Journal of Advances in Virology Research
Volume 3 | Issue 1

Research Article Open Access

A Novel Immune Response Associated Human Cytomegalovirus Microrna, Mir-US33-5p, Down-Regulates HCMV UL16

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Citation: Xiaochen Chang, Xinyi Wang, Yaozhong Shao, Xin Guo (2023) A novel immune response associated human cytomegalovirus microRNA, miR-US33-5p, down-regulates HCMV UL16. J Adv Virol Res 3(1): 301

Received Date: March 18, 2023 **Accepted Date:** May 17, 2023 **Published Date:** May 22, 2023

Abstract

Human cytomegalovirus (HCMV) is the only beta herpesvirus, which can encode microRNA (miRNA). HCMV utilizes miRNAs to regulate its own genes as well as the host genes during infection to achieve immune evasion, regulation of cellular processes and viral DNA replication. As one of the 26 HCMV miRNAs, HCMV miR-US33-5p has been reported to inhibit viral DNA syntheses and DNA replication via down regulation of a host gene Syntaxin 3. Here, we tested the luciferase activity of other 8 putative target mRNAs which were identified by Hybrid PCR, 7 of them showed decrease following over expression of HCMV miRNA-US333-5p. A viral gene HCMV UL16 was confirmed as a direct target of miR-US33-5p since both luciferase activity and protein level were decreased by miR-US33-5p over expression. HCMV UL16 plays a critical role in NK cell evasion by sequestrating ULBP1, 2, 6 and MICB in the endoplasmic reticulum (ER) and reducing their cell surface expression. Furthermore, HCMV miR-US33-5p overexpression induced ULBP1, 2 and MICB independent of other HCMV genes. Thus, we illustrated two ways for HCMV miR-US33-5p up regulates these NKG2D ligands expression, NK cell could be activated by reporter NKG2D binding to them.

Keywords: Human cytomegalovirus, HCMV miR-US33-5p, HCMV UL16, down regulation, NKG2D ligand.

Introduction

Human Cytomegalovirus (HCMV) also known as Human Herpesvirus 5 (HHV-5) is widespread throughout the world [1]. HCMV infection normally controllable by the immune system, persists as lifelong infection, however it can cause significant morbidity and mortality in newborns [2], as well as in transplant recipients and patients with AIDS [3–4].

As the only beta herpesvirus that could encode microRNA (miR), HCMV encodes at least 26 mature miRNAs from 16 precursors which are scattered along the 230 kb viral genome [5]. HCMV utilizes these miRNAs to regulate its own genes as well as the host cell genes during infection to achieve regulation of cellular processes, viral DNA replication or other biologic processes.

HCMV miR-US33 is encoded by sequence complementary to HCMV US29 gene [6], and was shown to promote apoptosis of aortic vascular smooth muscle cells by targeting EPAS1/SLC3A2 pathway [7]. It has been reported that HCMV miR-US33 inhibits the lytic HCMV replication and promote HCMV latent infection [8]. Our previous study illustrated that HCMV miR-US33-5p inhibits viral DNA synthesis and viral replication by down-regulating expression of the host Syntaxin3[9]. HCMV miR-US33-5p does not express during HCMV latency, however it is highly expressed after virus reactivation from latency and during productive infection [10].

We found that HCMV UL16 gene is one of putative targets of HCMV miR-US33-5p by Hybrid-PCR method [9]. In the present study, we confirmed that both mRNA and protein level of HCMV UL16 were down regulated by HCMV miR-US33-5p. HCMV UL16 encode a glycoprotein, which is not necessary for viral growth in vitro [11]. The UL16 glycoprotein reduces cell surface expression of NKG2D ligands ULBP-1, ULBP-2, and MICB, resulting in increased resistance to NK cell cytotoxicity [12-16]. This is one of strategies which developed by HCMV to evade innate and adaptive immune responses, and protected HCMV-infected cells from NK cell attack.

Here, we present evidence that HCMV UL16 is a direct target of HCMV miR-US33-5p, and our results provide insight into the mechanisms involved, and support a role for HCMV miR-US33-5p as an HCMV-encoded immune response associated molecular modulator.

Materials and Methods

Cell Culture and Virus Preparation

Human embryonic kidney cells (HEK 293) were maintained in Dulbecco′s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units/ml penicillin and streptomycin. HELF cells were maintained in minimal essential medium (MEM) supplemented with 10% FBS, 100 units/mL penicillin and streptomycin.

