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Research paper

Transcriptome altered by latent human cytomegalovirus infection on THP-1 cells using RNA-seq

Qi Zhang [a,b,c], Mei-Mei Lai [a,b,c], Yun-Yan Lou [a,b,c], Bin-Han Guo [a,b,c], Hui-Yan Wang [a], Xiao-Qun Zheng [a,b,c,*]

[a] Department of Laboratory Medicine, the Second Affiliated Hospital&Yuying Children's Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, China
[b] School of Laboratory Medicine, Wenzhou Medical University
[c] Key Laboratory of Laboratory Medicine, Ministry of Education, China

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ABSTRACT

Human cytomegalovirus (HCMV) has been recognized as a cause of severe, sometimes life-threatening disease in congenitally infected newborns as well as in immunocompromised individuals. However, the molecular mechanisms of the host-virus interaction remain poorly understood. Here, we profiled the expression of mRNAs and long noncoding RNAs (lncRNAs) in THP-1 cells using the emerging RNA-seq to investigate the transcriptional changes during HCMV latent infection. At 4 days post HCMV infection, a total of 169,008,624 sequence reads and 180,616 transcripts were obtained, respectively. Of these transcripts, 1,354 noncoding genes and 12,952 protein-coding genes were observed in Refseq database. Differential gene expression analysis identified 2,153 differentially expressed genes (DEGs) between HCMV-infected and mock-infected THP-1 cells, including 1,098 up-regulated genes and 1,055 down-regulated genes. These regulated genes were involved in pathways of apoptosis, inflammatory response and cell cycle progression, all of which may be implicated in viral pathogenesis. In addition, 646 lncRNAs (208 known lncRNAs and 438 novel lncRNAs) were upregulated and 424 (140 known and 284 novel) were downregulated in infected THP-1 cells. These findings have provided a dynamic scenario of DE candidate genes and lncRNAs at the virus-host interface and clearly warrant further experimental investigation associated with HCMV infection.

1. Introduction

Human cytomegalovirus (HCMV) is a ubiquitous, highly host-specific herpesvirus that causes severe, sometimes life-threatening disease in congenitally infected newborns as well as in immunocompromised individuals such as bone marrow allograft transplant recipients and AIDS patients (Boeckh and Geballe, 2011; Chen et al., 2012).The ability to reactivate with high viral load from latency in immunocompromised or immunosuppressed hosts endowed HCMV with high pathogenesis (Slobedman et al., 2004). To date, no effective vaccine or antiviral drugs is available to prevent or treat HCMV infection. The most important and effective factor to prevent the development of serious complications after HCMV infection dwells in cellular immunity in humans (Einsele et al., 2002; Saffert and Kalejta, 2006), which may involved transcriptional alterations. Whereas a virus can potentially modulate the level of cellular mRNA by various mechanisms, identification of the cellular transcription alterations that are repressed or activated after HCMV latent infection could unveil the dynamic virus-host interaction and facilitate the development of antiviral therapy.

Several high-throughput studies provided a broad catalog of the transcriptional response of the cell during lytic HCMV infection by analyzing temporal changes in total RNA levels (Zhu et al., 1998; Kenzelmann and Muhlemann, 2000; Browne et al., 2001; Simmen et al., 2001; Challacombe et al., 2004; Hertel and Mocarski, 2004). These studies identified a subset of differently expressed genes, which are involved in a variety of biological functions including innate immunity response, inflammation pathway, cell cycle regulation, cellular metabolism and cell adhesion, during lytic HCMV infection. However, the HCMV latency still remains untapped.

Previous studies in HCMV infection focused on the changes in mRNAs. During the last decade, the importance of long noncoding RNAs (lncRNAs) as mediators involved in chromatin structure, gene regulation, subcellular structural organization, as well as nuclear-cytoplasmic trafficking has begun to be recognized (Amaral and Mattick, 2008; Umlauf et al., 2008; Mercer et al., 2009). Recent studies have demonstrated the changes in host lncRNA expression in response to virus infection. In 2010, Yin et al.(Yin et al., 2013) observed the differential expression of more than 4,800 lncRNAs (2,990 lncRNAs were up-regulated, whereas 1,876 lncRNAs were down-regulated) in response to enterovirus 71(EV71) infection. Ouyanget al.(Ouyang et al., 2014)

Abbreviations: HCMV, human cytomegalovirus; lncRNAs, long noncoding RNAs; GO, gene ontology; MOI, multiplicity of infection; DEGs, differentially expressed genes; CNCI, Coding Noncoding Index; RPKM, reads per kilobase transcriptome per million reads.

* Correspondence author.
E-mail address: jszhengxq@163.com (X.-Q. Zheng).

