Analysis of human α-herpesvirus microRNA expression in latently infected human trigeminal ganglia

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Abstract

Analysis of cells infected by a wide range of herpesviruses has identified numerous virally encoded microRNAs (miRNAs), and several reports suggest that these viral miRNAs are likely to play key roles in several aspects of the herpesvirus life cycle. Here, we report the first analysis of human ganglia for the presence of virally encoded miRNAs. Deep sequencing of human trigeminal ganglia latently infected with two pathogenic α-herpesviruses, Herpes Simplex Virus 1 (HSV-1) and Varicella Zoster Virus (VZV), confirmed expression of five HSV-1 miRNAs, miR-H2 through miR-H6, which had been previously observed in mice latently infected with HSV-1. In addition, two novel HSV-1 miRNAs, termed miR-H7 and miR-H8, were also identified. Like four of the previously reported HSV-1 miRNAs, miR-H7 and miR-H8 are encoded within the second exon of the HSV-1 latency associated transcript. Although VZV genomic DNA was readily detectable in the three human trigeminal ganglia analyzed, we failed to detect any VZV miRNAs suggesting that VZV, unlike other herpesviruses examined so far, may not express viral miRNAs in latently infected cells.
Introduction

MicroRNAs (miRNAs) are a family of ~22 nt long non-coding RNAs that are capable of binding to, and inhibiting, the expression of specific target mRNAs (reviewed in 1). They are typically derived from one arm of RNA stem-loops found within non-coding regions of capped and polyadenylated transcripts (4, 26). Successive cleavage of these hairpin structures by the RNAse III enzymes Drosha in the nucleus (25), and Dicer in the cytoplasm (7, 20), generates a miRNA duplex structure of ~20 bp with 2-nt 3’-overhangs. One arm of this duplex is then loaded into the RNA induced silencing complex (RISC), where it is used as a guide to target complementary transcripts for inhibition (19, 28). In mammalian cells, miRNAs usually guide RISC to imperfectly complementary target sites, resulting in the translational arrest of bound mRNAs and a modest but detectable mRNA destabilization (12, 31, 43).

Due to their small size and non-immunogenic nature, miRNAs appear ideally suited for use as regulatory molecules by viruses and indeed, a number of human DNA viruses have now been reported to encode miRNAs, including many herpesviruses (39). Herpesviruses can be divided into three subfamilies, the α-, β- and γ-herpesviruses, based on replication characteristics, genomic organization, and preferred latency sites. Members of all three sub-families have been found to encode miRNAs, ranging from a low of 3 in the α-herpesvirus Herpes Simplex Virus 2 (HSV-2) (37, 38) to a high of 25 in Epstein-Barr virus (EBV) (5, 17, 33, 46). The fact that all herpesviruses examined to date express miRNAs suggests that they play important roles in the herpesvirus life cycle, and several studies have in fact demonstrated the down-regulation of cellular and/or viral mRNA targets by herpesvirus miRNAs (reviewed in 16).
Herpes simplex virus 1 (HSV-1) and varicella-zoster virus (VZV) are pathogenic human viruses that both belong to the α-herpesvirus subfamily. HSV-1, the prototypic α-herpesvirus, typically initiates productive replication in the mucosal epithelia of the face and establishes latency in neurons of multiple cranial nerve ganglia, including the trigeminal ganglia (TG) (35, 41). VZV replicates in the mucosal epithelia of the respiratory tract and establishes latency not only in the TG, but also in the dorsal root and autonomic ganglia (15). During latency, transcription of the HSV-1 genome is largely restricted to a single RNA – the latency associated transcript (LAT) (2, 36). Although the LAT is capped and polyadenylated, it does not appear to encode a protein. LAT is unusual in that the spliced ~6.3 kb LAT transcript is highly unstable, while the single ~2 kb intron accumulates to significant levels within latently infected cells, although its function remains unknown (13, 21).