Bacterial artificial chromosome of a characterized HCMV clinical low-passage isolate HAN (BAC HAN) has been constructed and labeled with green fluorescent protein (GFP)[17]. BAC HAN stock was prepared in HELF cells and the virus titer was determined by standard median tissue culture infective dose (TCID50) assays.

RNA Isolation

Total RNAs was isolated from cells using RNeasy mini-Kit (Qiagen) according to the manufacturer′s protocol. The quality and purity of the RNA preparations were estimated by electrophoresis on 1% agarose gel and the quantities were detected by ND-1000 spectrophotometer (Nanodrop Technologies).

Plasmid Construction

The 3′-untranslated region (3′-UTR) of putative targets were amplified from HCMV infected (72-hour post infection) HELF mRNA derived cDNA by specific primers. After purification and digestion, the fragments were cloned into pMIR-REPORT firefly luciferase (FFL) vector (Promega).

Table 1: Primers for amplification of cDNAs synthesized from 3′- UTRs of putative targets′ mRNA

OTU5 - sense	GGACTAGTCCGTCCTTCTAATTTGGC
OTU5 - antisense	CCCAAGCTTGTTTGCTCCTCCTCTTCG
Extl3 -sense	GGACTAGTTTTGCTTCCACCACCACT
Extl3 - antisense	CCCAAGCTTTCAGGGAAACTGACA
STX3 - sense	GGACTAGTCTGGCAGTCTCCTTGATT
STX3 - antisense	CCCAAGCTTGGCCTTTAGTTATTTTGTCT
AK3 - sense	GGACTAGTTGTATGGAGCTGAATGCC
AK3 - antisense	CCCAAGCTTACCGTGTTAGCCAGGATG
H1FX - sense	GGACTAGTCCCCACCCTCCTTGCCTTTG
H1FX - antisense	CCCAAGCTTGAAAGGTTTAAACGCTT
TIMP2 - sense	GGACTAGTGCTGGAATATGAAGTCTGA
TIMP2 - antisense	CCCAAGCTTGATAATACTGTT
Polymeraseⅱ - sense	GGACTAGTGATGGGAGCTATGAAGAC
Polymeraseⅱ- antisense	CCCAAGCTTACATATAAAGTGAGAATG
UL16 - sense	GGACTAGTAAGGATGGGATCTTTCTGG
UL16 - antisense	AGCTTTGTTTAAACTCCAATGTAAGAC

A mutant vector, pMIR-UL16-MUT, containing three-point mutations of the putative binding site of HCMV miR-US33-5p was generated from the pMIR-UL16-UTR using pyrobest DNA polymerase (TaKaRa) and a mutant primer of 5′ CAGAGCCACTGATGTCGGATCCGGCAGCGACTCCCGAAGA-3′ (Characters underlined are mutant nucleotides) according to the protocol of site-directed Gene Mutagenesis Kit (Beyotime). Inserts in all the constructs were sequenced on an ABI 3730 automated sequencer.

Dual-Luciferase Reporter Assays

To assess binding ability of HCMV miR-US33-5p to putative targets′ 3′-UTR, HEK293 cells were cultured in 24-well plates. Cells were co-transfected with 100 nM miRNA negative control (RiboBio), which has the lowest homology with any other known miRNAs, or HCMV miR-US33-5p mimics (RiboBio) together with 100 ng pRL-TK-Renilla-luciferase plasmid (Promega), respectively with 200 ng pMIR-REPORT vector, or constructed putative targets′ 3′-UTR using Lipofectamine 2000 (Lipo2000, Invitrogen) in triplicate wells.

Luciferase activities were measured using Dual Luciferase Reporter Assay System (Promega) at 48-hour post transfection according to the manufacturer′s instructions and the transfection efficiencies were normalized by the renilla luciferase activities in corresponding wells.

Western Blot

To evaluate if protein level of HCMV UL16 is down regulate by HCMV miR-US33, since there is no specific antibody of HCMV UL16, whole HCMV UL16 gene which was tagged by c-Myc had been cloned into Mlu I and Sal I sites of pBI-CMV2 Vector by using sense primer 5′-CGACGCGTATGGAGGAGCAGAAG CTGATCTCAGAGGAGGACCTG(c-Myc) GAGCGTCGCCGAGGTATGGTA-3′ and antisense primer 5′-ACGCGTCGACTCATCATGGCGTTTTATTAACAGTT-3′.