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reported that a long noncoding RNA-negative regulator of antiviral response (*NRAV*) could modulate antiviral responses through suppression of multiple critical interferon-stimulated genes transcription, including *IFITM3* and *MxA*, after influenza Avirus (IAV) infection. These findings suggest the widespread differential expression of lncRNAs in response to virus infection and their potential roles in regulating the host response, including innate immunity.

The recent developments in next-generation sequencing (NGS) technologies have enabled quantification of response in host cells to infections at transcriptomic level. As a NGS technology, RNA-seq not only outperforms the conventional microarray in sensitivity and specificity, but is also able to detect new genes, lncRNAs, rare transcripts, alternative splice isoforms and novel SNPs, which extends the limited analysis scope of microarrays (Marioni et al., 2008; Morozova et al., 2009; Nielsen et al., 2011). Therefore, this technology has gained rapidly expanding application in transcriptomic studies (Wang et al., 2009; Qian et al., 2014). The present study adopted RNA-seq to identify global changes of mRNAs and lncRNAs in host cell during HCMV experimental latent infection of THP-1 cells, in an attempt to derive insights into the mechanism underlying alterations in response to infection. To our knowledge, this is the first study to explore the comprehensive transcriptome profile of both noncoding RNAs and mRNAs during HCMV experimental latent infection. These results fill the gap of knowledge in the complexity of the virus-cell interactions, and promise to offer clues with which the control of HCMV infection may advance.

2. Materials and Methods

2.1. Cells culture and virus infections

THP-1 cell line was cultured in RPMI 1640 medium(GIBCO, Life Technologies) supplemented with 10% (v/v)fetal bovine serum, 100 units/ml Penicillin, and 100μg/ml Streptomycin (P/S) (GIBCO, Life Technologies) in incubator (5% CO2, at 37°C). To analyze cellular responses to HCMV infection, a THP-1 cellline was infected with HCMV Towne at a multiplicity of infection (MOI) of 5. RNAs were isolated and analyzed at 4 days through the infection cycle and were also obtained from mock-infected cells. HCMV Towne strain infection and latent infection cell modelwere performed as described previously(Fu et al., 2014; Zheng et al., 2015).

2.2. RNA extraction, Illumina library construction and sequencing

Total RNA from THP-1 cells was extracted using Trizol reagent (Invitrogen, USA). Quantification and quality evaluation were performed with Nanodrop 2000 (Thermo Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies), respectively. Because the majority of RNAs purified from eukaryotic cells are large, structured ribosomal RNAs (rRNA), the mRNA signal must be enriched. We used a kit developed for the removal of the large rRNAs (18s and 28s rRNAs) from the samples. Subsequently, they were used for mRNA purification and library construction with the Truseq™ RNA Sample Preparation Kit v2 (Illumina, SanDiego, CA, USA) according to the manufacturer's instructions. Our samples were named mock-infected and latent-infected THP-1 respectively that were sequenced on an Illumina HiSeq™2000 (Illumina) with pair-end libraries in Berry Genomics Bio-Technology Co. (Beijing, China).

2.3. RNA-seq reads mapping and transcriptome reconstruction

The spliced read aligner TopHat version V1.31 were used to map all sequencing reads to the human genome. All reads were mapped with TopHat (Trapnell et al., 2009) using the following parameters: segment-mismatches = 2, splice-mismatches = 0. Aligned reads from TopHat were assembled into transcriptome for each sample separately by Cufflinks. Hg 19 RefSeq (RNA sequences, GRCh37) was downloaded from the UCSC Genome Browser (http://genome.ucsc.edu)and all known noncoding genes from NONCODE 3.0 database (Bu et al., 2012). Cufflinks uses a probabilistic model to simultaneously assemble and quantify the expression level of a minimal set of isoforms and provides a maximum likelihood explanation of the expression data in a given locus. Cufflinks version V1.0.3 was run with default parameters (and 'min-frags-per-transfrag = 0'). The RNA-Seq reads used in this study have been deposited to NCBI Sequence Read Archive (SRA) database under accession number SRA458685.

2.4. Novel lncRNAs detection pipeline

We implement the following four steps to enhance the reliability of constructing expressed lncRNAs from transcripts obtained from our two samples: (1) Select transcripts with multi-exon; (2)Select transcripts which are longer than 200 bases; (3) Calculate the coding potential of each transcript using CNCI (Coding Noncoding Index) in-house software(Sun et al., 2013) to recover the transcripts which can be categorized as noncoding (CNCI, is a powerful signature tool that profiles adjoining nucleotide triplets to effectively distinguish protein-coding and non-coding sequences independent of known annotations; CNCI software is available at http://www.bioinfo.org/software/cnci); (4) Select transcripts that are located in the intron, intergenic and antisense regions from genes encoding known.

