Original Paper

Deep RNA Sequencing Reveals a Repertoire of Human Fibroblast Circular RNAs Associated with Cellular Responses to Herpes Simplex Virus 1 Infection

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Key Words

Circular RNA • MicroRNA • Gene • HSV-1 • KMB17 • Whole transcriptome

Abstract

Background/Aims: Circular RNAs (circRNAs), a new class of regulators of gene expression, are involved in diverse physiological and pathogenic processes. However, their role in cellular responses to virus infection is yet unclear. **Methods:** A human lung fibroblast cell line was infected or mock infected by herpes simplex virus 1 (HSV-1). Deep RNA sequencing was used to profile the global changes in circRNAs, genes, and miRNAs following HSV-1 infection. Altered circRNAs, genes, and miRNAs were validated using quantitative reverse transcription polymerase chain reaction (RT-qPCR). An integration analysis of circRNAs, genes, and miRNAs was applied to investigate the putative function of the dysregulated circRNAs. **Results:** A total of 536 circRNAs, 3,885 genes, and 207 miRNAs were significantly dysregulated after HSV-1 infection. An integration analysis of circRNAs, genes, and miRNAs revealed the alleged involvement of dysregulated circRNAs in cellular responses to HSV-1 infection via the circRNA-miRNA-gene regulatory axis. These genes regulated by circRNAs were enriched to NOD-like receptor/JAK-STAT signaling pathway, and pathways of apoptosis, cell cycle progression, and cell death, all of which may be implicated in the viral pathogenesis and cellular immunity. **Conclusions:** These data present a comprehensive view for circRNAs induced by HSV-1 and their interplay with miRNAs and genes during HSV-1 infection, thus offering new insights into the mechanisms of interactions between HSV-1 and the host.

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Introduction

Circular RNAs (circRNAs) are a new class of endogenous regulatory RNAs that have been recently recognized as critical regulators of gene expression and pathological processes [1, 2]. They are formed from pre-mRNAs by a back-splicing event that refers to the alternative circularization of the 5' end of the upstream exon to the 3' end of the downstream exon in order to produce a covalently closed loop [3, 4]. The covalently closed loop structures confer high resistance to RNA exonuclease or RNase R, rendering them more durability as compared to linear RNAs. The circRNAs are widely distributed with biological function relatively conserved from humans to *Drosophila* and Archaea that also present cell type-specific and tissue-specific expression [5-8]. Moreover, circRNAs exert a variety of regulatory roles in physiological and pathological processes, including transcriptional regulation [9], alternative splicing [10], regulation of parental gene translation [11], cell cycle regulation [12], protein sponge [13], and miRNA sponge [14]. Although diverse biological functions of circRNAs have been revealed widely, little is known about their associations with virus infection and the antiviral immunity of the host.

Herpes simplex virus 1 (HSV-1), a human neurotropic virus, can cause a variety of human diseases ranging from asymptomatic viral shedding to lethal encephalitis and disseminated disease [15, 16]. Worldwide, >80% of the population is affected by HSV-1 due to the establishment of lifelong viral infection in the neurons of sensory and autonomic ganglia [17]. As a member of the family Herpesviridae and subfamily Alpha Herpesviridae, HSV-1 possesses a double-strand linear DNA genome (152 kb) encoding >80 proteins [18, 19]. The initial infection of HSV-1 occurs primarily in the epithelial or mucosal cells, from where it causes a latent infection in neurons [20]. When HSV-1 establishes a latent infection, viral genome transcription is inhibited except for the latency-associated transcripts (LATs) [21]. When subjected to a variety of stimuli, virus can trigger the reactivation and lytic infection to cause the recurrent disease at or near the original site of inoculation [20, 22]. In a majority of the cell cultures, HSV-1 establishes a lytic infection, during which, various cellular stress responses, including apoptosis [23], antiviral immunity [24], and DNA damage response [25], are elicited in order to limit the HSV-1 infection and proliferation. As a common and critical human pathogen, HSV-1 has served as a robust model to study the viral-host interactions and antiviral defense. Accumulating evidence has shown that the virus infection can alter the host transcriptome and reshape the cellular environment for promoting the fitness and survival of the virus [26-28]. Although the mechanisms underlying the interactions between HSV-1 and its host are thoroughly investigated, whether circRNAs are involved in the interactions between HSV-1 and its host yet remain unknown. In this study, we systematically investigated the virus-responsive circRNAs and their putative roles during HSV-1 infection by whole-transcriptome sequencing and small RNA sequencing analyses. A large number of dysregulated circRNAs were detected after HSV-1 infection, which revealed their putative regulatory roles in cellular response to HSV-1 infection based on an integrated analysis of circRNAs, miRNAs, and genes. In summary, these results provided a novel insight into the functions of circRNAs in viral infection and cellular antiviral immunity and broadened our understanding of the mechanisms underlying the interaction between HSV-1 and its host cells.

Materials and Methods

Cells and viruses

Human fibroblast KMB17 strain (Institute of Medical Biology, CAMS, Kunming, China), a normal diploid cell line that originated from fetal lung tissue, was cultured in Minimum Essential Medium (MEM) supplemented with 10% bovine serum (Minhai Biotech, Beijing, China) at 37 °C in 5% CO₂. Wild-type HSV-1 (strain 17) was a kind gift from Professor Longding Liu, Institute of Medical Biology. The virus was propagated, and titers determined on Vero cells (ATCC) as described previously [29]. KMB17 cells were mock-infected or HSV-1-infected at a multiplicity of infection (MOI) of 1.

Whole transcriptome library construction and sequencing analysis

At 48 h post-infection, total RNA from HSV-1 infected and uninfected KMB17 cells was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), followed by digestion with DNase I (Epicentre) for 15 min at 37 °C. Then, rRNA was depleted in total RNA (5 µg) using a Ribo-Zero Gold Kit (Epicentre), and a cDNA library was generated according to the Illumina TruSeq protocol (Illumina, San Diego, CA, USA). Furthermore, the cDNA samples were fragmented, adaptor-ligated, amplified, and sequenced on an Illumina HiSeq 4000 platform (LC Sciences, Hangzhou, China), and 2 × 150 bp (PE150) paired-end reads were generated according to Illumina's standard protocol. The raw sequencing data and the processed data have been deposited at the Gene Expression Omnibus (GEO) database under the accession number [GSE103763](#).

