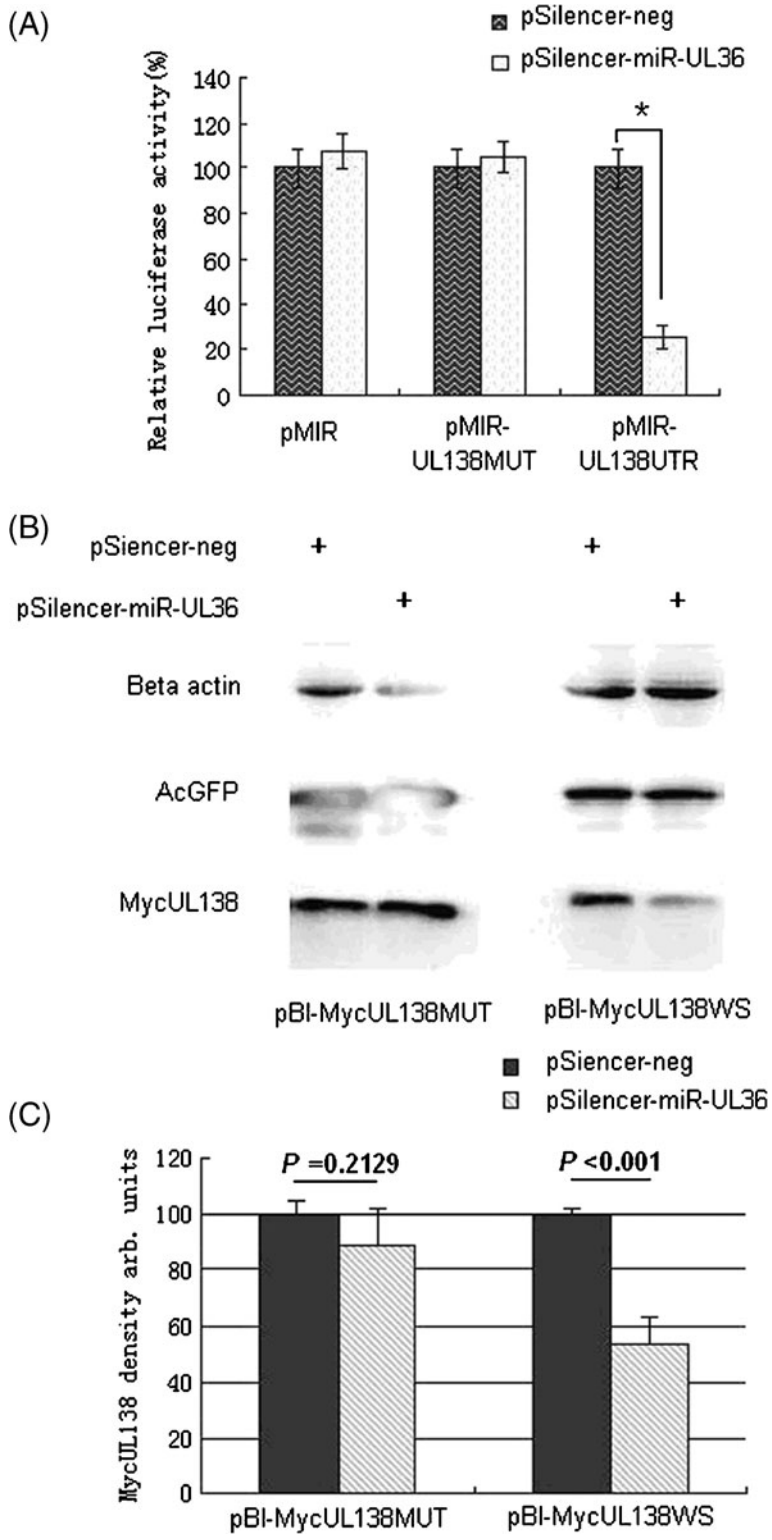
All experiments were shown to be reproducible. The results are presented as the mean±SD A Student's *t*-test was used to determine statistical significance with a cut-off value of $P \leq 0.05$.