2.5. Identification of differentially expressed genes

The gene expression was calculated using the RPKM method (reads per kilobase transcriptome per million reads) and a minimum RPKM value of 0.1 is required for expressed genes/isoforms (Mortazavi et al., 2008). The RPKM method is able to remove the influence of different gene lengths and sequencing discrepancies from the calculation of gene expression. Therefore, the calculated gene expression can be directly used to comparing the differences in gene expression among samples. Differentially expressed genes (DEGs) were defined as those with changes of at least 2-fold change between a pair of samples.

2.6. PCR validation

qPCR was performed to confirm the expression of mRNAs and lncRNAs by RNA-seq analysis. Briefly, cDNA was synthesized from total RNA (random hexamers) using Revert Aid First Strand cDNA Synthesis Kit (Thermo). Primers for 7 mRNAs and 7 lncRNAs were designed and synthesized (Tables S1 and S2). Then, qPCR was performed using a BIO-RAD CFX96 (BIO-RAD). The 20 μl PCR reactions included 1μl of cDNA product and 10 μl of FastStart Essential DNA Green Master (Roche). The reactions were incubated at 95°C for 5 min, followed by 40 cycles at 95°C for 5 s, 60°C for 30 s, and 72°C for 15 s. All reactions were run in triplicate. After reaction, the threshold cycle value (CT) data were determined using default threshold settings, and the mean CT was determined from the duplicate PCRs. The expression levels of mRNAs and lncRNAs were measured in terms of CT, and then normalized to GAPDH (endogenous gene) using -[delta][delta] CT.

2.7. Gene ontology (GO) and signaling pathway analysis

Pathway and GO analyses were applied to determine the roles of these closest coding genes in biological pathways or GO terms. GO (http://www.geneontology.org/) is an international classification system for standardized gene functions, offering a controlled vocabulary and a strictly defined conceptualization for comprehensively describing the properties of genes and their products within any organism. Signaling pathway analysis was based on Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) database. For the identification of the GO categories and pathways that the differentially expressed genes (DEGs) are predicted to participate in, all DEGs were

mapped to terms in the GO and KEGG database and searched for significantly enriched GO and KEGG terms compared to the genomic background.

3. Results and Discussion

3.1. Mapping of RNA-seq reads and transcriptome reconstruction

The original image data generated by the sequencing machine were converted into sequence data via base calling (Illumina pipeline CASAVA v1.8.2) and then a total of 169,008,624 valid reads were obtained by HiSeqTM 2000 (Illumina) 100 bppaired-end sequencing after a stringent filtering process (Table 1). The filtered reads were mapped to the human genome identifying a total of 180,616 transcripts and 33,243 genes in the two samples. The number of junctions, transcripts, protein-coding genes and noncoding genes for each sample are shown in Supplementary Tables S3 and S4.

3.2. Differential expression of mRNAs and lncRNAs inlatent-infected vs. mock-infected THP-1 cells

With RNA-seq, we totally detected 6,158 lncRNAs and 32,815 coding transcripts (Tables S3 and S4). The lncRNAs and mRNAs of above two-fold change in latent-infected vs. mock-infected THP-1 cells were statistically analyzed. The number of DEGs (up- and down-regulated) and lncRNAs for each sample is shown in Fig. 1 and Table 2. Fold change (FC) values ranged from 43.05 to -32.31 in DEGs, from 32.81 to -34.86 in lncRNAs (Supplementary Tables S5-S10 online). RNA-seq is able to provide quantitative read-out of mRNA expression levels of each sample.During viral infections, the interaction between viral and antiviral activities arouse rapid alterations in cellular gene expression, which had been shown to be effectively profiled by RNA-seq. In contrast to microarrays that just permit comparative analyses with relative expression values,

3.3. Validation of RNA-seq data by qRT-PCR

To verify the RNA-seq data, we performed qRT-PCR on a subset of 14 randomly chosen genes which represented 8 of the upregulated and 6 of the downregulated genes. The results of qRT-PCR indicated either an up- or down-regulation of transcription which correlate with the up- or down-regulation in RNA-seq. This validation demonstrated that theresults from RNA-seq and qPCR are highly concordant ($r = 0.87$, Pearson correlation)(Fig. 2). qRT-PCR tests confirmed the quality and robustness of the results.