Small RNA library construction and sequencing analysis

Total RNA from the same samples (2.5 µg) was used to prepare small RNA libraries using the stranded Illumina TruSeq Small RNA Preparation Kit according to the manufacturer's recommendations (Illumina). The cDNA samples were fragmented, adaptor-ligated, amplified, and sequenced on an Illumina HiSeq 2500 platform (LC Sciences, Hangzhou, China), and single-end sequencing (50 bp) reads were obtained. The raw sequencing data and all the processed data have been deposited at the GEO database under the accession number [GSE102470](#).

In silico identification and differential expression analysis of circRNAs

The circRNAs were identified according to the following computational pipeline: First, the raw reads were mapped to a human reference genome hg38 (GRCh38.p7) using TopHat (v2.1.0) to exclude the linear transcripts (ftp://ftp.ensembl.org/pub/release-85/fasta/homo_sapiens/) [30]. Second, the unmapped reads were aligned to the human reference genome hg38 (GRCh38.p7) to identify the fusion transcripts using TopHat-Fusion (v2.1.0). Third, the fusion transcripts were used for identifying the known and novel circRNAs by the circExplorer algorithm [3]. The known circRNAs were annotated in circBase database (<http://www.circbase.org/>). The novel circRNAs were identified based on the following criteria: (1) GU/AG signal flanking the splice sites; (2) mismatch, ≤ 2; (3) back-spliced reads, ≥ 1; and (4) distance between two splice sites, ≤ 100 kb. Finally, the expression of circRNAs was quantified based on the number of reads spanning the back-splicing junction, and their fragments per kb for a million reads (FPKM) were calculated using Cuffdiff (v2.1.1) [31]. The differential circRNAs expression was analyzed using a two-tailed Student's t-test and the SPSS Statistics v19.0 software package (IBM, NY, USA). The circRNAs were considered significantly differentially expressed only when the log₂ fold-change was ≥ 1 or ≤ -1 and $P < 0.05$.

Identification and differential expression analysis of genes

The transcripts assembly and quantification were performed using Cufflinks (v2.1.1) [32]. The differential mRNA expression was analyzed using the CuffDiff program of Cufflinks by calculating the FPKM of mRNAs in all samples. The false discovery rate (FDR) was calculated by correcting the P -value. The genes were considered significantly differentially expressed when the log₂ fold-change was ≥ 1 or ≤ -1 and $P < 0.05$.

Identification and differential expression analysis of miRNAs

The clean small RNA reads, obtained after excluding the low-quality reads and adaptor sequences in the raw reads, were used for miRNA identification. Briefly, the sequences were blasted to human reference genome hg38 (GRCh38.p7), the RFam, and Rfam to identify the putative mRNA, rRNA, tRNA, snRNA, snoRNA, and other ncRNAs. To identify the known miRNAs, the remaining sequences were aligned to the miRBase (release 21.0) using Bowtie [33]. Matched sequences with < 1 mismatch were known miRNAs. In addition, the unmatched sequences were used to predict the candidate novel miRNAs using miRDeep2 [34]. The hairpin RNA structures containing the unmatched sequences were predicated, complying with the criteria of pre-miRNAs in order to identify the potentially novel miRNAs. The expression level of miRNAs was determined by normalizing the reads to tags per million (TPM) counts. The TPM was calculated as follows: normalized expression, TPM = (actual miRNA count/number of total clean read) × 10⁶. The miRNA differential expression, based on the normalized TPM expression value, was analyzed using the Student's t-test. The significance threshold was set to 0.05 for each test.

Real-time quantitative PCR

To validate the results of RNA-seq, we performed RT-qPCR analysis for the differentially expressed circRNAs, genes, and miRNAs. For circRNAs, the different primers encompassing the back-splicing junctions of circRNAs and the convergent primers of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were designed (Table 1). For genes, the convergent primers for selected genes were cited from the PrimerBank (<https://pga.mgh.harvard.edu/primerbank/>) (Table 2). For miRNAs, the following primer sets were purchased from the All-in-One™ miRNA qPCR primers (GeneCopoeia, Rockville, MD, USA) and used in this study: hsa-miR-146a-3p (HmiRQP0195), hsa-miR-196a-5p (HmiRQP0284), hsa-miR-4741 (HmiRQP2402), hsa-miR-6876-35p (HmiRQP3848), hsa-miR-663b (HmiRQP0775), and hsa-miR-1247-5p (HmiRQP0079). Reverse transcription was performed using a universal adaptor reverse primer at 37 °C for 60 min, followed by inactivation of the enzyme at 85 °C for 5 min. Next, the total RNA was isolated from HSV-1 infected and uninfected KMB17 cells, and cDNA was synthesized using a Reverse Transcription Kit (Promega, Madison, WI, USA). Then, the qPCR analysis was carried out on a CFX96™ Real-Time PCR system (Bio-Rad, CA, USA) using GoTaq qPCR Kit (Promega, Madison, WI, USA) setting different parameters for each type of RNAs. For circRNAs, qPCR parameters were as follows: 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 35 °C for 15 s, and extension at 72 °C for 15 s. For genes, the qPCR parameters were as follows: 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 40 °C for 15 s, and extension at 72 °C for 15 s. For miRNAs, qPCR was performed using All-in-One™ miRNA qRT-PCR Detection System (GeneCopoeia) according to the manufacturer's protocol. Subsequently, the qPCR reaction was conducted on a CFX96™ Real-Time PCR system (Bio-Rad) under standard conditions using the following program: 95 °C for 10 min and 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 10 s. The Homo sapiens housekeeping gene *GAPDH* was used as an internal control for circRNAs and genes. snRNA *U6* (HmiRQP9001) served as an internal control for miRNAs. A melting curve analysis was performed to confirm the primer specificities for circRNAs, genes, and miRNAs. The relative abundance of circRNA and genes was normalized against the internal control (*GAPDH* or *snU6*).