Previously, we used deep sequencing of RNA harvested from murine TG latently infected with HSV-1 to demonstrate that HSV-1 expresses at least 5 miRNAs, 4 of which (miR-H2, miR-H3, miR-H4, and miR-H5) are derived from the unstable, exonic regions of LAT (40). A fifth miRNA, miR-H6, was found to lie in the opposite transcriptional orientation, just upstream of LAT and antisense to another HSV-1 miRNA, miR-H1, that is expressed exclusively during productive replication (11). Two of the HSV-1 LAT-derived miRNAs, miR-H2 and miR-H6, were found to down-regulate expression of the viral proteins ICP0 and ICP4, respectively (40). ICP0 and IPC4 are viral immediate early proteins that function as potent activators of productive HSV-1 replication (3, 18, 34), and it was hypothesized that their repression by miR-H2 and miR-H6 might facilitate the establishment and/or maintenance of the latent state in HSV-1-infected neurons (40).
Humans are the only natural hosts of HSV-1 and VZV, although cell lines of various origins will support productive HSV-1 or VZV replication in culture. Importantly, neither HSV-1 nor VZV latency can be established in vitro using currently available cell culture systems. Although various animal models can be latently infected with either HSV-1 or VZV by artificial means (30, 35, 45), these animal models do not fully recapitulate all the hallmarks of true latent infection in humans, suggesting that aspects of the human neuronal replication environment may be unique.

In order to identify viral miRNAs that are expressed by HSV-1 or VZV in latently infected human neurons in vivo, we used Solexa/Illumina technology to deep sequence cDNA libraries prepared from post-mortem human TG samples naturally infected with latent HSV-1 and/or VZV. Based on these data, we demonstrate the in vivo expression of the five previously reported HSV-1 miRNAs, miR-H2 to miR-H6, and identify two novel HSV-1 LAT-derived miRNAs, miR-H7 and miR-H8. Surprisingly, despite the successful recovery of large numbers of HSV-1 miRNAs from the same samples, no latently expressed VZV miRNAs were identified.

Materials and Methods

Human tissues. Both trigeminal ganglia were removed within 61 hrs after death from three subjects (clinical data provided in Table 1) who, at autopsy, did not show cutaneous signs of recent herpesvirus infection. The dura, nerve roots and connective tissue were trimmed aseptically, and the ganglia were quick frozen in liquid nitrogen.

Nucleic acid extraction and PCR analysis. Ganglionic tissue was powdered under liquid nitrogen, and ~30 mg was used for DNA extraction (DNeasy, Qiagen, Germantown, MD) of which 100 ng was analyzed for the presence of HSV-1 and VZV
DNA by fluorescence-based simultaneous amplification and product detection (real-time PCR). Based on the complete sequence of HSV-1 (NC_001806) and VZV (NC_001348), the following primers were used to assay for HSV-1: Forward primer-

TGGTATTGCCCAACACTTTCC, Reverse primer-GCGCCAGGCACACACAT, probe-

CGTGTGTCGCTGTGTGGT. The following primers were used to assay for VZV: Forward primer- CGAACACGTTCGCCATCAA, Reverse primer-

CCCGGTTCGTTAGTTTGG, probe-TCCAGGTGTAGTTGATACCA. Replicate DNA samples were amplified using the 7500-Fast real-time PCR system (Applied Biosystems, Foster City, CA) as described (9). The remaining powdered ganglionic tissue was dissolved in 7 ml Tri-reagent (Molecular Research Center, Inc. Cincinnati, OH) and stored at -80°C. RNA was extracted according to manufacturer’s directions.

**Solexa/Illumina sequencing and data analysis.** cDNA libraries for deep sequencing were prepared using the protocol outlined in Umbach et al, 2008 (40) adapted for the Solexa/Illumina sequencing platform. Twenty-five µg of total RNA from each right and left TG were pooled and used to prepare the cDNA libraries, using linkers described in the Solexa Digital Gene Expression kit but chemically modified as described in Lau et al, 2001(24). In particular, the custom 3’ linker obtained from IDT (Coralville, IA) was 5’ adenylated and 3’ blocked. The 5’ DNA/RNA hybrid linker was also obtained from IDT. Raw sequence data was returned in FASTA format and 3’ linker sequences removed. Final sequences were collapsed, indexed and size filtered for reads 18-24 nts in length in order to generate a final list for MegaBLAST analysis. Sequences were analyzed against the HSV-1 genome (NC_001806), the VZV genome (NC_001348), and the miRBASE
database (release 9.2) using the formatdb, megablast, blastoutparse and filter_alignment scripts found in the miRDeep software package (14).

Cell culture, virus infection and RNA extraction. Vero and HeLa cells were maintained in DMEM with 10% FBS. SY5Y cells were maintained in RPMI with 10% FBS. SY5Y, HeLa and Vero cells were infected with HSV-1 strain KOS at a multiplicity of infection (MOI) of 10. Cells were washed with serum-free media before being infected with a minimal volume of virus for 1.5 hrs at 37°C with occasional rocking. Virus was removed and cells were washed before 10% FBS media was replenished. Samples were harvested at various hours post-infection (hpi) and total RNA isolated using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer’s directions.

