Expression of Herpes Simplex Virus 1 MicroRNAs in Cell Culture Models of Quiescent and Latent Infection

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To facilitate studies of herpes simplex virus 1 latency, cell culture models of quiescent or latent infection have been developed. Using deep sequencing, we analyzed the expression of viral microRNAs (miRNAs) in two models employing human fibroblasts and one using rat neurons. In all cases, the expression patterns differed from that in productively infected cells, with the rat neuron pattern most closely resembling that found in latently infected human or mouse ganglia in vivo.

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fter primary infection of mammalian hosts, herpes simplex virus 1 (HSV-1) establishes a lifelong latent infection in neurons of sensory ganglia (reviewed in reference 1). A hallmark of latency is the abundant expression of the latency-associated transcripts (LATs) and specific virus-encoded microRNAs (miRNAs; reviewed in references 1 and 2). Some HSV-1 miRNAs are expressed preferentially during productive (“lytic”) infection in cell culture and others during latent infection in sensory ganglia. For example, HSV-1 miR-H1 is abundantly expressed only during lytic infection, while miR-H2, -H3, and -H4 are most predominantly expressed during latency (3–6). An exception is HSV-1 miR-H6, which is expressed at high levels during both lytic and latent infection (3–5, 7, 8). There is evidence suggesting that certain HSV-1 miRNAs can repress the expression of important HSV-1 activators of lytic gene expression (7, 9).

Addressing functions of miRNAs (and other gene products) for latent infection has been experimentally challenging, as studies of HSV latency have largely been limited to animal models. To address this challenge, cell culture models have been developed. To assess whether HSV-1 miRNA expression in three such models resembles that seen in latently infected ganglia, we extracted RNA, generated small-RNA libraries (TrueSeq small RNA sample preparation kit; Illumina), and sequenced the libraries on a HiSeq2000 sequencer (Illumina) at the Biopolymers Facility, Departments of Genetics, Harvard Medical School. Data sets were analyzed using miRanalyzer software (http://www.ncbi.nlm.nih.gov/pubmed/21515631) and aligned against known miRNAs from miRBase release 19 (10) by using Bowtie seed alignment, allowing for no mismatches within the first 18 nucleotides (11, 12). The total number of aligned sequence reads (read count) for each viral miRNA was normalized using read counts for cellular let-7a, whose expression does not change during HSV-1 infection (4, 6). Although the number of sequence reads does not necessarily reflect the actual abundance of a specific miRNA, it can be used to compare miRNA abundances among different samples (13–15).

We analyzed a model employing primary human fetal foreskin fibroblasts (HFF2; European Collection of Cell Cultures) infected at a multiplicity of infection (MOI) of ~3 at 38.5°C with HSV-1 strain 17syn+, a virus mutant derived from HSV-1 strain 17syn that carries mutations in genes encoding VP16, ICP0, and ICP4 (16–18). Under these conditions, the virus exhibits negligible lytic gene expression and does not produce infectious virus but can be reactivated by certain stimuli (18, 19). We analyzed infected cells at 1.5 and 16 h postinfection (hpi) (during establishment of quiescent infection) and at 8 days postinfection (dpi) (quiescent infection). We detected only four HSV-1 miRNAs (Fig. 1A). Of these, miR-H1 declined slightly during the experimental time frame, while miR-H4-5p increased slightly and miR-H3 and H4-3p increased substantially (Fig. 1A). Notably, we did not detect miR-H2 or miR-H6, which are relatively abundant in latently infected sensory ganglia (3, 4, 8) and whose expression is abolished by a 200-bp deletion that removes the LAT promoter (4). This expression pattern might be due in part to the activity of the long-short junction-spanning transcript promoter, which is ordinarily repressed by ICP4 (20, 21).

