



Minireview

The miRNAs of Herpes Simplex Virus (HSV)*

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Herpes simplex virus (HSV) is a group of common human pathogens with two serotypes HSV-1 and HSV-2. The prevalence of HSV is worldwide. It primarily infects humans through epithelial cells, when it introduces a latent infection into the nervous system. During viral latency, only a region known as the latency-associated transcript (LAT) is expressed. The discovery of HSV miRNAs helps to draw a larger picture of the infection and pathogenesis of the virus. This review summarizes miRNAs found in HSV-1 and HSV-2 so far. The functional studies of miRNAs in HSV to date indicate that they play a stage-specific role coordinated with viral proteins to maintain the virus life cycle.

Herpes simplex virus (HSV); miRNAs; Latency-associated transcript (LAT)

Introduction of Herpes Simplex Virus (HSV) and miRNAs

Herpes simplex virus (HSV) is a group of well-known human pathogens that has two serotypes, HSV-1 and HSV-2 which are large double-stranded DNA viruses. The two serotypes differ slightly in genome length, restriction fragment patterns, and G+C content^[32]. Both HSV-1 and HSV-2 vary in their effect, in mainly oral and genital ulcerations of varying severity. HSV-1 infection occurs especially in children while HSV-2 is the major cause of genital herpes in adults^[47]. Although the prevalence of HSV varies between countries, in general HSV-2 infection has less impact^[2,45,50]. As an example, in the United States, the overall age-adjusted seroprevalence of HSV-1 is 57.7% and that of HSV-2 is only 17%^[51].

The lifecycle of HSV starts on contact with a variety of human cells^[29]. When the epithelial cells are first invaded by HSV, lytic and replicative infection is caused. The virus then enters the endings of sensory neurons and the DNA of HSV is released in the nuclei of the neurons and switches to the latent infection form. In addition, the HSV particles are shed from the neurons to the innervated epithelium during lytic infection. Those particles are then released to the surrounding tissue to enable re-infection^[13].

During the productive development of HSV infection, several genes coding for structural and nonstructural components are expressed^[27]. For HSV-1, the lytic gene expression starts with immediate early (IE or α) genes followed by early (E or β), "leaky-late" (L_1 or $\gamma-1$), and late (L_2 or $\gamma-2$) genes^[12]. The successful replication of HSV results in severe functional and structural alterations of host cells, and leads to cell death^[13]. 10-14 days after the initial HSV replication period, the virus enters the latent phase as shown in animal models^[13]. HSV latency exists in the sensory ganglia and trigeminal ganglia close to the site of primary infection^[13]. Once HSV reaches the latent phase, only a small portion of DNA, known as the latency-associated transcript (LAT) coding for a

Received: 2012-06-28, Accepted: 2012-10-24

* Foundation items: This work was supported by the National Natural Sciences Foundation of China (No. 30670094 and 30700028) and Youth Science Research Foundation of PUMC (No. 2012X23).

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family of RNAs, is expressed^[36,46]. The primary transcript of LAT is 8.3 kb in length; it is then spliced to produce a 2.0 kb intron, that is further spliced to a 1.5 kb intron^[52]. While it is the only product expressed in HSV latency, LAT has not yet been found to code for any protein^[18,30,31]. Although surprisingly, LAT is found not to be essential for latency establishment, maintenance or reactivation^[14,33,34]; it must play a key role in this. It is still not clear how LATs lead to the recurrent HSV infection, but the recent discovery of LAT-encoded miRNAs enhances the understanding of HSV latency^[7,37,39,41,43].