3. Results

3.1 *Ectopic expression of hcmv-miR-UL36 resulted in alteration of HCMV DNA synthesis*

MRC-5 cells transfected with pSilencer-miR-UL36 or pSilencer-neg were infected with H strain. The prevalence of viral DNA copies post-HCMV infection was measured by real-time PCR at varying time points. The copy numbers of viral DNA in cells transfected with pSilencer-miR-UL36 at 24 HPI were more than threefold higher than those in cells transfected with pSilencer-neg (P=0.002) (figure 1). No significant difference was observed between cells transfected with pSilencer-miR-UL36 and those transfected with pSilencer-neg after 48 HPI.

Figure 3. Down-regulation of HCMV UL138 protein by hcmv-miR-UL36. (**A**) As a putative target, HCMV UL138 3"UTR was validated for its ability to inhibit expression of a luciferase reporter construct in the presence of hcmv-miR-UL36 (pSilencer-miR-UL36). Results were shown as percentage expression of the negative control sample (pSilencer-neg) following correction for transfection levels according to the control of renilla luciferase expression. Values are mean±standard deviation for triplicate samples. P<0.05. (**B**) HEK 293 cells were co-transfected with pSilencer-miRUL36 or a control vector, and pBI-MycUL138WS or pBI-MycUL138MUT, respectively. Cells were collected 48 HPI and subjected to Western blot analysis using the indicated antibodies. (**C**) The amounts of each protein presented in figure 3B were quantified by densitometry. Results are shown as percentage expression of the negative control sample (pSilencer-neg).


(A)
pSilencer-neg
pSilencer-miR-UL36
Relative luciferase activity(%)
140
120
100
80
60
40
20
0
*
pMIR
pMIR-UL138MUT
pMIR-UL138UTR
(B)
pSiencer-neg
pSilencer-miR-UL36
Beta actin
AcGFP
MycUL138
pBI-MycUL138MUT
pBI-MycUL138WS
(C)
pSiencer-neg
pSilencer-miR-UL36
MycUL138 density arb. units
P =0.2129
P <0.001
120
100
80
60
40
20
0
pBI-MycUL138MUT
pBI-MycUL138WS

3.2 *Putative targets of hcmv-miR-UL36 were identified by hybrid PCR*

Fifteen unique sequences were obtained from the 57 sequenced clones. Corresponding original mRNAs were successfully identified in GenBank (*http://www.ncbi.nlm.nih.gov/GenBank*). Detailed information regarding the identified putative target mRNAs is presented in table 1. Four putative target mRNAs, including HCMV UL138 mRNA, were from the HCMV genome. The remaining putative target mRNAs were from the human genome.

A sequence in the 3′UTR of HCMV UL138 mRNA was identified as a candidate target site for hcmv-miR-UL36 (figure 2A). The predicted binding site for hcmv-miR-UL36 was located 105 nucleotides (nt) upstream of the polyA structure of HCMV UL138 mRNA. A schematic representation of hcmv-miR-UL36 binding to the 3′UTR sequence of HCMV UL138 is shown in figure 2B.

3.3 *Expression of HCMV UL138 protein was functionally down-regulated by hcmv-miR-UL36*

To further determine whether the 3′UTR sequence of HCMV UL138 represents a functional target site for hcmv-miR-UL36, the 3′UTR sequence and its deletion mutant at the predicted binding site were validated by luciferase reporter assays. As shown in figure 3A, a significant inhibition of luciferase activity (74.6%) was observed in cells transfected with pMIR-UL138UTR in the presence of pSilencer-miR-UL36, compared to the control cells (P=0.014). Hcmv-miR-UL36 had no significant inhibitory effects on the luciferase activities of pMIR and pMIR-UL138MUT. These results demonstrate that the 3′UTR sequence of HCMV UL138 is a specific and functional binding site for hcmv-miR-UL36.