In the second model, originally developed by McMahon and Walsh (22), serum-starved HFF cells were infected with wild-type (WT) HSV-1 strain KOS at an MOI of ~1 and incubated at 42°C until 72 hpi, at which point we detected no infectious virus or expression of the late protein, gC (data not shown). In this model, lytic replication of the virus can be triggered by exogenous expression of HSV-1 ICP0 (22). We detected expression of one or both (guide and passenger) strands of most HSV-1 miRNAs but at low levels (fewer than 10 reads), except for miR-H3 at 72 hpi and miR-H4-3p and miR-H6 at 18 and 72 hpi (Fig. 1B). There were more reads for miR-H1, a lytic miRNA, than for miR-H2, a latent miRNA. The expression of most miRNAs increased between 18 and 72 hpi, although miR-H6 levels increased only ~1.5-fold (Fig. 1B). Overall, this model appears to exhibit relatively low-level expression of both lytic and latent miRNAs.

In the third model, we investigated primary neurons derived from rat superior cervical ganglia (SCG; obtained using an IACUC-approved protocol). We analyzed infected cells at 1.5 and 16 hpi and at 8 days postinfection (dpi) (quiescent infection). We detected only four HSV-1 miRNAs (Fig. 1A). Of these, miR-H1 declined slightly during the experimental time frame, while miR-H4-5p increased slightly and miR-H3 and H4-3p increased substantially (Fig. 1A). Notably, we did not detect miR-H2 or miR-H6, which are relatively abundant in latently infected sensory ganglia (3, 4, 8) and whose expression is abolished by a 200-bp deletion that removes the LAT promoter (4). This expression pattern might be due in part to the activity of the long-short junction-spanning transcript promoter, which is ordinarily repressed by ICP4 (20, 21).

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approved protocol) that were infected with HSV-1 Patton strain GFP-Us11 at an MOI of 1 in the presence of acyclovir (ACV) and nerve growth factor (NGF) (23–25). Infectious virus and lytic gene expression are repressed, and virus can be reactivated by interruption of NGF signaling (23, 24). In latently infected neurons (7 dpi), we detected a relatively low number of reads for the lytic miRNA, miR-H1, and much higher numbers of reads for latent miRNAs H2, H3, H4, H6, H7, and H8 (Fig. 1C). The abundant expression of these miRNAs resembles the expression pattern previously found in HSV-1 latency in vivo (3–5, 7). In addition, the proportion of HSV-1 miRNAs in the total number of reads that could be aligned to the HSV-1 genome (mapped reads) was much higher in latently infected rat neurons (~0.2%) than in the other models (~0.01%) (data not shown). Moreover, in the rat neuron model, most HSV-1-specific reads corresponded to HSV-1 miRNAs, whereas in the other models we also detected a large number of reads representing degradation products of lytic mRNAs (data not shown). Similarly, using quantitative stem-loop reverse transcription-PCR, as described previously (4), we found much higher levels of miR-H2 and miR-H4-3p than miR-H1 in this model, regardless of whether we used HSV-1 Patton strain GFP-Us11 or WT strains 17syn or KOS (Fig. 2). Taken together, these data indicate a program of miRNA expression in latently infected rat neurons in cell culture that appears more physiologically relevant to latency in vivo than that in the fibroblast systems. The more restricted program of gene expression in the in1374-infected HFF2 model compared to that in the rat neuron model may reflect cell type differences in host factors important for expression of latency-specific miRNAs, greater repression of viral transcription generally in quiescently infected fibroblasts than in neurons (26), and/or, possibly, an alternative version of the latency program.

Although cell culture models of latency cannot entirely reproduce in vivo conditions, such models are easily accessible, permit a relatively high MOI, and allow a variety of analyses that are extremely difficult in animal models. The complex nature of HSV-1 latency, including the expression of miRNAs, should be considered when selecting the appropriate model. The rat neuron model may be particularly useful for examining how mutations or oligonucleotides altering expression or function of latency-specific miRNAs affect parameters such as viral gene expression and reactivation.

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