Gene ontology enrichment and pathway analysis

Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed for differentially expressed genes. GO terms and KEGG pathways with corrected *P*-values ≤ 0.05 indicated significant enrichment.

Integrated analyses of circRNAs, miRNAs, and genes

To perform an integration analysis of circRNA-miRNA-gene, top 10 HSV-1-induced circRNAs were selected. First, the complementary pairs between miRNAs and circRNAs and between miRNAs and genes were obtained by miRNA binding site analysis using TargetScan [35] and miRanda (v3.3a) [36]. Those genes that were significantly enriched in the “cell growth and apoptosis” and “cellular immunity” pathways were selected for further analysis. Three circRNAs and apoptosis/immunity-related genes with TargetScan scores > 150 and miRanda minimum free energy $\Delta G \leq -20$ kcal/mol were selected as miRNA targets. Second, the relationship among circRNA, miRNAs, and genes was analyzed by a Python script. Third, the circRNA-miRNA-gene network was constructed and presented using Cytoscape (v3.4.0) [37].

Table 1. Primers of circRNAs used for RT-qPCR

Primer name	Sequence	Amplicon size (bp)
circRNA6783-F	CAACCAGAGGGAACAC	154
circRNA6783-R	TCCTGTTCCACCATCCT	
circRNA3683-F	TGTAATCACTGGGAGAAT	168
circRNA3683-R	TTAGGGCTGAGGTTTGTG	
circRNA7231-F	GGACTTGGCTAATGAGAA	226
circRNA7231-R	CTGGACTTAAGTGGGGT	
circRNA3046-F	CACCTAGGACCTTCCAC	137
circRNA3046-R	TGAATCATCAGATGGTCTC	
circRNA7752-F	AGGGATAACTACCGATT	178
circRNA7752-R	CTAGGTCCATCATAAGCA	
GAPDH-F	CATGTACGTTGCTATCCAGGC	250
GAPDH-R	CTCCTTAATGTCACGCACGAT	

Table 2. Primers of genes used for RT-qPCR

Gene	Primer name	Sequence	Product (bp)
MAN1C1	MAN1C1-f	CTATAAGCGTTATGCAATGGGGA	120
	MAN1C1-r	TAGAGGGTATCGAGGGAGTCA	
HTR7	HTR7-f	AACAGATCAACTACGGCAGAGT	106
	HTR7-r	CACACGGAGATCACCA	
IL24	IL24-f	TTGCCTGGGTTTACCCTGC	117
	IL24-r	AAGGCTTCCACAGTTTCTGG	
ERO1LB	ERO1LB-f	TTCTGGATGATTGCTTGTGTGAT	127
	ERO1LB-r	GGTCGCTTCAGATTAACTTGT	
LMOD1	LMOD1-f	GGTGAACGTCAACAACCTCAGA	94
	LMOD1-r	GGCGAACAGCTTAACACAGT	
PTHLH	PTHLH-f	AAGGTGGAGACGTACAAAGAGC	109
	PTHLH-r	CAGAGCGAGTTCGCGGTTT	
MMP3	MMP3-f	CTGGACTCCGACACTCTGGA	79
	MMP3-r	CAGGAAGGTTCTGAAGTGACC	
LAMB3	LAMB3-f	CCAAAGGTGCGACTGCAATG	125
	LAMB3-r	AGTTCTTGCTTCGGGTGTTG	
BIRC3	BIRC3-f	TTTCCGTGGCTCTTATTCAAAT	96
	BIRC3-r	GCACAGTGGTAGGAACCTTCTAT	
GAPDH	GAPDH-f	ACAACCTTGGTATCTGTGAAGG	101
	GAPDH-r	GCCATCAGGCCACAGTTTC	

Statistical analyses

Quantile normalization and subsequent data processing were performed using the R software package (v3.3.3). Data were expressed as the mean $\pm$ standard deviation (SD). Student's t-test was used to evaluate the differences among groups. $P < 0.05$ was considered as statistically significant.

Results

Transcriptome-wide identification and characterization of circRNAs in HSV-1 infected and uninfected human fibroblasts

For comprehensive profiling of circRNAs in HSV-1-infected and uninfected KMB17 cells, the rRNA-depleted RNA obtained from six HSV-1-infected and uninfected cell samples were used for whole transcriptome sequencing. As a result, multiple back-spliced reads (about 1% of the total reads) were obtained by mapping canonical and non-canonical fusion-junctions to the human reference genome. These included 1, 009, 970 (1.33%), 737, 922 (1.08%), and 960, 581 (1.33%) back-spliced reads in three control cell samples, and 1, 145, 752 (1.52%), 943, 186 (1.19%), and 746, 927 (1.03%) in three HSV-1 infected cell samples, respectively. Based on these back-spliced reads, we identified 22, 906 putative circRNAs from 6, 522 parental genes with at least one back-spliced read in a single RNA library. These circRNA candidates comprised of 2, 377 intron-derived circRNAs (ciRNAs) and 20, 529 exon-derived circRNAs (circRNA), indicating that most of the circRNAs (up to 90%) were transcribed from gene-containing regions. Furthermore, only 284 circRNAs were annotated in circBase database (<http://www.circbase.org/>), and others represented novel circRNAs. The length of the circRNAs varied from 63–17, 180 nt. Numerous circRNAs seemed to be expressed in all samples, as only 62 circRNAs were detected in the three HSV-1-infected cell samples, suggesting that these circRNAs were strongly induced as virus-responsive circRNAs during HSV-1 infection. Moreover, we also observed that most of the circRNAs possessed a small number of back-spliced reads (< 50 reads) (Fig. 1A). A Circos plot showed that these circRNAs were widely distributed among all chromosomes, including sex chromosomes (Fig. 1B). Clustering analysis of highly expressed circRNAs (FPKM > 10) suggested differential expression patterns of circRNAs in HSV-1-infected and uninfected cells (Fig. 1C). To further investigate the expression features of the circRNAs, the relationship between the circRNA reads and their parental gene abundance was analyzed. Interestingly, we found that the genes with a higher number of circRNA reads also showed higher expression levels of

Fig. 1. Identification and characterization of circRNAs expressed in HSV-1-infected and uninfected KMB17 cells. (A) The number of circRNAs containing different back-spliced reads in KMB17 cells. (B) The Circos plots depicting the abundance all circRNA transcripts on different chromosomes across all samples. The expression of circRNAs was visualized based on their FPKM expression value. (C) Heatmap of the maximally expressed circRNAs (FPKM > 10) across all samples. (D) The correlation of the FPKM values between circRNA reads and their parental genes. The box plot represents the FPKM value in the $\log_2$ scale of circRNA parental genes (Y-axis) corresponding to different circRNA reads in the $\log_2$ scale (X-axis).

their linear transcripts (Fig. 1D), thereby indicating a consistent expression pattern between circRNAs and their parental genes. Taken together, these results provided a valuable clue for the identification of circRNAs that might be involved in the regulation of interactions between HSV-1 and host cells.