To analyze whether HCMV UL16 protein expression is affected by HCMV miR-US33-5p, HEK 293 cells cultured in 60 mm tissue culture plates were transfected with pBI-CMV2 Vector or pBI-c-Myc-UL16 respectively with miRNA negative control and HCMV miR-US33-5p mimics. Proteins were separated on 12% SDS-PAGE gels and transferred onto polyvinylidene fluoride (PVDF) membranes. The protein levels of c-Myc-UL16 and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were detected using specific antibodies of anti-cMyc (Santa Cruz) and anti-GAPDH (Santa Cruz), respectively. The expressions of HCMV miR-US33-5p in the HEK 293 cells was detected by quantitative real-time PCR as described above.

Construction of HCMV UL16 Deleted Mutant

UL16 deletion mutant was constructed from BAC HAN by homologous recombination. A fragment encoding kanamycin resistance gene was amplified from the previously described plasmid pGEM-oriV/Kan using forward and reverse UL16 Del primers (sense : 5′-TCCATGCTGACGTAGGTACCGACTGGGGTCAA AAGCCTGGGTACTTATGACTGTCTCTTATACACATCTCAACCATC-3′

Antisense: 5′-ACATGGCCTTCTTATAGCAGCGTGAACGTTGCACGTGGCCTTT GCGGTTACTGTCTCTTATACACATCTCAACCCTG-3′; sequence homologous to UL16 flanking regions was underlined). Purified PCR production was electro-transferred (electroporated?) into competent bacteria DY380 that contained BAC HAN construct. Kanamycin-resistant? colonies were picked and the deletion of HCMV UL16 was approved by PCR and sequencing directly.

BAC HAN ΔUL16 plasmid was extracted from the identified bacteria DY380 colony using Nucleo BondXtra Midi Kit (Macherey--Nagel) following the manufacturer′s protocol and was transfected into HELF cells by electroporation. The cells were then maintained under standard cell culture conditions until cytopathic effects (CPEs) and GFP signal were clearly apparent. The reconstituted virus was named as HCMV ΔUL16, and the virus stock was prepared as described previously (reference).

Viral Dna QUANTIFICATION

HELF cells were transfected with miRNA negative control, HCMV miR-US33-5p mimics, inhibitor for hcmv-miR-US33-5p or negative control. Six hours later, the cells were infected with HCMV BAC HAN or HCMV ΔUL16 at MOI of 1 and harvested at indicated time using phosphate buffered saline (PBS). DNA was extracted from the infected cells using General Allgen Kit (Cwbio). Quantitative PCR was performed by using CMV DNA detection kit (Fluorescence Quantitative PCR, ZJ Bio-Tech) kit on a ABI prism 7300 (Applied Biosystems).

Quantitative Real-Time PCR

To evaluate NKG2D ligands expression, cells were grown in 6 well plates. Total RNAs were extracted from the cultured, post transfected or infection HEK 293 cells and HELF cells. Reverse transcription (RT) was performed using PrimeScriptTM RT Master Mix (Takara) and HCMV miR-US33-5p specific primer or primer of small nucleolus RNA (snRNA) U6 (Applied Biosystems) with TaqMan miRNA reverse transcription kit (Applied Biosystems). Quantitative PCR was carried out with Power SYBR® Green PCR Master Mix (Applied Biosystems), specific NKG2D ligands primers, TaqMan probes of HCMV miR-US33-5p and snRNA U6 (Applied Biosystems) following the manufacture′s protocol using Applied Biosystems 7300 Fast Real-Time PCR System. The relative expression level of NKG2D ligands and HCMV miR-US33-5p were normalized to that of ULBP3 and snRNA U6 in corresponding samples by $2^{-\Delta\Delta CT}$ method.

Table 2: Specific NKG2D ligands primers for RT-qPCR

MICA-sense	5′-ATCTTCCCTTTTGCACCTCC-3′
MICA-antisense	5′-AACCCTGACTGCACAGATCC-3′
MICB-sense	5′-CTGCTGTTTCTGGCCGTC-3′
MICB-antisense	5′-ACAGATCCATCCTGGGACAG-3′
ULBP1-sense	5′-GCGTTCCTTCTGTGCCTC-3′
ULBP1-antisense	5′-GGCCTTGAACTTCACACCAC-3′
ULBP2-sense	5′-CCCTGGGGAAGAAACTAAATGTC-3′
ULBP2-antisense	5′-ACTGAACTGCCAAGATCCACTGC-3′
ULBP3-sense	5′-AGATGCCTGGGGAAAACAACTG-3′
ULBP3-antisense	5′-GTATCCATCGGCTTCACACTCAC-3′
GAPDH-sense	5′-GAGTCAACGGATTTGGTCGT-3′
GAPDH-antisense	5′-GATCTCGCTCCTGGAAGATG-3′

Statistics

Data are shown as mean ± SE. Statistical significance was determined by Student's t-test. $P < 0.05$ was considered to be statistically significant.