Stem-loop RT-PCR. HSV-1 miRNA expression levels were analyzed using the TaqMan MicroRNA Assay system (Applied Biosystems, Foster City, CA), according to manufacturer’s directions, using 10 ng of total RNA per RT reaction. All miRNA levels are given as fold expression compared to an uninfected control sample (27). In these experiments, RNA from subject 3, which was VZV positive, but HSV-1 negative, was used as the HSV-1 uninfected control. All values were normalized against levels of the ubiquitous cellular miRNA miR-16 and all samples were run in triplicate. Custom primers for detecting each miRNA were designed by Applied Biosystems against the most common isoform of each miRNA recovered during deep sequencing.

Results

Deep sequencing of small RNAs from human TG latently infected with HSV-1 and/or VZV. TG were harvested from 3 recently deceased humans who did not present any symptoms of viral reactivation at the time of death (Table 1). The left and right TG of
each subject were individually assayed for the presence of both the HSV-1 and VZV genome by real-time PCR; both TG of subjects 1 and 2 were found to harbor HSV-1 and VZV genomic DNA, while subject 3 contained only VZV DNA (Table 2).

Deep sequencing returned 3,086,881 usable reads from subject 1, 9,152,800 reads from subject 2 and 4,063,968 reads from subject 3. As neurons comprise only ~10% of all cells in the TG, and only a small percentage of neurons are actually latently infected by either HSV-1 or VZV (10), the vast majority of sequences recovered proved, as expected, to be human cellular miRNAs. Members of the let-7 family were the predominant species detected, in particular isoforms let-7a, let-7b, let-7f and let-7c (data not shown).

Identification of HSV-1 miRNAs expressed in vivo. Deep sequencing of TG from subject 1 recovered all five HSV-1 miRNAs previously reported to be expressed in latently infected murine neurons (40), except miR-H5, while all five latency miRNAs, including miR-H5 (albeit only one copy), were recovered from subject 2 (Table 3). The HSV-1 miR-H1 miRNA, which has previously only been detected in cells undergoing productive HSV-1 replication (11, 39), was not recovered in this analysis of latently infected human TG.

In addition to finding all HSV-1 latency miRNAs previously reported (40), two new LAT-derived miRNAs were also recovered and are designated here as miR-H7 and miR-H8 (Table 3). miR-H7 was recovered from both subjects 1 and 2, while miR-H8 was only recovered from subject 2. The loci encompassing both miRNAs can be folded into the stem-loop structures typical of miRNA precursors (25, 44), and recovery of mature miRNAs derived from both the 5’ (5p) and 3’ (3p) arms confirms that, when annealed,
they display the 2-nt 3’-overhangs characteristic of miRNA duplex intermediates (Fig. 1A) (25), suggesting that these are indeed genuine miRNAs. The genomic locations of these miRNAs place them within LAT, antisense to intron 1 of ICP0 (Fig. 1B). No HSV-1 miRNAs were recovered from subject 3, whose TG did not contain detectable HSV-1 genomic DNA (Table 2).

Expression of HSV-1 miRNAs in latently and productively infected cells. The presence of all HSV-1 miRNAs was analyzed by qRT-PCR in the original HSV-1+ TG samples used for deep sequencing (Fig. 2A). miR-H2 through miR-H7 were found to be present at readily detectable levels, with miR-H2 being the most abundant miRNA in subject 1, and miR-H4-3p the most abundant miRNA in subject 2 (Fig. 2A). Despite being able to recover miR-H8, albeit infrequently, from subject 2, this miRNA was not detectable by qRT-PCR using RNA recovered from either subject 1 or subject 2. Surprisingly, miR-H1 was detected, although at a low level, in subject 1, suggesting either that some neurons were undergoing viral reactivation at the time of post-mortem sample collection, despite the absence of a visible rash (Table 1), or that low levels of miR-H1 are expressed in latently HSV-1 infected cells.

HSV-1 miRNA expression was also assessed by qRT-PCR in productively infected SY5Y cells, a neuronal cell line of human origin. At both 6 and 18 hpi, all nine HSV-1 miRNAs, miR-H1 through miR-H8, were detectable, with miR-H1 being the most abundant, as expected (11, 39), and miR-H8 being the least abundant (Fig. 2B). miR-H6, which is induced during productive replication (40), was also detected at elevated levels. Concordant with LAT expression, which is low at the start of the productive replication cycle but increases through the course of infection (23), the amount of each HSV-1
miRNA increased significantly as infection progressed from 6 to 18 hpi. Examination of infected HeLa and Vero cells demonstrated that all nine HSV-1 miRNAs were also expressed in these cell lines during productive replication (Fig. 2C and D).