Cellular circRNAs in response to HSV-1 infection

Recent studies indicated that circRNAs function as miRNA sponges that can regulate the gene expression indirectly, which can affect multiple pathological processes [38-40]. Thus, to investigate the putative function of circRNAs during HSV-1 infection, we compared the expression profiles of circRNAs in HSV-1-infected and uninfected KMB17 cells by RNA-seq analysis. The results showed that a total of 536 circRNAs, including 188 upregulated and 348 downregulated circRNAs, were significantly dysregulated in HSV-1-infected cells (Fig. 2A). Interestingly, the downregulated circRNAs (64.93%) were more common than upregulated circRNAs (35.07%) in the RNA-seq data, suggesting a predominant role for the downregulated circRNAs in the case of virus infection. The hierarchical cluster analysis indicated distinguishable circRNA expression patterns among different samples (Fig. 2A). The differentially expressed circRNAs were present on various chromosomes based on their sequencing counts (Fig. 2B). Importantly, we detected abundant virus-responsive circRNAs, such as circRNA1450, circRNA13804, circRNA13855, circRNA13896, and circRNA13902, which were significantly dysregulated in HSV-1-infected cells. Therefore, we focused on those circRNAs induced by HSV-1 in the subsequent functional analysis of circRNAs. Furthermore, to validate the results of RNA-seq, we measured the expression levels of the randomly selected 5 circRNAs (circRNA3046, circRNA3683, circRNA6783, circRNA7752, and circRNA7231) by RT-qPCR using the primers designed to amplify the circRNA-specific back-splice junctions. As expected, the randomly selected 5 circRNA exhibited an expression trend consistent between RNA-seq and RT-qPCR (Fig. 2C). Collectively, these results revealed a comprehensive expression profile of dysregulated circRNAs in HSV-1-infected cells, suggesting a potential involvement of these dysregulated and virus-responsive circRNAs in the regulation of host-virus interactions. However, further studies are essential in determining the putative roles and mechanism of circRNAs during HSV-1 infection.

Dysregulated genes induced by HSV-1

To understand the cellular responses to HSV-1 infection, we identified the differentially expressed genes (DEGs) between HSV-1-infected and mock-infected cells. In order to improve the accuracy of the current gene quantification, the data from the three biological replicates were merged, and PPKM (fragments per kilobase per million reads) values calculated. We detected 3,885 differentially expressed protein-coding genes, which comprised of 1,377 upregulated and 2,508 downregulated genes. Interestingly, a large number of DEGs were found to be associated with “cell growth and death” and “immune response” induced by HSV-1. Genes, such as *RELA*, *TRAF3*, *NFKBIA*, *DHX58*, *TRIM25*, *NFKB1*, *POLR3GL*, *PYCARD*, *NFKBIB*, *IL1B*, *SOCS2*, and *STAT3* were associated with “immune response” and were significantly dysregulated by HSV-1 infection. The genes, such as *TGFB2*, *CDK1*, *TP53*, *CDC25B*, *PCNA*, *RBL1*, *CCNA2*, *SKP2*, *CCNB1*, *E2F3*, and *CDK6*, were associated with “cell growth and death” and significantly dysregulated upon HSV-1 infection. The hierarchical clustering analysis revealed differences in the expression of genes between HSV-1-infected and mock-infected cells (Fig. 3A). Furthermore, the transcriptome dynamics of both HSV-1-infected and mock-infected cells were visualized using MA-plots (the log₂ fold-change in the expression plotted against the mean of normalized counts (Fig. 3B). To verify the RNA-seq data, we performed RT-qPCR on a subset of 9 randomly selected genes. As expected, the fold-changes in the target gene expression in HSV-1 infected cells showed similar expression trends in the qPCR and RNA-seq data as compared to the mock-infected cells (Fig. 3C). In summary, these results indicated that HSV-1 could increase and decrease the expression levels of a number of host genes, suggesting a putative role for these dysregulated genes in cellular responses to HSV-1 infection.

Fig. 2. Dysregulated circRNAs after HSV-1 infection. (A) Heatmap of differentially expressed circRNAs induced by HSV-1 in KMB17 cells. Red color indicates high expression level, and blue color indicates low expression level. (B) The global distribution of dysregulated circRNAs in human chromosomes. (C) Verification of dysregulated circRNAs by RT-qPCR. Divergent primers were designed to amplify the back-spliced junction sequences for circRNA candidates. qPCR data were normalized against that of human GAPDH. The differences among groups were analyzed using the t-test (two-tailed and unpaired). P-value < 0.05 (*), < 0.01 (**) or < 0.001 (***) was considered as statistically significant.