Results

HCMV Mir-US33-5p Down Regulate Mrna of Multi Host and Viral Genes.

Our previous study found 12 putative target mRNAs from 58 random sequenced clones by using hybrid-PCR and BLAST (http://blast.ncbi.nlm.nih.gov/ Blast.cgi) analysis. 8, 3′-UTR sequences out of 12 putative target mRNAs were cloned into pMIR-REPORT firefly luciferase (FFL) vector. Binding ability of HCMV miR-US33-5p to 3′-UTR sequence of each mRNA was validated by luciferase reporter assays. As shown in figure 1A, the normalized renilla luciferase activity of most putative mRNAs was significantly lower (20% - 60%) in cells co-transfected with HCMV miR-US33-5p than that of cells co-transfected with miRNA negative control, only except pMIR-OTU5-UTR. The identified binding site of HCMV miR-US33-5p to HCMV UL16 mRNA 3′-UTR were predicted by RNAhybrid (http://bibiserv. techfak.uni-bielefeld.de/rnahybrid/submission.html) (figure 1B). The inhibitory effect of HCMV miR-US33-5p on luciferase activity found in pMIR-UL16-UTR (30%) disappeared in pMIR-UL16-UTR -MUT (figure 1A), which contained the sequence with triple point mutations at the putative binding site (figure 1B). These results demonstrate that HCMV miR-US33-5p can directly bind to the predicted binding site in the 3′-UTR of HCMV UL16 mRNA.

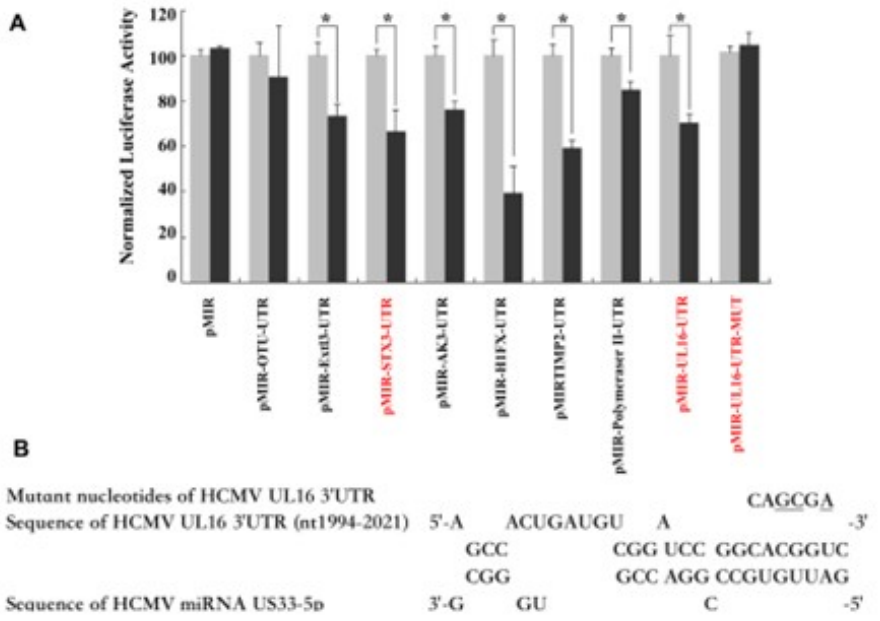

Figure1: Analysis of luciferase activities in HEK293 cells transfected with different putative mRNA 3′-UTR. (A) Dual luciferase assays in HEK 293 cells cotransfected respectively with pMIR-REPORT vector (pMIR), vectors containing the wild type of putative mRNA 3′-UTR and mutant type of HCMV UL16 3′-UTR (pMIR-UL16-MUT) together with pRL-TK-Renilla-luciferase plasmid, and respectively with miRNA negative control and hcmv-miR-US33-5p mimics. The transfection efficiencies were normalized by the renilla luciferase activities in corresponding wells. The assays were performed in triplicate wells, ∗ $P < 0.05$. (B) schematic diagram of preicted binding site of HCMV miR-US33-5p in the HCMV UL16 3′-UTR and mutant nucleotides (underlined) of HCMV UL16 3′-UTR. The predicted binding site of hcmv-miR-US33-5p is located at nucleotides (nt) 1994–2021 of STX3 3′-UTR, in which the first nucleotide of HCMV UL16 mRNA is defined as nt 1.