The regulatory effect of hcmv-miR-UL36 on HCMV UL138 protein expression was then examined using Western blot analysis. pBI-MycUL138WS or pBI-MycUL138MUT was co-transfected into HEK293 cells with pSilencer-miR-UL36 or pSilencer-neg. The Myc intensity was measured to determine the protein expression level of HCMV UL138. HCMV UL138 protein expression level was significantly reduced in cells co-transfected with pSilencer-miR-UL36 and pBI-MycUL138WS in comparison to those in controls (P=0.004) (figure 3B). HCMV UL138 protein densitometer values were normalized to that of AcGFP expression. Hcmv-miR-UL36 caused an approximately 46.2% decrease in the protein level of HCMV UL138, as determined by densitometry (figure 3C). Our observations confirm that the expression of HCMV UL138 protein could be specifically inhibited by ectopically expressed hcmv-miR-UL36.

4. Discussion

The only HCMV intronic miRNA, hcmv-miR-UL36, is encoded by the intron of an anti-apoptosis gene UL36 (Grey *et al.* 2005; Pfeffer *et al.* 2005). With ectopic expression of hcmv-miR-UL36, we detected viral DNA levels at different time points following HCMV infection. The copy numbers of viral DNA at 24 HPI in cells transfected with pSilencer-miR-UL36 was more than three times that in cells transfected with pSilencer-neg. This result demonstrates that hcmv-miR-UL36 may be a newly described HCMV-encoded miRNA affecting HCMV replication.

It has been previously speculated that hcmv-miR-UL36 may regulate the viral DNA synthesis by binding to target genes involved in HCMV replication. We successfully identified 15 putative hcmv-miR-UL36 targets using hybrid PCR, from either the human or HCMV genomes. Among them, two genes (HCMV UL84 and UL138) have been reported to be involved in viral DNA replication (Gebert *et al.* 1997; Xu *et al.* 2004; Goodrum *et al.* 2007; Petrucelli *et al.* 2009). HCMV UL138 is a 510 nt gene within the UL/b′ region of the genome, which is unique to clinical or low-passage strains (Cha *et al.* 1996; Dolan *et al.* 2004). The UL138 protein is a type I membrane protein that localizes to the Golgi apparatus (Goodrum *et al.* 2007; Petrucelli *et al.* 2009) and was reported as the first virus-encoded protein to promote HCMV latency. Recombinant viruses lacking the UL138 gene replicated productively in hematopoietic progenitor cells (HPCs) infected *in vitro* (Goodrum *et al.* 2007). In the present study, a 74.6% decrease in the luciferase activity of pMIR-UL138UTR and a 46.2% decrease of HCMV UL138 protein expression were observed in the presence of hcmv-miR-UL36. We confirmed that hcmv-miR-UL36 could specifically repress HCMV UL138 expression through the predicted binding site in the 3′UTR of UL138. Hcmv-miR-UL36 expression has previously been detected firstly at 24 HPI with immediate early kinetics, and subsequently accumulating over time (Grey *et al.* 2005; Pfeffer *et al.* 2005). The functional relevance of UL138 regulation by hcmv-miR-UL36 is currently unclear, but it may be involved in establishing a balance between the lytic replication and latency of HCMV.

Our study identified that over-expression of hcmv-miR-UL36 resulted in an increase of HCMV DNA synthesis at 24 HPI. No significant difference was observed between cells transfected with pSilencer-miR-UL36 and cells transfected with pSilencer-neg after 48 HPI. However, it cannot be excluded that this observation may be due to the exhaustion of transfected hcmv-miR-UL36 or the activation of another pathway that also affects the viral DNA replication. This, therefore, means that our results from transfected cells are limited in showing the effects of hcmv-miR-UL36. Much more validation

is required via further studies, including the instruction of HCMV mutant bacterial artificial chromosome (BAC) clones.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81171580 and 81201274), the Specialized Research Fund for the Doctoral Program of Higher Education (20112104110012) and the Outstanding Scientific Fund of Shengjing Hospital.