Cellular miRNA in response to HSV-1 infection

To investigate the influence of HSV-1 infection on the expression of cellular miRNAs, six small RNA libraries from the same samples as transcriptome sequencing were constructed and sequenced. A comparison of the miRNA expression profile in HSV-1-infected and mock-infected cells revealed that a total of 142 known and 65 novel miRNAs were significantly dysregulated, of which, 128 were upregulated and 79 down-regulated. Especially, the expression level of hsa-miR-7974 and hsa-miR-4449 are altered markedly with -5.12-fold- and 6.16-fold-change, respectively. The differential expression patterns of miRNAs in HSV-1-infected and mock-infected cells were presented by a cluster heatmap (Fig. 4A). To further confirm the results of RNA-seq, we estimated the differential expression of hsa-miR-146a-3p, hsa-miR-196a-5p, hsa-miR-4741, hsa-miR-6876-35p, hsa-miR-663b, and hsa-miR-1247-5p by RT-qPCR. These RT-qPCR results were consistent with those of the RNA-seq results,

Fig. 3. Dysregulated genes after HSV-1 infection. (A) Heatmap of differentially expressed genes induced by HSV-1 in KMB17 cells. Red color indicates a high expression level, and blue color indicates a low expression level. (B) MA-plots show transcriptome dynamics of both HSV-1 infected and uninfected KMB17 cells. The $\log_2$ fold-change in the expression (Y-axis) was plotted against the mean of normalized counts (X-axis). (C) Validation of dysregulated genes by RT-qPCR. qPCR data was normalized against the human gene GAPDH. Differences among groups were analyzed using the t-test (two-tailed and unpaired). A P-value of < 0.05 (*), < 0.01 (**) or < 0.001 (***) was considered statistically significant.

suggesting that the expression profiles of these cellular miRNAs were in agreement with those observed in the RNA-seq assay (Fig. 4B). Overall, these results suggested that the expression of host miRNAs was altered by HSV-1.

Functional enrichment and pathway analysis of the dysregulated genes

To gain insight into the functions of the dysregulated genes and the biological processes involved in the HSV-1 infection, the dysregulated genes were mapped to the terms in the GO and KEGG databases. The GO analysis identified diverse and enriched biological processes, including “toll-like receptor signaling pathway”, “cell death”, “regulation of growth”, “cell cycle”, “cell apoptosis”, and “signal transduction” that were associated with the cellular response to HSV-1 (Fig. 5A), thereby indicating that viral infection elicited a strong cellular response. Moreover, the KEGG pathway analysis indicated that dysregulated genes were primarily enriched in the pathways associated with the host immune response, such as cancer, inflammatory response, and cell metabolism (Fig. 5B). Intriguingly, 19 dysregulated genes (*BIRC3*, *RELA*, *HSP90B1*, *NFKBIA*, *MAPK3*, *PYCARD*, *CCL2*, *NLRP1*, *NFKBIB*, *IL1B*, *MAPK11*, *CXCL1*, *NFKB1*, *CXCL2*, *MAPK10*, *TNFAIP3*, *IL6*, *TRIP6*, and *RIPK2*) were enriched in

Fig. 4. Dysregulated miRNAs after HSV-1 infection. (A) Heatmap of differentially expressed miRNAs induced by HSV-1 in KMB17 cells. Red color indicates high expression level, and blue color indicates low expression level. (B) Validation of dysregulated miRNAs by RT-qPCR. The qPCR data were normalized against that of cellular snU6.

Fig. 5. GO functional enrichment and KEGG pathway analysis of the dysregulated genes. (A) Top 20 maximally enriched GO terms of dysregulated genes induced by HSV-1. The X-axis represents the rich factor; the Y-axis represents the GO term. The point size represents the number of genes enriched in a specific GO term. (B) Top 20 most enriched KEGG pathways of dysregulated genes induced by HSV-1. The X-axis represents the rich factor; the Y-axis represents the pathway names. The point size indicates the number of genes enriched in a specific pathway.

the NOD-like receptor signaling pathway. Similarly, a total of 27 dysregulated genes (*IL24*, *CLCF1*, *IL23A*, *SOCS2*, *SPRY2*, *IL4R*, *CREBBP*, *SOCS1*, *CSF3*, *STAT3*, *GRB2*, *IL11*, *SPRED2*, *LIF*, *SPRY1*, *IL7R*, *OSMR*, *PIK3R1*, *TSLP*, *CSF2*, *LIFR*, *SPRY4*, *PIM1*, *IL6*, *MYC*, *JAK2*, *IL13RA1*, and *IL13RA2*) were enriched in the JAK-STAT signaling pathway. Some other dysregulated genes

were enriched in the pathways related to inflammatory response and cancer. Overall, these results suggest that the dysregulated genes were involved in pathways implicated in the cellular immune response and viral pathogenesis, including inflammatory response and cell cycle progression.

Integrated in silico analyses of circRNAs, miRNAs, and genes reveal the putative involvement of circRNAs in virus-host interactions

In order to postulate the in-depth potential function of the significantly dysregulated circRNAs in cellular responses to virus infection, an integrated analysis of circRNA-miRNA-gene based on their differential expression profiles was performed. As a result, a global regulatory landscape of circRNAs in viral pathogenesis and cellular antiviral immunity was presented. Firstly, the regulatory roles of HSV-1-induced circRNAs were analyzed in viral pathogenesis. Since the pathogenicity of the virus is mainly reflected in the impact on cell growth and death, we analyzed the regulatory roles of circRNAs in “cell growth and death” pathways and found that several genes associated with “cell growth and death” such as *CDC6*, *IRAK3*, *CREBBP*, *SKP2*, *TGFB2*, and *IRAK4* were potentially regulated by HSV-1-induced circRNAs (Fig. 6). Interestingly, one circRNA could potentially regulate multiple genes. For instance, 17 genes (*GADD45G*, *PRKAR1B*, *RELA*, *TP53*, *CDC6*, *CDKN2B*, *IRAK2*, *ANAPC1*, *CDC6*, *CREBBP*, *IRAK4*, *CDKN2B*, *E2F3*, *IRAK3*, *MCM5*, *NFKBIA*, and *TNFRSF10B*) were potentially regulated by circRNA14189. Correspondingly, one gene could be potentially regulated by multiple circRNAs: for instance, circRNA14556, circRNA15053, and circRNA15655 putatively regulated *IRAK3*, which was mediated by cellular miRNAs based on a circRNA-miRNA-gene axis. These data suggested that the cell growth and death pathways might be regulated by HSV-1-induced circRNAs by a circRNA-miRNA-gene axis. Furthermore, to ascertain whether HSV-1-induced circRNAs regulate the immune response, we analyzed

Fig. 6. Regulatory roles of the top 10 circRNAs induced by HSV-1 on genes and pathways associated with “cell growth and death” based on the circRNA-miRNA-genes axis.