Expression of Recombinant HCMV UL16 Is Down-Regulated By Over-Expressed HCMV Mir-US33-5p

The regulatory effect of HCMV miR-US33-5p on HCMV UL16 protein expression was examined by western blot. As the limit use of HCMV UL16 antibody, the whole HCMV UL16 mRNA was cloned into PBI-CMV2 vector with a c-Myc tag in N-terminal end?. Over-expression of HCMV miR-US33-5p markedly reduced the PBI-c-Myc-UL16 protein in both HEK-293 cells (figure 2A and 2B) and HCMV infected HELFs (figure 2D and 2E). Moreover, the reduction was abolished in cells transfected with inhibitor of HCMV miR-US33-5p, which can efficiently inhibit the expression of HCMV miR-US33-5p (figure 2C and 2F). The probe of HCMV miR-US33-5p was used to test its expression in each condition by RT-qPCR (figure 2C and 2F). These results indicate that over-expressed HCMV miR-US33-5p can specifically inhibit the expression of c-Myc-UL16 protein.

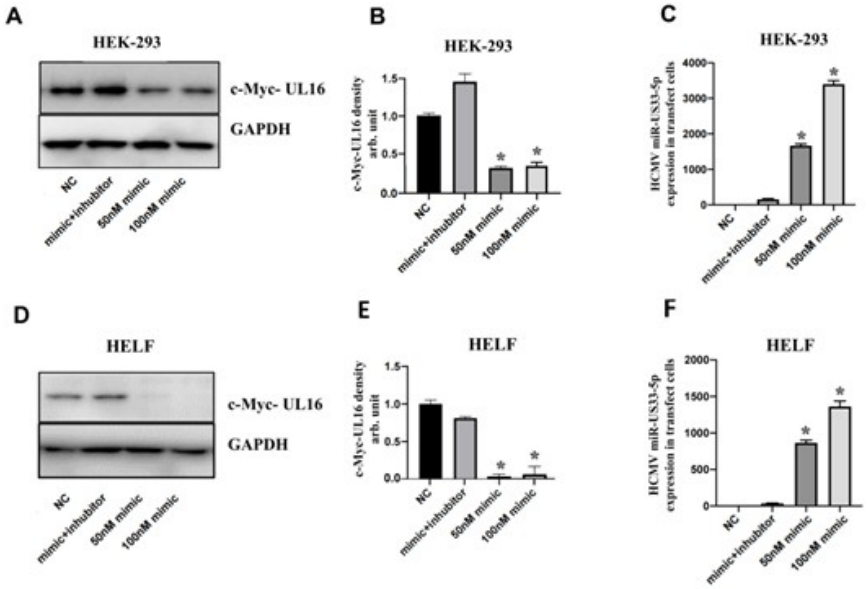

Figure 2: Western blot analyses of c-Myc-UL16 protein expression in HEK-293 cells and HCMV infected HELF cells. (A and D) Over-expressions of c-Myc-UL16 in HEK-293 cells or HCMV infected HELF cells (MOI: 1) transfected respectively with miRNA negative control, HCMV miR-US33-5p and inhibitor for HCMV miR-US33-5p were detected at 48 hours post transfection by Western blot. The relative expressions of c-Myc-UL16 were normalized to those of GAPDH detected in the corresponding samples. The experiment was repeated three times, and data from a single representative experiment are shown. (B and E) The relative expression of c-Myc-UL16 in the cells transfected with miRNA negative control defined as 1, $* P < 0.05$. (C and F) Expressions of HCMV miR-US33-5p in the transfected HEK-293 cells and infected HELF cells were detected by quantitative real-time PCR. The numbers of HCMV miR-US33-5p were normalized to those of snRNA U6 in the corresponding samples by $2-\Delta\Delta CT$ method. The assays were performed in triplicate wells, $* P < 0.05$.