**No VZV miRNAs were identified in latently infected human TG.** Despite being able to recover large numbers of latency-associated HSV-1 miRNAs (Table 3), no VZV-derived small RNAs were identified from any of the three human subjects analyzed here. In agreement with these findings, Solexa/Illumina deep sequencing of small RNAs derived from TG recovered from rhesus macaques latently infected with simian varicella virus (SVV), the primate equivalent of VZV, yielded 1,420,064 usable reads, but again did not yield any small RNAs of SVV origin (J. L. Umbach, I. Messaoudi and B. R. Cullen, unpublished observations). Together, these results suggest that both VZV and SVV fail to express viral miRNAs during latency *in vivo.*

**Discussion**

Although HSV-1 and VZV both readily establish productive infections in culture, these viruses are only able to establish latency in primary neurons, an environment that has so far proven impossible to fully recapitulate in culture. While HSV-1, but not VZV, can also establish latency *in vivo* in the sensory ganglia of laboratory animals such as mice and rabbits, these model systems do not faithfully mimic the latent infections seen in humans, the only natural host for HSV-1. We therefore felt that it was important to assess the miRNA profile of these viruses in naturally infected human neurons by using deep sequencing to analyze RNA samples derived from human TG latently infected with HSV-1 and/or VZV.
Using latently infected human TG obtained post-mortem, we were able to identify two new HSV-1 miRNAs (Fig. 1A and Table 3). One of these novel HSV-1 miRNAs, miR-H7, was verified to exist in both latently and productively infected cells by qRT-PCR (Fig. 2), while the other miRNA, miR-H8, was only detected in productively infected cells, even though it was initially sequenced from latently infected TG (Table 3, Fig. 2). Overall, there was only a modest level of correlation in miRNA expression levels as determined by deep sequencing (Table 3) or qRT-PCR (Fig. 2A). This may reflect differences in linker ligation efficiency during cDNA synthesis, differential PCR amplification of the cDNA library during sample preparation and sequencing and/or the differential annealing efficiency of the primers used for the qRT-PCR analysis.

The genomic locations of miR-H7 and miR-H8 place both antisense to the first intron of *ICP0* (Fig. 1B). This location suggests that they are unlikely to downregulate ICP0 expression as reported for miR-H2, which lies antisense to an exonic region of *ICP0* (Fig. 1B), as RNA interference is thought to operate exclusively in the cytoplasm (42). However, there is a report of alternative splicing of *ICP0* intron 1, which generates up to 4 different introns of variable size, although the significance and function, if any, of these alternatively spliced *ICP0* mRNAs is unknown (6). It is possible that miR-H7 and miR-H8 either act directly on these splice variants or, conversely, act on currently unknown cellular mRNA targets, as previously reported for several other herpesviral miRNAs (16).

Analysis of the mature HSV-1 miRNA sequences recovered revealed a significant level of sequence heterogeneity at both the 5’, and especially at the 3’, ends of some of the mature HSV-1 miRNAs (Table 4). The 5’ region of miRNAs, especially nucleotides 2
to 8, has been shown to be particularly important for target mRNA binding, and full sequence complementarity to this so-called “seed” region is usually, but not invariably, essential for mRNA translational inhibition (1). Therefore, even small sequence differences at the 5′ end of the miRNA, as seen with miR-H2-3p, miR-H6-3p and miR-H7-5p (Table 4), have the potential to affect the identity of the mRNAs targeted by these viral miRNAs. A comparison to cellular miRNA sequences obtained from the same RNA samples in the same sequencing run showed very little sequence variation at the 5′ end, with 96.0% of the 3,634,951 individual reads of let-7a, and 96.3% of the 1,489,388 reads of let-7b recovered from these two HSV-1 positive small RNA samples, showing the same, predicted 5′ end (data not shown). Therefore, it is unlikely that the sequence variation observed at the 5′ end of miR-H2-3p and miR-H6-3p, but not at the 5′ end of miR-H3-3p or miR-H4-5p (Table 4), is due to degradation during RNA isolation or some other sequencing artifact. It is also notable that the minor 5′ sequence variants observed with miR-H6-3p and miR-H7-5p are actually 1 nt longer than the consensus sequence (Table 4), which is clearly inconsistent with exonucleolytic RNA degradation. Of note, because miR-H2-3p lies antisense to its only known target, ICP0 (Fig.1B), a 1-nt change at the 5′ end would not affect the level of miRNA complementarity to this transcript. In contrast, a 1-nt addition at the 5′ end of miR-H6, which has been reported to target ICP4 mRNAs via the seed region (40), would be predicted to disrupt this interaction and, hence, may attenuate ICP4 downregulation.