the interactions between circRNAs and immunity-related genes and observed that a large number of immunity-related genes, such as *IL11*, *SOCS2*, *SPRY1*, *MAP3K1*, *IL24*, *TRIM25*, *IRAK4*, *TNFAIP3*, and *TRIP6*, were regulated by HSV-1-induced circRNAs via the circRNA-miRNA-gene axis (Fig. 7). Because these genes could be significantly enriched in various immune response pathways, including the RIG-I-like receptor signaling pathway, Toll-like receptor signaling pathway, JAK-STAT signaling pathway, and cytosolic DNA-sensing pathway, it can be speculated that HSV-1-induced circRNAs are involved in cellular antiviral immunity by affecting the immunity-related genes. Taken together, these results suggested that HSV-1-induced circRNAs potentially regulate the cellular immune response and cell growth/death by functioning as miRNA sponges. Although these *in silico* results illuminate the mechanism underlying the circRNAs-mediated regulation of viral pathogenesis and cellular antiviral immunity, additional *in vitro* and *in vivo* investigations are essential to further substantiate the role and machinery of the significantly dysregulated circRNAs.

Discussion

As a novel type of endogenous noncoding RNA, circRNAs represent a recent research hotspot in the field of RNA. Although the functions of circRNAs are yet largely unclear, accumulating evidence indicates their involvement in modulating the disease progression [41-44]. Thus, it is vital to understand the biological functions of circRNAs by investigating their expression patterns and interactions with other types of RNAs. However, little is known about the expression patterns of circRNAs and their interaction with other types of RNAs

during viral infection. In the present study, we employed whole-transcriptome sequencing and small RNA sequencing to profile and identify the dysregulated circRNAs, genes, and miRNAs in human embryo lung fibroblasts (KMB17) following HSV-1 infection. These results provided valuable clues for an improved understanding of the roles and mechanisms underlying circRNAs in HSV-1 pathogenesis and cellular immunity.

Transcriptional profiling is known as a powerful tool for elucidating the host-virus interactions during infection. Recently, several studies focused on the transcriptome responses to virus infection, thereby suggesting that virus infection could remodel the host transcriptome landscape [45-47]. Thus, the virus can achieve the proliferation and cellular fitness by altering the host transcriptome responses, such as cellular responses associated with antiviral immunity and cell death/apoptosis. This remodeling effect of the virus on the host transcriptome is a common strategy exploited by the virus in host-virus interactions and has been observed in various viruses [48-50]. Notably, a recent study showed that HSV-1 infection could not only result in changes in gene expression but also in alternative polyadenylation and splicing in the host transcriptome by influencing the host RNA processing [51]. However, several concerns about the alteration of RNA transcripts at the whole-transcriptome level, as a result of HSV-1 infection, especially the circular RNA transcriptome, have not yet been elucidated. The current results provide genome-wide profiling and identification of dysregulated circRNAs, genes, and miRNAs that would facilitate further studies on the roles and mechanisms of circRNAs during HSV-1 infection. Furthermore, at the whole transcriptome level, virus infection can also alter the small RNA profiles of the host. Some small RNAs could be induced or exploited by viruses to favor the viral infection and persistence by attenuating the immune response, ceasing cell apoptosis, and promoting cell growth [52-54]. Interestingly, this strategy, as employed by a virus, was observed in HSV-1 and its host interactions [55-57]. Based on the roles of miRNAs during HSV-1 infection, we presumed that circRNAs functioned as miRNA sponges to indirectly regulate the host transcriptional responses. The integration analysis of multiple RNA expression profiles revealed the regulatory roles of circRNAs and specific links to other RNAs, by the circRNA-miRNA-gene regulatory axis, and those genes associated to viral pathogenesis and cellular immunity (Figs. 6 and 7).

Notably, a recent study demonstrated the involvement of circRNAs in viral infection. Xiang Li et al. found that biogenesis and function of circRNAs were regulated by immune factors NF90/NF110 upon viral infection [58]. This finding strongly supported the theory that circRNAs are involved in the regulation of interactions between the virus and its host cell. Consistent with this hypothesis, we also discovered abundant circRNAs that served as virus-responsive circRNAs and were induced by HSV-1 infection. Functional analysis suggested their roles in viral pathogenesis and cellular immunity effectuated by the circRNA-miRNA-gene regulatory axis. In summary, the present study not only describes the altered expression of circRNAs, genes, and miRNAs during HSV-1 infection of KMB17 cells but also raises issues as follows: How does HSV-1 induce expression changes of circRNAs? What are the precise roles and mechanisms underlying the circRNAs explicitly induced by HSV-1? Although the putative roles of circRNAs were analyzed by an integrated analysis of circRNAs, genes, and miRNAs, the mechanisms underlying the action of circRNAs in HSV-1 infection have not yet been validated experimentally. Therefore, the changes in host cell circRNAs in response to HSV-1 infection might serve as a putative mechanism that coordinates the gene expression and determines the outcome of viral infection. Nevertheless, future studies are aimed at understanding the regulatory roles and mechanisms of these circRNAs in the etiology of HSV-1 infection.

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Disclosure Statement

The authors declare that no conflict of interests exists.