3.4. Assignment of GO terms and KEGG pathways

To understand the functions of the DEGs and the biological processes involved in HCMV infection, all of the DEGs were mapped to terms in the GO and KEGG databases. The three main, independent GO categories are biological processes, molecular functions, and cellular components. The details are shown in Fig. 3. According to the results of GO analysis, the GO terms obviously differentiate up-regulated genes and down-regulated genes. The results of KEGG pathway analysis indicated that the unigenes were related to 21 signaling pathways, particularly to those in the cancer and MAPK signaling pathways (Table 3). Those differentially expressed genes are involved in pathways implicated in viral pathogenesis including apoptosis, inflammatory response and cell cycle progression. The dynamic alterations in the expression profile reflect human cells' response to HCMV infection as an attempt to antagonize viral replication and spread (see supplementary Tables S11-S12, supplementary material online, for full details of GO terms).

Table 1
The reads of latent-infected and mock-infected THP-1 cells libraries mapping with reference genome.

	latent-infected		mock-infected	
	reads number	percentage	reads number	percentage
Total reads	83,921,512	100.00%	85,087,112	100.00%
Total basepairs	8,392,151,200	100.00%	8,508,711,200	100.00%
Total mapped reads	47,490,950	56.59%	37,434,119	44.00%
Perfect match	22,382,356	26.67%	15,592,328	18.33%
<=2bp mismatch	47,394,990	56.48%	37,364,061	43.91%
Unique match	44,870,347	53.47%	35,471,872	41.69%
Multi-position match	2,620,603	3.12%	1,962,247	2.31%
Total unmapped reads	36,430,562	43.41%	47,652,993	56.00%

3.5. Cell-death induced by HCMV infection

Apoptosis is an essential biological process in multicellular organisms induced in response to many extrinsic stimuli (such as oxidative stress and inflammatory reaction) and was considered as an infection-associated immunopathology (Lovegrove et al., 2007; Wyllie, 2010). Apoptosis of virus-infected cells plays an essential role in the immune system, as its removal of viral survival environment controls the proliferation of intracellular pathogens (Tortorella et al., 2000). Apoptosis can be initiated via intrinsic pathway and the receptor-mediated pathway, which are mediated through endoplasmic reticulum and mitochondrion, respectively(Fulda and Debatin, 2006). Multiple previous studies indicated that human cells infected with HCMV undergo apoptosis(Browne et al., 2001; Challacombe et al., 2004).In this study, we found that 47 pro-apoptosis genes were up-regulated and 12 anti-apoptosis genes were down-regulated (especially *BCL-2*, 2.91-fold)at 4 days HCMV post infection (Table 4). Most of the up-regulated genes were involved in the mitochondrial pathway, including *Bcl-2-binding component 3* (*BBC3*), *CASP8* (2.58- and 2.49-fold, respectively). *CASP8*is one of the most crucial molecules for cell death induction(Barnhart et al., 2003). It may directly cleave downstream effector, such as *caspase-3* and link the receptor to the mitochondrial pathway by cleavaging Bid to initiate a mitochondrial amplification loop(Adams and Cory, 2007). *BBC3*, a pro-apoptotic protein that effects as a p53 up-regulated modulator of apoptosis (PUMA), has the ability to inhibit the interaction between the anti-apoptotic molecules, *Bcl-2* and the pro-apoptotic molecules, *Bax* and *Bak* and then result in apoptosis(Han et al., 2001; Nakano and Vousden, 2001). Many other genes involved in tumor necrosis factor (TNF) signaling pathways (*TNFRSF10B* and *TNFSF14*) and p53 signal transduction (*ABL1*, *CDKN1A*, *CHEK2*and *HIPK2*). This indicated that the TNF signal pathway and the p53 signal pathway were activated upon HCMV infection, but the detailed mechanisms still need further investigation.

As a matter of fact, many viruses, including HCMV, attempt to inhibit the apoptotic pathway as to create a favorable environment for viral replication(Zhu et al., 1995; Goldmacher et al., 1999; Browne et al., 2001; Brune et al., 2001; Challacombe et al., 2004; Michaelis et al., 2004). In our study, we found that 25 mRNAs that could result in apoptosis were down-regulated (especially *CASP3*, 6.49-fold), and 22 mRNAs encoding anti-apoptotic proteins were up-regulated, suggesting that complex adjustments in apoptotic signals may occur during HCMV latent infection. Sarkar et al. (Sarkar et al., 2009) demonstrated that cytokines (IL1β, TNF-α and IFN-γ) upregulate several anti-apoptotic genesthrough NF-κB-mediated signalling, including BCL2A1, BIRC3, TNFIAP3,CFLAR and TRAF1. In our study, we found IL1β and BCL2A1 upregulate 7.53 and 3.67 foldchange, respectively.