HCMV UL16 Is Not Necessary During the Process of HCMV Mir-US33-5p Down Regulates HCMV Replication

We previously demonstrated that HCMV miR-US33-5p could down regulate HCMV replication[9]. To evaluate the function of this HCMV miR-US33-5p new target HCMV UL16 during HCMV infection, a reconstituted HCMV Han BACs, UL16 deleted mutant (HCMV ΔUL16) were used. HELFs were infected either HCMV han BACs (HCMV) or HCMV ΔUL16 (MOI: 1). As shown in figure 3A, B and C, GFP signal, HCMV protein level (IE1) and viral load have no significant difference at indicated time. However, HCMV miR-US33-5p shows comparable suppression of HCMV DNA replication in both HCMV ΔUL16 infected cells and HCMV HAN BACs infected cells (figure 3C). These results suggest that the absence of HCMV UL16 has no impact on the down regulating of viral replication by miR-US33-5p.

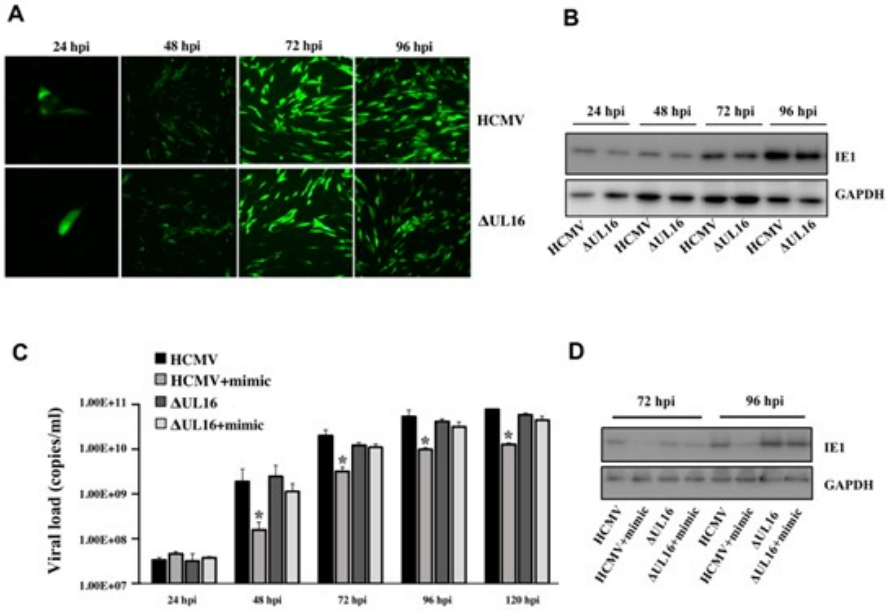

Figure 3: HCMV UL16 is not necessary during the process of HCMV miR-US33-5p down regulates HCMV replication. (A) HCMV Han BACs or HCMV ΔUL16 BACs infected HELF cells (MOI: 1) were used to take GFP signal at indicated time points. (B) An immunoblot was performed for detection of viral protein IE1 at indicated time points. (C) Viral DNA replication in infected HELF cells (MOI:1) with HCMV miR-US33-5p (mimic) or without HCMV miR-US33-5p were determined by real-time PCR in . Data shown are average ± SD of quadruplicate values from two independent experiments ∗ $P < 0.05$. (D) An immunoblot was performed for detection of viral protein IE1 at indicated time points.

HCMV Mir-US33-5p Induces Some of NKG2D Ligands Expression.

The UL16 glycoprotein reduces cell surface expression of NKG2D ligands ULBP-1, ULBP-2, and MICB, resulting in increased resistance to NK cell cytotoxicity[12-16]. 5 different NKG2D ligands (MICA, MICB, ULBP1, ULBP2 and ULBP3) in HCMV infected HELF cells, transfected HEK-293 cells were examined by RT-qPCR.

As shown in figure 4A, HCMV induced all 5 different NKG2D ligands at varying degrees. There is almost no MICB expression in normal HELF cells, however, HCMV infection stimulated MICB expression with the highest change fold (figure 4B).

To differentiate the HCMV infection-inducedstimulation of these NKG2D ligands from the miRNA posttranscriptional regulation by HCMV miR-US33-5p, over-expressed HCMV miR-US33-5p non-infected HEK-293 cells were harvest to measure the expression of these NKG2D ligands. HCMV miR-US33-5p up regulated ULBP1, ULBP2 as well as MICB in both HEK-293 cells and HELF cells, (figure 4C and E). Similar to HCMV infection, transfection of HCMV miR-US33-5p reversed the relative non-expression with the highest change fold (figure 4D and F). All these data collectively illustrate that miR-US33-5p is an independent molecule which could stimulate ULBP1, ULBP2 and MICB expression in the absence of any other HCMV gene.