miRNAs are a family of non-coding RNAs which are of ~22 nucleotides in length. They post-transcriptionally function to regulate various biological processes from proliferation, apoptosis, development and immune system regulation, to oncogenesis^[5,21,23,48,49]. So far, there are more than 14,000 miRNAs identified in all species as recorded in the Sanger miRBase 2011 Release 18 and it is estimated that 1-5% of animal genomes are miRNAs^[1,26]. The “seed” region (the 5' nucleotides 2-8 of the mature miRNA) of miRNAs is thought to be the key component of miRNAs as interaction of miRNAs with their target's 3'-untranslated region (3'-UTR) is guided by this region^[20]. The interaction leads to the formation of miRNA induced silencing complexes (miRISC), which inhibit protein

production through various mechanisms involving target mRNA degradation and translational inhibition^[1,26]. Viral miRNAs, especially those in the herpes-virus family, are thought to down-regulate directly or cooperate with viral proteins to target host immune defense genes in order to invade the host immune system as a survival mechanism^[35]. The discovery of viral miRNAs gives a new understanding of the interaction between virus and host, how the virus infects its host and the pathogenesis of virus^[44].

The Discovery of HSV miRNAs

miRNAs in HSV-1 were first reported by Cui *et al.*, in 2006^[7]. They screened the hairpin-structured miRNA precursors from the complete genome sequence of HSV-1, limited other conditions including GC contents and locations step by step to finally get 13 miRNA precursors encoding 24 miRNA candidates. In order to confirm the expression of the HSV-1 miRNAs during productive infection, they were able to detect the precursor and mature form of a miRNA located ~450bp upstream of the transcription start site of the LAT, which was named HSV-1 miR-H1^[7]. Up to now, 17 HSV-1 miRNAs known as HSV-1 miR-H1 to H8, H11-H18, and most recently, miR-92944 have been discovered^[7,16,25,39,41,43]. Table 1 summarizes the

Table 1. HSV-1 miRNAs

Name	Mature Sequence	Position, Orientation	References
HSV-1 miR-H1	UGGAAGGACGGGAAGUGGAAG	118316-118401, +	7, 16
HSV-1 miR-H2	CCUGAGCCAGGGACGAGUGCGACU	121871-121892, +	16, 41, 42
HSV-1 miR-H3	CUGGGACUGUGCGGUUGGGAC	125775-125794, +	16, 39, 41, 42
HSV-1 miR-H4-5p	GGUAGAGUUUGACAGGCAAGCA	125882-125903, +	16, 39, 41, 42
HSV-1 miR-H4-3p	CUUGCCUGUCU AACUCGCUAGU	125927-125948, +	16, 39, 41, 42
HSV-1 miR-H5-5p	GGGGGGUUCGGGCAUCUCUAC	126727-126748, +	16
HSV-1 miR-H5-3p	GUCAGAGA UCCAAACCCUCCGG	126769-126790, +	16, 41, 42
HSV-1 miR-H6-5p*	GGUGGAAGGCAGGGGGUGUA	7983-8003, -	16
HSV-1 miR-H6-3p	CACUCCCCGUCCUCCA UCCC	8023-8043, -	16, 41, 42
HSV-1 miR-H7	AAAGGGGUCUGCAACCAAAGG	123421-123441, +	16, 41
HSV-1 miR-H8	UAUAUAGGGUCAGGGGUUC	123810-123829, +	16, 41
HSV-1 miR-H11	UUAGGACAAAGUGCGAACGC	62483-62502, +	16
HSV-1 miR-H12	UUGGGACGAAGUGCGAACGCUU	146241-146262, -	16
HSV-1 miR-H13	UUAGGGCGAAGUGCGAGCACUGG	132006-132028, +	16
HSV-1 miR-H14-5p	AGUCGCACUCGUCCUGGCUCAGG	4477-4500, -	16
HSV-1 miR-H14-3p	UCUGUGCCGGGCGCGUGCGAC	4520-4540, -	16
HSV-1 miR-H15	GGCCCCGGGCCGGGCCGCCACG	7131-7152, -	16
HSV-1 miR-H16	CCAGGAGGCUGGGAUCGAAGGC	69042-69063, +	16
HSV-1 miR-H17	UGGCGCUGGGGCGCGAGGCGG	150661-150681, -	16
HSV-1 miR-H18	CCCGCCCGCCGACGCCGGGACC	131837-131859, +	16
HSV-1 MIR-92944	TGGCTCGGTGAGCGACGGTC	92925-92944, +	25