The lack of detectable VZV-derived miRNAs in any of the three VZV-infected TG samples was unexpected, given that all members of the herpesviruses superfamily examined thus far, including primate, murine and avian herpesviruses, have been found...
to encode multiple viral miRNAs (39). It is possible that, despite analyzing over $1.6 \times 10^7$
cDNA sequence reads, this analysis was still not sensitive enough to detect VZV
miRNAs, even though ~3000 HSV-1 miRNAs were recovered from the same samples
(Table 3). As is commonly the case in co-infected TG, the HSV-1 viral genomic DNA
load was modestly higher than that of VZV (Table 2) (10), but this minor difference
seems unlikely to explain the lack of VZV miRNAs reported here. Interestingly, a
previously reported computational analysis of the genomes of HSV-1, HSV-2 and VZV,
as well as several other herpesviruses, predicted that HSV-1 and HSV-2 would encode
multiple viral miRNAs but that VZV was unlikely to produce viral miRNAs in infected
cells (32).

It should also be noted that the mechanism underlying VZV latency is thought to
differ significantly from that seen in HSV-1. During HSV-1 latency, the only transcript
expressed at significant levels is the LAT, which is contained within the repeat regions of
the viral genome (Fig. 1B). Although the VZV genome shares significant homology with
HSV-1, VZV lacks sequences complementary to much of the HSV-1 long internal (IR_L)
and terminal (TR_L) repeat regions (Fig. 1B) (29), and is therefore not predicted to encode
a LAT (10). In addition, VZV differs from HSV-1 in that it expresses at least 5 viral
protein-coding mRNAs during latency, albeit at low levels (8, 9, 22). As most of the
HSV-1 miRNAs are LAT-derived, it is therefore perhaps not surprising that VZV does
not appear to express any latency-specific viral miRNAs. The apparent lack of VZV
latency miRNAs, however, does not preclude the possibility of VZV miRNAs expressed
during productive replication, as is indeed observed with HSV-1 miR-H1 (11) (Fig. 2).
Acknowledgments

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We thank Dr. Ilhem Messaoudi for providing SVV-infected simian trigeminal ganglia and IDT Inc. for the 3’ linker used during miRNA cloning.
References


Figure Legends

Fig. 1. Two novel HSV-1 miRNAs recovered from latently infected humans. A) Predicted secondary structures of the primary miRNA stem-loops of the novel HSV-1 miRNAs miR-H7 and miR-H8. Black triangles indicate sites of Drosha cleavage, grey arrows indicate sites of Dicer cleavage. The recovered, mature 5p and 3p miRNA strands are bolded. B) Schematic of the HSV-1 genome expanded to show details of the LAT locus. Relative sizes, locations and orientations of other viral transcripts are also indicated. Sequence coordinates of viral miRNAs are given according to the HSV-1 strain 17 syn+ genome (NC_001806). IR, internal repeat; TR, terminal repeat; U1, unique long; U5, unique short. The novel HSV-1 miRNAs miR-H7 and miR-H8 both map antisense to an intronic region in the viral ICP0 gene.

Fig. 2. HSV-1 miRNA expression determined by qRT-PCR in latently and productively infected cells and tissues. All values are indicated as fold expression over an HSV-1 uninfected control sample and were normalized to a cellular miRNA, miR-16. A) HSV-1 miRNA expression levels in latently infected human TG from subject 1 (black bars) and subject 2 (grey bars). Despite recovery during deep sequencing, miR-H8 was not detected in either TG sample. Subject 3 served as the negative control. B) HSV-1 miRNA expression profile of SY5Y cells productively infected with HSV-1 strain KOS (MOI = 10) 6 hpi (black bars) or 18 hpi (grey bars), C) Similar to B, except this shows HeLa cells 18 hpi, D) Similar to B, except Vero cells at 24 hpi were analyzed.
Table 1. Clinical description of the tissue donors