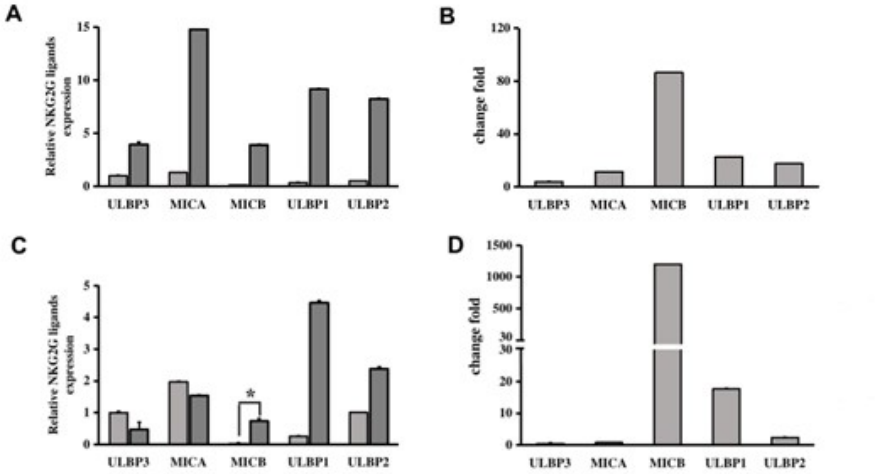

Figure 4: HCMV UL16 (A) HELF cells were infected by HCMV (MOI: 1) and harvest for test NKG2D ligands expression by real-time PCR. Data shown are average ± SD of quadruplicate values from two independent experiments ∗ $P < 0.05$. (B) Change fold of NKG2D ligands in HELF cells were calculated. (C) HEK-293 cells with or without HCMV miR US33-5p were collected to test NKG2D ligands expression by real-time PCR. Data shown are average ± SD of quadruplicate values from two independent experiments ∗ $P < 0.05$. (D) Change fold of NKG2D ligands in HEK-293 cells were calculated.

Discussion

Since the discovery of HCMV miRNAs[6, 18-20], biological functions of certain miRNAs have been studied, including those of miR-UL112-1[21–24], miR-US25-1[25], miR-US25-2-3p[26], miR-UL36[27], miR-UL70-3p and miR-UL148D[28, 29]. HCMV utilizes miRNAs to regulate its own genes as well as the genes of the host cell during infection to achieve immune evasion, regulation of cellular processes, viral DNA replication and countering of cellular apoptosis.

In this study, we examined luciferase activity of 8 putative target mRNAs 3′-UTR, 7 of them were significantly decreased by HCMV miR-US33-5p. The protein encoded by Homo sapiens exostoses (multiple)-like 3 (Extl 3) mRNA is important for both skeletal development and hematopoiesis[30]. Homo sapiens syntaxin 3 (STX3) is positive relative to HCMV replication[31]. Homo sapiens adenylate kinase 3 (AK3) regulates adenine nucleotide metabolism, maintaining intracellular nucleotide metabolic homeostasis[32]. Homo sapiens H1 histone family, member X (H1FX) is preferentially expressed in immature embryonic cells and plays some roles in cells with neural properties[33]. Homo sapiens tissue inhibitor of metalloproteinase-2 (TIMP2) not merely as an antiprotease but as a protein that could influence many signaling processes, including aging and inflammation processes[34]. Homan sapiens RNA polymerase RNA II could initiates a distinct subset of HCMV early-late and late promoters transcription in late infection[35]. Thus, we speculate that HCMV miR-US33-5p alter host cell homeostasis by down-regulating expression of its targets to establish cell environment which facilitates productive viral replication.

As one of the earliest found HCMV miRNA, miR-US33, is encoded by sequence complementary to US29 gene, divided into miR-US33-3p and miR-US33-5p[6]. It has been reported that over expression of HCMV miR-US33 can inhibit the lytic viral replication and down regulate US29 mRNA in the context of HCMV infection[8]. Our previous study demonstrated that HCMV miR-US33-5p inhibits viral DNA synthesis and viral replication by down-regulating expression of host Syntaxin 3[9]. miR-US33-5p is high express during HCMV lytic infection, but could not be detected during latency infection even the early stage. Therefore we hypothesized that one of functions of HCMV miR-US33-5p is to facilitate establishment or maintenance of HCMV latency for

lifelong survival within host.