NOTE: * Complementary to HSV-1 miR-H1

Table 2. HSV-2 miRNAs

Name	Mature Sequence	Position, Orientation	References
HSV-2 miR-H2	UCUGAGCCUGGGUCAUGC CGCA	122976-122997, +	16, 37
HSV-2 miR-H3	UUUGGGAGUCUGCGGUUGGGAG	126681-126702, +	16, 37, 43
HSV-2 miR-H4-5p	AGUUCACUCGGCACGCAUGCA	126864-126884, +	16, 37, 43
HSV-2 miR-H4-3p	CUUGCCUAGCGAACUCACCCGU	126901-126921, +	16, 37, 43
HSV-2 miR-H5	GGGGGGGCUCGGGCCACCUGACC	127593-127615, +	16
HSV-2 miR-H6	AAUGGAAGGCGAGGGGAUGC	8385-8404, -	16
HSV-2 miR-H7-5p	AAAGGGGUCCGUAACCAAAGG	124592-124612, +	16, 43
HSV-2 miR-H7-3p	UUUGGAUCCGACCCUCGUC	124625-124645, +	16
HSV-2 miR-H9-5p	CUCGGAGUGGAGUCGCGGU	124340-124359, +	16
HSV-2 miR-H9-3p	UUCCCACCUCGGUCUCCUCCUC	124378-12439, +	43
HSV-2 miR-H10	GGGUGCGGGGUGGGCGG	34890-34907, +	43
HSV-2 miR-H11	UUAGGACAAAGUGCGAACGCUU	62937-62958, +	16
HSV-2 miR-H12	UUAGGACGAAGUGCGAACGCUU	148910-148931, -	16
HSV-2 miR-H13	UUAGGGCAAAGUGCGAGCACUG	132847-132868, +	16
HSV-2 miR-H19	UUCGCUAGGCAAGCACGGACUG	332-353, -	16
HSV-2 miR-H20	UUUGGUUACGGACCCUUCU	2640-2660, -	16
HSV-2 miR-H21	AUAACGUCAUGCUGUCUACGG	11703-11723, +	16
HSV-2 miR-H22	AGGGGUCUGGACGUGGGUGGGC	80041-80062, +	16
HSV-2 miR-H23	AGGCCGUGGAGCUUGCCAGC	94211-94230, +	16
HSV-2 miR-H24	CUCCGGCGCCUCCCCCGCCCU	126502-126524, +	16
HSV-2 miR-H25	CUGCGCGCGGAGACCGGGAC	132997-133017, +	16

HSV-1 miRNAs with their major mature form sequences, localization and orientations.

Among the 17 mature miRNAs, more than 50% of them are within the LAT region (H1-5, H7-8, H14, and H15), indicating that LAT functions as a primary miRNA precursor^[16, 42]. Interestingly, HSV-1 miR-H6, as first reported by Umbach *et al.*, 2008, was found to lie antisense to an RNA stem-loop from the opposite strand of HSV-1 genome within the LAT promoter and shows extensive sequence complementarity with miR-H1^[42].

Three of the HSV-2 miRNAs, known as HSV-2 miR-H2, H3 and H4 were first reported by Tang *et al.*, 2009^[37]. 15 more HSV-2 miRNAs were reported later by Umbach *et al.*, and Jurak *et al.*, in 2010 to make up the total miRNAs of HSV-2 to 18^[16, 43]. Table 2 shows the HSV-2 miRNA family with their mature sequences, position and orientation.

HSV-1 miR-H1 is highly expressed in productive infection^[16]. No homolog of miR-H1 is found in HSV-2. In total, 9 miRNAs are thought to be conserved between the two viruses in position and/or sequence, especially within their seed regions. Table 3 compares these miRNAs with the seed region marked in bold. Among all the homologs, apart from miR-H4-5p, all the others share more than 70% similarity between HSV-1 and HSV-2. This is especially true of the seed

regions of those homologues, which are the target sites for 3'-UTR, and are very similar (more than 5/7 identical). Interestingly, two studies both published in 2010 gave different sequences for HSV-2 miR-H4-5p, which are both shown in Table 3^[16, 43].