3.6. Inflammatory response in THP-1 cells via HCMV infection

Many genes with a role in the immune system were perturbed by viral infection (Table 4). Assignment of GO terms included positive regulation of response to stimulus, positive regulation of immune system process, positive regulation of immune response and activation of immune response. In the course of HCMV infection in the host, on the

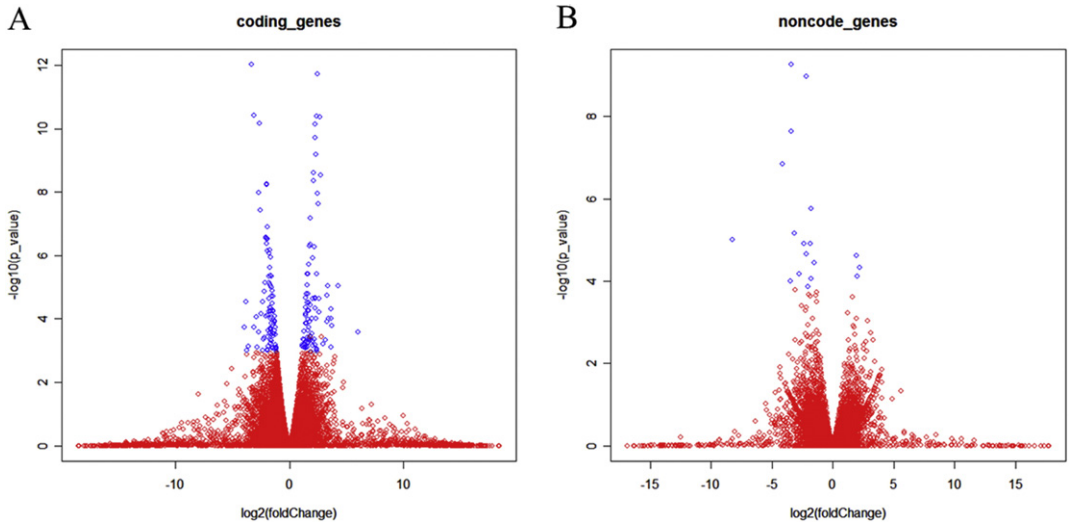

Fig. 1. Significantly differentially expressed coding genes (A) and noncode genes (B).The volcano plot (Kal's test) showing the relationship between the p-values of Kal's test and the magnitude of the difference in expression values of the two samples. Differentially expressed coding genes or noncode genes were highlighted in blue dot.

one hand, host can induce non-specific and specific immune response to clear HCMV. We found that several genes encoding regulators of NF-κB function were up-regulated, such as *IL-1β*, *RELA*, *ICAM1* and *CARD8* (7.53-, 2.09-, 4.21- and 2.06-fold, respectively). NF-κB is one of the key players in stimulating the transcription of genes involved in cellular immuneresponse, as well as cell adhesion. Thus, it plays an important role in the antiviral defense (Ghosh and Karin, 2002). A previous study revealed that NF-κB pathway could be induced by lytic HCMV infection(Challacombe et al., 2004). *IL-1β* is an important mediator of the inflammatory response and it is one of the known inducers of *RELA* activity, supporting its participation in immune response. *RELA*, also known as p65, is a REL-associated protein involved in NF-κB heterodimer formation, nuclear translocation and activation. In general, *RELA* participates in adaptive immunity and responses to invading pathogens via NF-κB activation (Li and Verma, 2002). *CARD8*, is involved in pathways leading to activation of caspases or nuclear factor kappa-B (NF-κB) in the context of apoptosis or inflammation, respectively. NF-κB triggers transcription of various genes that were critical to inflammation, such as cytokines, chemokines and cell adhesion molecules including *ICAM1* (Collins et al., 1995; Ledebur and Parks, 1995). Our results show that the expression levels of *ICAM1* was elevated. *ICAM1* can mediate leukocyte adhesion, and then transmigrate leukocytes into tissues resulting in inflammation(Yang et al., 2005). P2RX7 increasedin our study (3.13 foldchange). The product ofP2RX7 gene belongs to the family of purinoceptors for ATP.P2X7 receptors play an important role in regulating inflammatory responses during acute viral infection (adenoviral vector) in ATP-mediatedinflammation (Lee et al., 2012).

Continuous interactions between viruses and hosts have made the viruses evade host immune defenses during their co-evolution. Consequently, viruses have manipulated host immune-control mechanisms to facilitate their propagation. HCMV is well-known for its ability to evade normal immune response pathways such as antigen presentation(Wiertz et al., 1997; Michelson, 1999; Loenen et al., 2001). Functional studies using purified complement components demonstrated that up-regulation of *CD55* (5.26-fold) suppressed the activity of cell-associated C3 convertases on HCMV-infected cells. Furthermore, increased *CD55* expression protected infected cells from complement-mediated lysis, an effect which directly correlated with the length of HCMV infection(Spear et al., 1995; Spiller et al., 1996). These negatively impacting cellular functions in the immune system, would severely compromise the host response to the infection.