References

Ambros V 2004 The functions of animal microRNAs. *Nature* **431** 350–355

Brennecke J, Hipfner DR, Stark A, Russel RB and Cohen SM 2003 Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell* **113** 25–36

Cha TA, Tom E, Kemble GW, Duke GM, Mocarski ES and Spaete RR 1996 Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. *J. Virol.* **70** 78–83

Crough T and Khanna R 2009 Immunobiology of human cytomegalovirus: from bench to bedside. *Clin. Microbiol. Rev.* **22** 76–98

Dolan A, Cunningham C and Hector RD 2004 Genetic content of wild-type human cytomegalovirus. *J. Gen. Virol.* **85** 1301–1312

Dostie J, Mourelatos Z, Yang M, Sharma A and Dreyfuss G 2003 Numerous microRNPs in neuronal cells containing novel microRNAs. *RNA* **9** 180–186

Dunn W, Trang P, Zhong Q, Yang E, van Belle C and Liu F 2005 Human cytomegalovirus expresses novel microRNAs during productive viral infection. *Cell. Microbiol.* **7** 1684–1695

Gebert S, Schmolke S, Sorg G, Flöss S, Plachter B and Stamminger T 1997 The UL84 protein of human cytomegalovirus acts as a transdominant inhibitor of immediate-early-mediated transactivation that is able to prevent viral replication. *J. Virol.* **71** 7048–7060

Goodrum F, Reeves M, Sinclair J, High K and Shenk T 2007 Human cytomegalovirus sequences expressed in latently infected individuals promote a latent infection in vitro. *Blood* **110** 937–945

Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC and Nelson J 2005 Identification and characterization of human cytomegalovirus-encoded microRNAs. *J. Virol.* **79** 12095–12099

Grey F, Meyers H, White EA, Spector DH and Nelson J 2007 A human cytomegalovirus-encoded microRNA regulates expression of multiple viral genes involved in replication. *PLoS Pathog.* **3** e163

Grey F, Tirabassi R, Meyers H, Wu G, McWeeney S, Hook L and Nelson JA 2010 A viral microRNA down-regulates multiple cell cycle genes through mRNA 5'UTRs. *PLoS Pathog.* **6** e1000967

Huang Y, Qi Y, Ruan Q, Ma Y, He R, Ji Y and Sun Z 2011 A rapid method to screen putative mRNA targets of any known microRNA. *Virol. J.* **8** 8

Filipowicz W 2005 RNAi: the nuts and bolts of the RISC machine. *Cell* **122** 17–20

Ho M 1990 Epidemiology of cytomegalovirus infections. *Rev. Infect. Dis.* **12** S701–S710

Khoshnevis M 2002 Cytomegalovirus infections. *Dermatol. Clin.* **20** 291–299

Petrucelli A, Rak M, Grainger L and Goodrum F 2009 Characterization of a novel golgi apparatus-localized latency determinant encoded by human cytomegalovirus. *J. Virol.* **83** 5615–5629

Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grässer FA, van Dyk LF, Ho CK, Shuman S, Chien M, Russo JJ, Ju J, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zavolan M and Tuschl T 2005 Identification of microRNAs of the herpesvirus family. *Nat. Methods* **2** 269–276

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR and Ruvkun G 2000 The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* **403** 901–906

Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF and Cannon MJ 2006 Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. *Clin. Infect. Dis.* **43** 1143–1151

Stark TJ, Arnold JD, Spector DH and Yeo GW 2012 High-resolution profiling and analysis of viral and host small RNAs during human cytomegalovirus infection. *J. Virol.* **86** 226–235

Stern-Ginossar N, Saleh N, Goldberg MD, Prichard M, Wolf DG and Mandelboim O 2009 Analysis of human cytomegalovirus-encoded microRNA activity during infection. *J. Virol.* **83** 10684–10693

Whitman AG, Bryan BA, Dyson OF, Patel DK, Ramasamy D, Anantharaman S, Reber AJ and Akula SM 2005 AIDS related viruses, their association with leukemia, and Raf signaling. *Curr. HIV Res.* **3** 319–327

Xu P, Vernooy SY, Guo M and Hay BA 2003 The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. *Curr. Biol.* **13** 790–795

Xu Y, Cei SA, Huete AR and Pari GS 2004 Human cytomegalovirus UL84 insertion mutant defective for viral DNA synthesis and growth. *J Virol.* **78** 10360–10369

MS received 22 March 2013; accepted 28 June 2013

Corresponding editor: SHAHID JAMEEL