Functions of HSV miRNAs

As a group of newly developed miRNAs, there are so far only a few related functional studies. Unlike those miRNAs encoded within the LAT region, which are expressed abundantly during latent infection, HSV-1 miR-H1, H6 and 92944 were found to be expressed abundantly in the HSV-1 productive infection samples^[16, 17, 25]. A 2012 study demonstrated that HSV-1 miR-H6 suppresses the expression of infected cell polypeptide 4 (ICP4) protein and decreases interleukin 6 production in human cornea epithelia (HCE)^[8]. *ICP4* gene is an IE gene of HSV-1, which upregulates early and late genes and downregulates IE genes, as well as driving the virus through the productive replication cycle^[8, 40]. It was also demonstrated that ICP4 is involved in HSV-1 reactivation during latency through LATs^[22, 42]. Tang *et al.* 2009 showed that, LAT-encoded miRNAs are negatively regulated by ICP4^[37]. Thus, ICP4 is essential for maintaining latency. An important

cytokine, IL-6, induces a pro- or anti-inflammation response in nature^[15]. It has been reported that IL-6 is influenced by HSV-1^[28]. Therefore, miR-H6 might be useful for prevention of HSV-1 lytic infection and inhibiting corneal inflammation^[8]. However, in HSV-2, miR-H6 is not reported to associate with ICP4 expression^[38]. Tang *et al.*, 2011 found that in HSV-2, miR-H6 is a latency-associated and transcript-associated miRNA, but by itself is not able to

establish latency^[38]. Although no target has been found for the newly reported HSV-1 miR-92944, Munson and Burch, 2012 reported that during the early stage of cell infection with miR-92944 mutant virus, the expression of miR-H1 was diminished^[25]. There is no report on the function of HSV-1 specific miRNA miR-H1 yet, but as the seed region is conserved with miR-H6, they might share some similarity in function^[16].

Table 3. miRNA homologues expressed by HSV-1 and HSV-2

HSV-1 and HSV-2 miRNA Homolog	Sequence	Alignment Score	Location	Proposed target (Reference)
miR-H2 HSV-1 HSV-2	CCUGAGCCAGGGACGAGUGCGACU 24 UCUGAGCCUGGGUCAUGCGCGA-- 22 ***** * * * *	72%	Within 2nd LAT exon; complementary to ICP0 3rd exon	ICP0 (37, 42)
miR-H3 HSV-1 HSV-2	-CUGGGACUGUGCGGUUGGGAC 21 UUUGGGAGUCUGCGGUUGGGAG 22 ***** *	80%	Within 2nd LAT exon and L/STs; complementary to ICP34.5 ORF	ICP34.5 (39)
miR-H4-5p HSV-1 HSV-2	GGUAGAGUUUGAC-AGGCAAGCA 22 AGUUCA-CUCGGC-ACGCAUGCA 21 * * * * * * * * *	52%	Within 2nd LAT exon and L/STs; complementary to ICP34.5 5'UTR	ICP34.5 (37)
miR-H4-5p ⁺ HSV-1 HSV-2	GGUAGAGUUUGACAGGCAAGCA--- 22 ---AGAGUUCACUCGGCACGCAUGC 22 ***** * * * *	68%	-	-
miR-H4-3p HSV-1 HSV-2	CUUGCCUGUCUAACUCGCUAGU 22 CUUGCCUAGCGAACUCACCCGU 22 ***** * * * *	72%	Within 2nd LAT exon and L/STs; complementary to ICP34.5 5'UTR	ICP34.5 (37)
miR-H5-5p HSV-1 HSV-2	GGGGGGGUUCGGGCAUCUCU-AC- 22 GGGGGGGCUCGGGCCAC-CUGACC 23 ***** * * * *	72%	Within 2nd LAT exon and L/STs	Unknown
miR-H6-5p HSV-1 HSV-2	GGUGGAAGGCAGGGGGUGUA 21 AAUGGAAGGCGAGGGGAUG-C 20 ..*****..* * * *	70%	Opposite strand and upstream of LATs; complementary to HSV-1 miR-H1	ICP4 (8)
miR-H7-5p HSV-1 HSV-2	AAAGGGGUCUGCAACCAAAGG 21 AAAGGGGUCCGUAACCAAAGG 21 ***** * * * *	90%	Within 2nd LAT exon; complementary to ICP0 1st intron	Unknown
miR-H11 HSV-1 HSV-2	UUAGGACAAAGUGCGAACGC-- 20 UUAGGACAAAGUGCGAACGCUU 22 ***** *	100%	oriL	Unknown
miR-H12 HSV-1 HSV-2	UUGGGACGAAGUGCGAACGCUU 22 UUAGGACGAAGUGCGAACGCUU 22 * * * * * * * * *	95%	oriS	Unknown
miR-H13 HSV-1 HSV-2	UUAGGGCGAAGUGCGAGCACUGG 23 UUAGGGCAAAGUGCGAGCACUG- 22 ***** * * * *	95%	oriS	Unknown