Table 2
The numbers of difference coding and non-coding transcripts in latent-infected THP-1 cells libraries compared to mock-infected group.

Group	coding gene	non-coding	novel non-coding
Up-expressed	1,098	208	438
Down-expressed	1,055	140	284

3.7. Cell cyclearrestby HCMV infection in THP-1 cells

Cell cycle and checkpoint control are intimately related to the outcome of herpesvirus infection. The complexity inherent in this virus-host interaction is becoming even more apparent (Goodrum et al., 2012). Seventy-three mRNAs encoding proteins with likely roles in cell cycle regulation were regulated by the virus (Table 4). The activation of protein kinases-cyclin dependent kinases (cdks) could trigger progression through the successive phases of the cell cycle. Whereas, the activities and specificities of these kinases are determined by their association with various cyclins and inhibitors which are differentially expressed during the cell cycle. Prevailing hypothesis proposes that proper temporal order of cell cycle was regulated by two major checkpoints(Hartwell and Weinert, 1989; Enoch and Nurse, 1991;

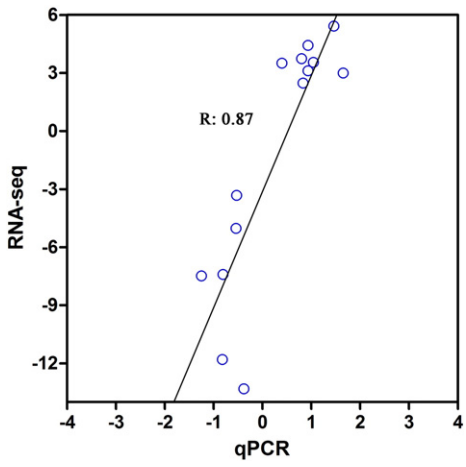

Fig. 2. Validation of RNA-seq data by qRT-PCR. X-axis: -[delta] [delta] CT values from qPCR comparing HCMV-infected THP-1 cells or mock-infected cells. Y-axis: $\log_2$ (foldchange) between infected-or mock-infected THP-1 cells via RNA-seq. Pearson correlation coefficient (R) based on all genes is shown in black.