References

- Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO: Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 2012;7:e30733.
- Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N: Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013;495:333-338.
- Zhao J, Li L, Wang Q, Han H, Zhan Q, Xu M: CircRNA expression profile in early-stage lung adenocarcinoma patients. *Cell Physiol Biochem* 2017;44:2138-2146.
- Zheng J, Li Z, Wang T, Zhao Y, Wang Y: Microarray expression profile of circular RNAs in plasma from primary biliary cholangitis patients. *Cell Physiol Biochem* 2017;44:1271-1281.
- Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L: Complementary sequence-mediated exon circularization. *Cell* 2014;159:134-147.
- Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H: Circular RNA: A new star of noncoding RNAs. *Cancer Lett* 2015;365:141-148.
- Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO: Cell-type specific features of circular RNA expression. *PLoS Genet* 2013;9:e1003777.
- Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE: Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 2013;19:141-157.
- Danan M, Schwartz S, Edelheit S, Sorek R: Transcriptome-wide discovery of circular RNAs in Archaea. *Nucleic Acids Res* 2012;40: 3131-3142.
- Kramer MC, Liang D, Tatomer DC, Gold B, March ZM, Cherry S, Wilusz JE: Combinatorial control of Drosophila circular RNA expression by intronic repeats, hnRNPs, and SR proteins. *Genes Dev* 2015;29: 2168-2182.
- Song CL, Wang JP, Xue X, Liu N, Zhang XH, Zhao Z, Liu JG, Zhang CP, Piao ZH, Liu Y, Yang YB: Effect of Circular ANRIL on the Inflammatory Response of Vascular Endothelial Cells in a Rat Model of Coronary Atherosclerosis. *Cell Physiol Biochem* 2017;42:1202-1212.
- Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G: Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* 2015;22:256-264.
- Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evtantal N, Memczak S, Rajewsky N, Kadener S: circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell* 2014;56:55-66.
- Chao CW, Chan DC, Kuo A, Leder P: The mouse formin (Fmn) gene: abundant circular RNA transcripts and gene-targeted deletion analysis. *Mol Med* 1998;4:614-628.
- Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB: Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res* 2016;44:2846-2858.
- Ebbesen KK, Kjems J, Hansen TB: Circular RNAs: Identification, biogenesis and function. *Biochim Biophys Acta* 2016;1859:163-168.
- Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J: Natural RNA circles function as efficient microRNA sponges. *Nature* 2013;495:384-388.
- Nicoll MP, Proença JT, Efsthathiou S: The molecular basis of herpes simplex virus latency. *FEMS Microbiol Rev* 2012;36:684-705.

- 19 Al-Dujaili LJ, Clerkin PP, Clement C, McFerrin HE, Bhattacharjee PS, Varnell ED, Kaufman HE, Hill JM: Ocular herpes simplex virus: how are latency, reactivation, recurrent disease and therapy interrelated? *Future Microbiol* 2011;6:877-907.
- 20 Su C, Zhan G, Zheng C: Evasion of host antiviral innate immunity by HSV-1, an update. *Virol J* 2016;13:38.
- 21 Kelly BJ, Fraefel C, Cunningham AL, Diefenbach RJ: Functional roles of the tegument proteins of herpes simplex virus type 1. *Virus Res* 2009;145:173-186.
- 22 Rajcáni J, Durmanová V: Early expression of herpes simplex virus (HSV) proteins and reactivation of latent infection. *Folia Microbiol (Praha)* 2000;45:7-28.
- 23 Lee S, Ives AM, Bertke AS: Herpes Simplex Virus 1 Reactivates from Autonomic Ciliary Ganglia Independently from Sensory Trigeminal Ganglia To Cause Recurrent Ocular Disease. *J Virol* 2015;89:8383-8391.
- 24 Nicoll MP, Hann W, Shivkumar M, Harman LE, Connor V, Coleman HM, Proença JT, Efsthathiou S: The HSV-1 Latency-Associated Transcript Functions to Repress Latent Phase Lytic Gene Expression and Suppress Virus Reactivation from Latently Infected Neurons. *PLoS Pathog* 2016;12:e1005539.
- 25 Padgett DA, Sheridan JF, Dorne J, Berntson GG, Candelora J, Glaser R: Social stress and the reactivation of latent herpes simplex virus type 1 *Proc Natl Acad Sci U S A* 1998;95:7231-7235.
- 26 Prasad A, Remick J, Zeichner SL: Activation of human herpesvirus replication by apoptosis. *J Virol* 2013;87:10641-110650.
- 27 Lafaille FG, Pessach IM, Zhang SY, Ciancanelli MJ, Herman M, Abhyankar A, Ying SW, Keros S, Goldstein PA, Mostoslavsky G, Ordoñas-Montanes J, Jouanguy E, Plancoulaine S, Tu E, Elkabetz Y, Al-Muhsen S, Tardieu M, Schlaeger TM, Daley GQ, Abel L, Casanova JL, Studer L, Notarangelo LD: Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature* 2012;491:769-773.
- 28 Smith S, Reuven N, Mohni KN, Schumacher AJ, Weller SK: Structure of the herpes simplex virus 1 genome: manipulation of nicks and gaps can abrogate infectivity and alter the cellular DNA damage response. *J Virol* 2014;88:10146-10156.
- 29 Deshiere A, Joly-Beauparlant C, Breton Y, Ouellet M, Raymond F, Lodge R, Barat C, Roy MA, Corbeil J, Tremblay MJ: Global Mapping of the Macrophage-HIV-1 Transcriptome Reveals that Productive Infection Induces Remodeling of Host Cell DNA and Chromatin. *Sci Rep* 2017;7:5238.
- 30 Cao J, Wu L, Jin M, Li T, Hui K, Ren Q: Transcriptome profiling of the Macrobrachium rosenbergii lymphoid organ under the white spot syndrome virus challenge. *Fish Shellfish Immunol* 2017;67:27-39.
- 31 Dong S, Behura SK, Franz AWE: The midgut transcriptome of Aedes aegypti fed with saline or protein meals containing chikungunya virus reveals genes potentially involved in viral midgut escape. *BMC Genomics* 2017;18:382.
- 32 Wu L, Zhang X, Che Y, Zhang Y, Tang S, Liao Y, Na R, Xiong X, Liu L, Li Q: A cellular response protein induced during HSV-1 infection inhibits viral replication by interacting with ATF5. *Sci China Life Sci* 2013; 56:1124-1133.
- 33 Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL: TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 2013;14:R36.
- 34 Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L: Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat Biotechnol* 2013;31:46-53.
- 35 Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L: Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 2012;7:562-578.
- 36 Langmead B, Salzberg SL: Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357-359.
- 37 Friedländer MR, Mackowiak SD, Li N, Chen W, Rajewsky N: miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Res* 2012;40:37-52.
- 38 Lewis BP, Burge CB, Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005;120:15-20.
- 39 Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS: MicroRNA targets in Drosophila. *Genome Biol* 2003;5:R1.
- 40 Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13:2498-2504.