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hrs from Death to Autopsy</th>
<th>Age</th>
<th>Gender</th>
<th>Cause of Death</th>
<th>Rash</th>
<th>Immunosuppressive Therapy in last 30 days</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>74</td>
<td>M</td>
<td>Sepsis with PCP(^a) pneumonia</td>
<td>No</td>
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<tr>
<td>2</td>
<td>22.5</td>
<td>57</td>
<td>M</td>
<td>Pancreatic cancer</td>
<td>No</td>
<td>Unknown</td>
</tr>
<tr>
<td>3</td>
<td>22.7</td>
<td>45</td>
<td>M</td>
<td>Myocardial infarction</td>
<td>No</td>
<td>No</td>
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</table>

\(^a\): pneumocystis carinii pneumonia
Table 2. Analysis of viral load in human trigeminal ganglia by real-time PCR

<table>
<thead>
<tr>
<th>Subject</th>
<th>Trigeminal ganglion</th>
<th>Copy number/100 ng DNA</th>
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<tr>
<td></td>
<td></td>
<td>HSV-1</td>
</tr>
<tr>
<td>1</td>
<td>right</td>
<td>879</td>
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<td>left</td>
<td>562</td>
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<tr>
<td>2</td>
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<td>521</td>
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<tr>
<td></td>
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<td>481</td>
</tr>
<tr>
<td>3</td>
<td>right</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>0</td>
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Table 3. HSV-1 miRNA expression profile in human trigeminal ganglia latently infected with HSV-1

<table>
<thead>
<tr>
<th>HSV-1 miRNA</th>
<th>5p freq. (^a)</th>
<th>3p freq. (^a)</th>
<th>5p freq. (^a)</th>
<th>3p freq. (^a)</th>
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</thead>
<tbody>
<tr>
<td>miR-H2</td>
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<td>15</td>
<td>4</td>
<td>127</td>
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<tr>
<td>miR-H3</td>
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</tr>
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</tr>
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<td>40</td>
<td>23</td>
</tr>
<tr>
<td>miR-H8</td>
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<td>0</td>
<td>2</td>
<td>1</td>
</tr>
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</table>

\(^a\): Frequency of each miRNA recovered from deep sequencing. The numbers shown indicate derivation from either the 5' (5p) or 3' (3p) arm of the predicted miRNA stem-loop precursor.
Table 4. Major species of mature HSV-1 miRNAs.

<table>
<thead>
<tr>
<th>HSV-1 miRNA</th>
<th>Sequence</th>
<th>Length (nt)</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-H2-3p</td>
<td>CTGAGCCAGGGAGGAGGTGGGA</td>
<td>21</td>
<td>18/142 (13%)</td>
</tr>
<tr>
<td></td>
<td>CTGAGCCAGGGAGGAGGTGGGACT</td>
<td>23</td>
<td>46/142 (32%)</td>
</tr>
<tr>
<td></td>
<td>TGAGCCAGGGAGGAGGTGGGACT</td>
<td>22</td>
<td>16/142 (11%)</td>
</tr>
<tr>
<td>miR-H3-3p</td>
<td>CTGGGACTGTGCGGTTGGG</td>
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<td>491/1216 (40%)</td>
</tr>
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<td></td>
<td>CTGGGACTGTGCGGTTGGG</td>
<td>19</td>
<td>138/1216 (11%)</td>
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<td></td>
<td>CTGGGACTGTGCGGTTGGG</td>
<td>20</td>
<td>244/1216 (20%)</td>
</tr>
<tr>
<td></td>
<td>CTGGGACTGTGCGGTTGGG</td>
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<td>301/1216 (25%)</td>
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<tr>
<td>miR-H4-5p</td>
<td>GGTAGAGTTTGACAGGCAAG</td>
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<td>217/1508 (14%)</td>
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<td></td>
<td>GGTAGAGTTTGACAGGCAAGCA</td>
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<td>1054/1508 (70%)</td>
</tr>
<tr>
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<td>CAGAGATCAAACCTCCG</td>
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</tr>
<tr>
<td>miR-H6-3p</td>
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<td></td>
<td>CACTTCCCGTCTTCCATCCC</td>
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<td>39/62 (63%)</td>
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<tr>
<td>miR-H8-5p</td>
<td>TATATAGGTCAGGGGTTTC</td>
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<td>2/2 (100%)</td>
</tr>
</tbody>
</table>

a: Major species of each HSV-1 miRNA comprising ≥10% of the total mature miRNA population are included. Data were pooled from subjects 1 and 2.