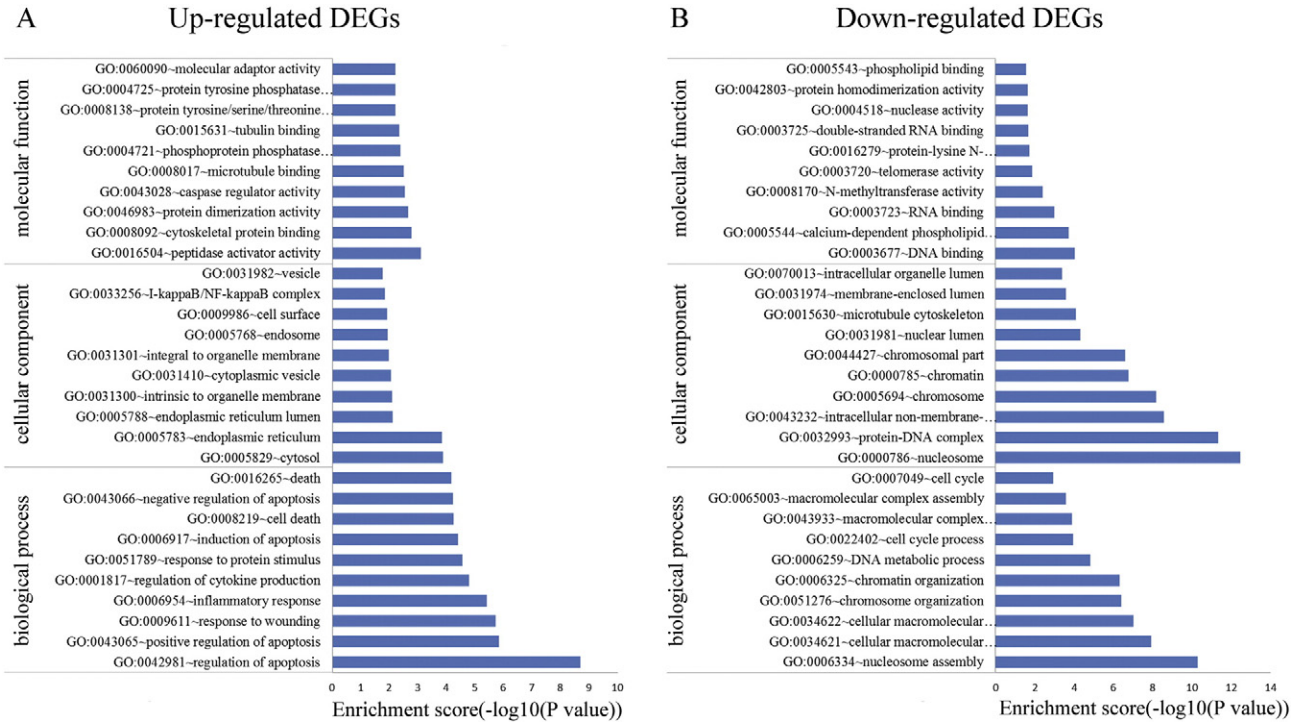

Fig. 3. Gene ontology assignments for differentially expressed genes (DEGs) upon HCMV infection. The DEGs upon HCMV infection that matched various ontology (GO) categories (top 10) of biological process, cellular component and molecular function. The x-axis indicates the GO terms and the y-axis indicates enrichment score (-log10 (P value)). A, GO analysis for the up-regulated genes upon HCMV infection. B, GO analysis for the down-regulated genes upon HCMV infection.

Pines, 1993). One occurs at the G1/S transition and controls the initiation of DNA replication, and the other occurs at the G2/M transition just prior to mitosis and cell division. Some previous studies indicated that THP-1 cells infected with HCMV undergo cell cycle arrest. Lu M et al. (Lu and Shenk, 1996) have revealed a block in late G1, and Jault et al.(Johnson et al., 1993) described a block in G2/M. In our study, HCMV infection tends to inhibit cell cycle progression at multiple points, including the transition from G1 to S and G2 to M, consistent with previous research results.

Cyclins expressed during the G1 phase promote the progression from G1 to S phase and include mainly the D-type cyclins and cyclin E(Pines, 1993). Many genes encode cell cycle regulators, especially cyclin-dependent kinase inhibitor 1. Cyclin-dependent kinase inhibitor 1 (*CDKN1A*, 2.11-fold), also known as p21, is a cyclin-dependent kinase inhibitor that inhibits the complexes of *CDK2* and *CDK1* and up-regulated more than 2 fold change. p21 is a potent cyclin-dependent kinase inhibitor (CKI). The p21 (CIP1/WAF1) protein binds to and inhibits the activity of cyclin-CDK2, -CDK1, and -CDK4/6 complexes, and thus functions as a regulator of cell cycle progression at G1 and S phase (Gartel and Radhakrishnan, 2005). *Cyclin A* (3.03-fold) plays an important role in promoting the progression from G2 to M phase. In our study, *TGFβ1* (2.51-fold) were up-regulated. The inhibited expression of cyclin A was primarily modulated by *TGFβ1* and may result in a block in G2/M (Djaborkhel et al., 2000). HCMV mediated perturbations that result in inhibition of cell cycle progression at multiple points to maximize its benefits in the host cell.

Table 3
Important KEGG pathways of coding genes influenced by HCMV infection.

Pathway	Unigenes with pathway annotations	Pathway ID
Systemic lupus erythematosus	23	hsa05322
MAPK signaling pathway	44	hsa04010
Apoptosis	28	hsa04210
p53 signaling pathway	19	hsa04115
NOD-like receptor signaling pathway	16	hsa04621
Neurotrophin signaling pathway	23	hsa04722
Pathways in cancer	49	hsa05200
Epithelial cell signaling in Helicobacter pylori infection	15	hsa05120
Wnt signaling pathway	24	hsa04310
Natural killer cell mediated cytotoxicity	32	hsa04650
Hematopoietic cell lineage	13	hsa04640
B cell receptor signaling pathway	15	hsa04662

3.8. New lncRNA discovery

We further focus on the long noncoding RNAs to address the influence in non-coding region. Finally, 2,107 novel lncRNAs were found to be expressed (Supplementary Table S13) and the numbers of exon distribution among novel lncRNAs are shown in Fig. 4. Regulation role of lncRNAs was recently observed in infection with severe acute respiratory syndrome coronavirus (SARS-CoV), HIV-1 and influenza virus, and have been suggested to impact host defenses and innate immunity (Peng et al., 2010; Zhang et al., 2013). Further studies to identify the functions of these differently expressed lncRNAs during HCMV infection may well provide novel insights into the virus-host molecular interface as well as possible therapeutic targets.

4. Conclusion

In this study, we profiled the expression of mRNAs and lncRNAs in host cell using the emerging RNA-seq to investigate the transcriptional changes in HCMV latent infection. A great number of genes (mRNAs and lncRNAs) that were differentially expressed upon HCMV infection were obtained and functionally annotated. As our findings are mainly derived from bioinformatic analysis which may not be adequate to provide solid evidence, their functional relevance would need to be further established at molecular and cellular levels experimentally. Given the global analysis of changes in lncRNAs and mRNA levels provides a catalog of genes that are modulated as a result of the host-virus interaction, it is highly likely that further study of these genes may lead to

Table 4
List of the differentially expressed genes response to HCMV infection on apoptosis, immunity and cell cycle.