NOTE: miR-H4-5p: HSV-2 sequence based on Jurak *et al.*^[16]. miR-H4-5p⁺: HSV-2 sequences based on Umbach *et al.*^[43]. Sequences of orthologous miRNAs expressed by HSV- (top sequence) and HSV-2 (bottom sequence) aligned using ClustalW2^[11, 19].

Among those miRNAs sharing structural similarities between two serotypes, miR-H2 was demonstrated to possess an antisense orientation to a viral IE transcriptional activator ICP0 protein^[37, 42]. *ICP0* is an IE gene that expresses the ICP0 protein, a promiscuous activator, which has been found to enhance expression of IE, E and L HSV gene classes and enhance expression of any gene function as a basal level transcription exhibitor^[3, 9, 24]. Moreover, ICP0 plays an essential role in both lytic infection and reactivation from latency^[4]. By perfectly complementing *ICP0* mRNA, miR-H2 is thought to inhibit the expression of the ICP0 protein at the translational level^[42]. In addition, as ICP0 helps the virus to enter the productive replication cycle^[4, 10], such inhibition of miR-H2 might lead to entering and maintaining latency^[42].

Both miR-H3 and miR-H4 are in an antisense orientation to a key neurovirulence factor, the protein kinase R inhibitor ICP34.5^[37, 39, 42]. However, miR-H3 maps to the ICP34.5 coding region in an antisense direction, while miR-H4 maps to the antisense strand of the ICP34.5 5'-UTR transcript^[37]. Nevertheless, the two miRNAs have the same target, ICP34.5, and act to reduce the expression of ICP34.5 protein^[37, 39]. *ICP34.5* is shown to be a γ 1 L gene^[6], and is believed to be required by HSV as a late acquisition^[37]. While the ICP34.5 down-regulating elements including miRNAs miR-H3 and miR-H4 are required as the full expression of ICP34.5 starts, it is thought that LAT-encoded miRNAs affect the HSV infection spreading to other neurons or direct the establishment of latency^[37, 39].

It is therefore hypothesized that during productive infection, non LAT-encoded miRNAs express and result in suppression of the LAT-encoded miRNAs production. When HSV later enters the latent infection, the expression of non LAT-encoded miRNAs is suppressed, and LAT-encoded miRNAs start to express and regulate the two other proteins ICP0 and ICP34.5 in order to maintain latency and later on, through other mechanisms to enable recurrent infection. Those HSV miRNAs are suggested to be stage-specific in function co-operating with other viral proteins to maintain the virus life cycle^[17].

This review summarizes all reported miRNAs in HSV-1 and HSV-2, comparing the structural and functional similarities across the two herpes family viruses to provide a better understanding of viral

miRNAs as future targets for treatment. Three of the LAT-encoded miRNA homologues are shown to suppress viral protein expression in latent and recurrent infection. The HSV-1 miR-H6 is thought to regulate the IE protein ICP4 before the virus enters into latency. However, functions of most HSV miRNAs remain unclear; especially those that have no homolog between the two serotypes. Further functional studies are required to confirm these hypotheses and to understand the functions of other HSV miRNAs that are not yet fully clarified.

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