In our study, both mRNA and protein level of a viral gene, HCMV UL16, are down regulated by HCMV miR-US33-5p. Thus HCMV UL16 is considered to be a direct target of HCMV miR-US33-5p. HCMV UL16 encode a glycoprotein, which is not necessary for viral growth in vitro[11]. It is consistent with our result, the GFP signal, viral protein and DNA copies have no significant difference between HCMV BAC Han infected HELFs and HCMV ΔUL16 infected HELFs. Even though HCMV UL16 is one of direct targets of HCMV miR-US33-5p, it is still not a key during the process of HCMV miR-US33-5p down regulates HCMV replication.

HCMV infection is well controlled by NK cells which could express a mosaic of inhibitory and activating receptors[36]. One of the activating receptors NKG2D is remarkably be recognized by at least 8 distinct ligands, the major histocompatibility complex class I (MHC-I) chain-related molecules (MICA and MICB) and the UL16-binding proteins 1–6 (ULBP1-ULBP6)[37-39], which can be induced on the cell surface during HCMV infection, only except of ULBP4[36]. At the same time, HCMV also engages bunch of viral genes to counter this up regulation, such as UL16[12-16], UL142[40] and miR-UL112[21], resulting in increased resistance to NK cell cytotoxicity.

HCMV UL16 plays critical role in NK cell evasion by sequestering ULBP1, 2, 6 and MICB in the endoplasmic reticulum (ER) and reduce their cell surface expression[12-16], but not ULBP3 or MICA[13]. Here we found that HCMV miR-US33-5p could induce ULBP1, 2 and MICB expression in HEK-293 cells and HELF cells without any other HCMV gene, meanwhile the expression of ULBP3 or MICA is either decreased or has no change. Thus, we presume that there are two ways for HCMV miR-US33-5p up regulate ULBP1, 2 and MICB expression (figure 5). One is caused by arresting HCMV UL16 and then followed by more unconjugated ULBP1, 2 and MICB transfer to cell surface. The other is directly stimulate ULBP1, 2 and MICB. As a result of ULBP1, 2 and MICB increasing expression, NK cell could be activated by reporter NKG2D binding to them.

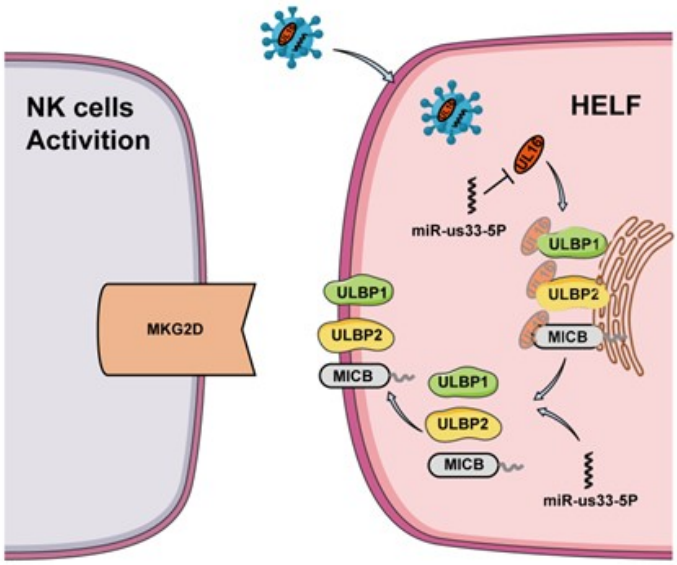

Figure 5: Model of HCMV miR-US33-5pstimulates ULBP1, 2 and MICB expression. One is caused by arresting HCMV UL16 and then followed by more unconjugated ULBP1, 2 and MICB transfer to cell surface. The other is directly stimulate ULBP1, 2 and MICB. As a result of ULBP1, 2 and MICB increasing expression, NK cell could be activated by reporter NKG2D binding to them.

HCMV has the ability of not only lytic infection but also has ability to establish life-long infection, also known as latency, which is a dormant infection lacking pathology and viral replication[41]. Based on the fact that miR-US33-5p down regulates viral DNA synthesis and replication[9], as well as the expression of HCMV UL16 and stimulates the expression of ULBP1, 2 and MICB, we

suspect that miR-US33-5p could be one of factors used by HCMV to evade innate and adaptive immune responses, resulting in latency of the virus. More experiments should be done to demonstrate the hypothesis.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81701993).

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