Response	Associated Genes
Apoptosis	
Positive regulation of apoptosis(UP[a])	*ABL1, ABR, ADAMTSL4, APC, APOE,ARHGAP4, BBC3, BCL2L11, BCL3, CAPN10, CASP1, CASP8, CD5, CDKN1A, CEBPβ, CHEK2, DDIT3, DEDD, EIF5A, FGD4, HIPK2, HMOX1, IL1β, INHBA, JUN, MAP3K5, MMP9, NFκBIL1, NOTCH1, NQO1, NR3C1, NUPR1, P2RX7, PHLDA1, PLEKHG5, PSEN2, RAD9A, RIPK3, SCAND1, SMAD3, SQSTM1, TGFβ1, TIAL1, TICAM1, TNFRSF10B, TNFSF14, WWOX*
Negative regulation of apoptosis(DN[b])	*ADNP, ANXA4, APBB2, ASNS, BCL2, BNIP3, CACNA1A, CASP3, CHD8, DLX1, EYA1, MEF2C, MPO, NME1, NUP62, PRLR, RASA1, RTEL1, SKP2, SOD2, TERT, XRCC4, YBX3, YWHAZ*
Anti-apoptosis(UP)	*ANXA1, APOE, BCL2A1, CEBPβ, HMOX1, HSP90β1, HSPA1A, HSPA1B, HSPA5, IER3, IκBKβ, IL1β, IRAK1, NFκB1, PRNP, RELA, SOCS3, SPHK1, SQSTM1, THBS1, VEGFA, VIMP*
Immune response	
Positive regulation of immune system process(UP)	*C1R, C3AR1, CD46, CD5, CD55, CD74, CDKN1A, CR1, EBI3, FCER1G, FYN, ICAM1, IL1β, IL4R, IRAK1, MAP3K7, P2RX7, PSEN2, RARα, RELA, SLC11A1, TGFβ1, THBS1, TICAM1, TNFSF14, VEGFA*
Virus-host interaction(UP)	*DDX39B, HIPK2, IRF7, SMAD3, TGFβ1*
Positive regulation of cytokine production(UP)	*AGPAT1, BCL3, CARD8, CASP1, FCER1G, IL1β, MAP3K7, P2RX7, RARα, SLC11A1, SMAD3, TGFβ1, THBS1, TICAM1, UCN*
Cell cycle	
Cell cycle arrest(UP)	*APC, CDKN1A, CXCL8, DDIT3, FOXO4, GAS7, INHBA, PPP1R15A, RASSF1, SESN2, SMAD3, TGFβ1, THBS1*
Cell cycle(DN)	*AIF1, ANAPC11, APBB1, APBB2, ARHGEF2, AURKA, BCL2, CASP8AP2, CCNA1, CDK11B, CDK20, CENPA, CEP55, CHEK1, CHTF8, DSN1, FANCD2, FBXO43, GFI1, GTSE1, HAUS2, HBP1, ILF3, KIF11, KMT2E, MAPK13, MLF1, MLH1, NEK6, OIP5, PBRM1, PIWIL4, PKD1, PLK1, PML, PRC1, PSMA4, PSMB8, PSRC1, RAD17, RAD51B, RAD51D, RAD54L, RASSF1, RCC1, SEPT9, SESN3, SIRT2, SKA2, SKP1, SKP2, SMARCB1, SMC4, SUN2, TERF1, TRNP1, TTK, ZNF655*

[a] UP denotes up-regulated genes; [b] DN means down-regulated genes.

breakthroughs in the understanding and treatment of cytomegalovirus-related diseases.

Competing interests

The authors have declared that no competing interests exist.

Author contributions

X-QZ, QZ and B-HG conceived and designed the experiments, M-ML and Y-YL performed the experiments and QZ, H-YW and X-QZ analyzed the data. All authors reviewed the manuscript.

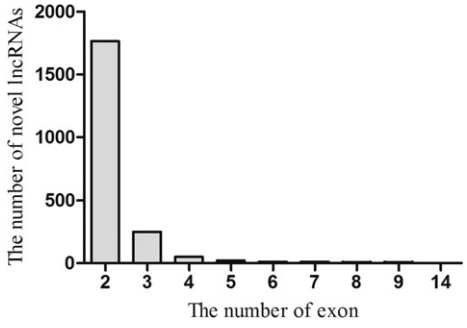

Fig. 4. The numbers of exon distribution among novel lncRNAs.

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