- 41 Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, Liang L, Gu J, He X, Huang S: Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun* 2016;7:11215.
- 42 Liu Q, Zhang X, Hu X, Dai L, Fu X, Zhang J, Ao Y: Circular RNA Related to the Chondrocyte ECM Regulates MMP13 Expression by Functioning as a MiR-136 'Sponge' in Human Cartilage Degradation. *Sci Rep* 2016;6:22572.
- 43 Peng L, Chen G, Zhu Z, Shen Z, Du C, Zang R, Su Y, Xie H, Li H, Xu X, Xia Y, Tang W: Circular RNA ZNF609 functions as a competitive endogenous RNA to regulate AKT3 expression by sponging miR-150-5p in Hirschsprung's disease. *Oncotarget* 2017;8:808-818.
- 44 Liu L, Li L, Ma X, Yue F, Wang Y, Wang L, Jin P, Zhang X: Altered Circular RNA Expression in Patients with Repeated Implantation Failure. *Cell Physiol Biochem* 2017;44:303-313.
- 45 Liu W, Zhang J, Zou C, Xie X, Wang Y, Wang B, Zhao Z, Tu J, Wang X, Li H, Shen J, Yin J: Microarray Expression Profile and Functional Analysis of Circular RNAs in Osteosarcoma. *Cell Physiol Biochem* 2017;43:969-985.
- 46 Ouyang Q, Wu J, Jiang Z, Zhao J, Wang R, Lou A, Zhu D, Shi GP, Yang M: Microarray Expression Profile of Circular RNAs in Peripheral Blood Mononuclear Cells from Rheumatoid Arthritis Patients. *Cell Physiol Biochem* 2017;42:651-659.
- 47 Li H, Hao X, Wang H, Liu Z, He Y, Pu M, Zhang H, Yu H, Duan J, Qu S: Circular RNA Expression Profile of Pancreatic Ductal Adenocarcinoma Revealed by Microarray. *Cell Physiol Biochem* 2016;40:1334-1344.
- 48 Wang Y, Zhang H, Lu Y, Wang F, Liu L, Liu J, Liu X: Comparative transcriptome analysis of zebrafish (*Danio rerio*) brain and spleen infected with spring viremia of carp virus (SVCV). *Fish Shellfish Immunol* 2017;69:35-45.
- 49 Gao FX, Wang Y, Zhang QY, Mou CY, Li Z, Deng YS, Zhou L, Gui JF: Distinct herpesvirus resistances and immune responses of three gynogenetic clones of gibel carp revealed by comprehensive transcriptomes. *BMC Genomics* 2017;18:561.
- 50 Martin KM, Barandoc-Alviar K, Schneweis DJ, Stewart CL, Rotenberg D, Whitfield AE: Transcriptomic response of the insect vector, *Peregrinus maidis*, to Maize mosaic rhabdovirus and identification of conserved responses to propagative viruses in hopper vectors. *Virology* 2017;509:71-81.
- 51 Zheng Y, Wang Y, Ding B, Fei Z: Comprehensive Transcriptome Analyses Reveal that Potato Spindle Tuber Viroid Triggers Genome-Wide Changes in Alternative Splicing, Inducible trans-Acting Activity of Phased Secondary Small Interfering RNAs, and Immune Responses. *J Virol* 2017;91.
- 52 Chen W, Yi L, Feng S, Liu X, Asim M, Zhou Y, Lan J, Jiang S, Tu J, Lin L: Transcriptomic profiles of striped snakehead fish cells (SSN-1) infected with red-spotted grouper nervous necrosis virus (RGNNV) with an emphasis on apoptosis pathway. *Fish Shellfish Immunol* 2017;60:346-354.
- 53 Wang L, Tang N, Gao X, Guo D, Chang Z, Fu Y, Akinyemi IA, Wu Q: Understanding the immune system architecture and transcriptome responses to southern rice black-streaked dwarf virus in *Sogatella furcifera*. *Sci Rep* 2016;6:36254.
- 54 Hu B, Li X, Huo Y, Yu Y, Zhang Q, Chen G, Zhang Y, Fraser NW, Wu D, Zhou J: Cellular responses to HSV-1 infection are linked to specific types of alterations in the host transcriptome. *Sci Rep* 2016;6:28075.
- 55 Chen J, Shi X, Zhang X, Wang A, Wang L, Yang Y, Deng R, Zhang GP: MicroRNA 373 Facilitates the Replication of Porcine Reproductive and Respiratory Syndrome Virus by Its Negative Regulation of Type I Interferon Induction. *J Virol* 2017;91.
- 56 Ni S, Yan Y, Cui H, Yu Y, Huang Y, Qin Q: Fish miR-146a promotes Singapore grouper iridovirus infection by regulating cell apoptosis and NF- κ B activation. *J Gen Virol* 2017;98:1489-1499.
- 57 Yin D, Wang Y, Sai W, Zhang L, Miao Y, Cao L, Zhai X, Feng X, Yang L: HBx-induced miR-21 suppresses cell apoptosis in hepatocellular carcinoma by targeting interleukin-12. *Oncol Rep* 2016;36:2305-2312.
- 58 Ru J, Sun H, Fan H, Wang C, Li Y, Liu M, Tang H: MiR-23a facilitates the replication of HSV-1 through the suppression of interferon regulatory factor 1. *PLoS One* 2014; 9:e114021.
- 59 Pan D, Flores O, Umbach JL, Pesola JM, Bentley P, Rosato PC, Leib DA, Cullen BR, Coen DM: A neuron-specific host microRNA targets herpes simplex virus-1 ICP0 expression and promotes latency. *Cell Host Microbe* 2014;15:446-56.
- 60 Hill JM, Zhao Y, Clement C, Neumann DM, Lukiw WJ: HSV-1 infection of human brain cells induces miRNA-146a and Alzheimer-type inflammatory signaling. *Neuroreport* 2009;20:1500-1505.
- 61 Li X, Liu CX, Xue W, Zhang Y, Jiang S, Yin QF, Wei J, Yao RW, Yang L, Chen LL: Coordinated circRNA Biogenesis and Function with NF90/NF110 in Viral Infection. *Mol Cell* 2017;67:214-227.