Review

Océane Sorel[a] and Benjamin G. Dewals*

MicroRNAs in large herpesvirus DNA genomes: recent advances

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Abstract: MicroRNAs (miRNAs) are small non-coding RNAs (ncRNAs) that regulate gene expression. They alter mRNA translation through base-pair complementarity, leading to regulation of genes during both physiological and pathological processes. Viruses have evolved mechanisms to take advantage of the host cells to multiply and/or persist over the lifetime of the host. *Herpesviridae* are a large family of double-stranded DNA viruses that are associated with a number of important diseases, including lymphoproliferative diseases. Herpesviruses establish lifelong latent infections through modulation of the interface between the virus and its host. A number of reports have identified miRNAs in a very large number of human and animal herpesviruses suggesting that these short non-coding transcripts could play essential roles in herpesvirus biology. This review will specifically focus on the recent advances on the functions of herpesvirus miRNAs in infection and pathogenesis.

Keywords: herpesvirus; microRNAs; pathogenesis.

Introduction

MicroRNAs (miRNAs) are single-stranded RNAs of 22–25 nucleotides in length that play important roles in the regulation of gene expression during both physiological and pathological processes. The first cellular miRNAs were identified two decades ago (1). Since this discovery, the global knowledge regarding the importance of miRNAs has grown exponentially. Cellular miRNAs are known to participate in many biological processes such as cell differentiation, metabolism, homeostasis, apoptosis and cancer. For the last decade, it has also been reported that several viruses also encode and express miRNAs. A general overview of viral miRNAs will be given in this review with an emphasis on the recent findings concerning herpesvirus-encoded miRNAs and their roles during infection and pathogenesis.

General overview of viral miRNAs

Viruses replicating in the nucleus of infected cells benefit from access to the host miRNA generation machinery and are, therefore, more likely to encode miRNAs. In addition, viruses establishing long-term infections often encode viral miRNAs as they provide valuable properties for altering cellular gene expression while remaining invisible for the host immune response (2). These include DNA viruses such as herpesviruses, polyomaviruses, ascoviruses, baculoviruses, iridoviruses and adenoviruses (3–8). However, viral miRNAs are not restricted to DNA viruses and have been found in at least one retrovirus: bovine leukemia virus (BLV) (9).

The sequences of cellular miRNAs as well as their target mRNA sequence sites are highly conserved, which is suggestive of similar functions (10). In contrast, although the location of miRNA loci in the genome structure of closely related viruses is generally conserved (11), there is hardly any conservation of sequence between viral miRNAs (12, 13). These observations suggest that viral miRNAs have co-evolved with their natural host to become host-specific. Besides the high divergence of sequence between viral miRNAs, some orthologs of cellular host miRNAs have also been described. Viruses use miRNAs as efficient tools to regulate expression of both cellular and viral genes, and viruses have also developed mechanisms

[a]Current address: Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA
***Corresponding author: Benjamin G. Dewals,** Fundamental and Applied Research in Animals and Health (FARAH), Immunology-Vaccinology, Faculty of Veterinary Medicine (B43b), Department of Infectious and Parasitic Diseases, University of Liège, B-4000 Liège, Belgium, e-mail: bgdewals@ulg.ac.be
Océane Sorel: Fundamental and Applied Research in Animals and Health (FARAH), Immunology-Vaccinology, Faculty of Veterinary Medicine (B43b), Department of Infectious and Parasitic Diseases, University of Liège, B-4000 Liège, Belgium

to avoid being targeted by cellular miRNAs. Thus, some viruses have evolved strategies to negatively regulate cellular transcripts through mimicking host miRNA functions by providing identical miRNA recognition sequences (14, 15). The complex interaction between host and viruses involve viral miRNAs which usually favor virus persistence in the infected host through multiple mechanisms, such as inhibiting cell apoptosis, evading the immune response and regulating host and viral genes that often leads to the repression of the viral lytic cycle and promotion of latency (16).

Viral miRNAs biogenesis

The great majority of viral miRNAs follow a canonical pathway for their biogenesis (17). In this pathway, miRNAs are initially transcribed by the RNA polymerase II (Pol II) in the nucleus as a long, capped and polyadenylated precursor called primary-miRNA (pri-miRNA). Then, pri-miRNAs are trimmed into precursor-miRNA hairpin intermediates (pre-miRNAs) by the nuclear RNAse III Drosha. Subsequently, pre-miRNAs are transported in the cytoplasm to be cleaved by the RNAse III Dicer into miRNA duplexes. Duplexes are then separated and one is selected to become the mature single-stranded miRNA whereas the complementary sequence [called the 'star' (*) or 'passenger' miRNA] is normally degraded (18), although there is some evidence for unique functions and regulatory mechanisms of passenger strands of miRNAs (19). Following this process, the mature miRNA is incorporated into an effector complex: the RNA-induced silencing complex (RISC).

In addition to the canonical miRNA biogenesis pathway, alternative mechanisms can generate miRNAs or miRNA-like small RNAs (20, 21). To date, several different non-canonical mechanisms were discovered regarding cellular miRNAs biogenesis (20). Surprisingly, none of these unusual cellular mechanisms were described for viral miRNAs. In contrast, some viruses seem to have developed Drosha-independent miRNA biogenesis pathways. Two herpesviruses follow the same overall process to generate miRNAs: saimiirine herpesvirus 2 (SaHV-2) and murid herpesvirus 4 (MuHV-4) pre-miRNAs are transcribed as small nuclear RNA (snRNA)-pre-miRNAs and transfer RNA (tRNA)-pre-miRNAs (TMERs) chimeras, respectively (22–24). Then, SaHV-2 pre-miRNAs are processed by Integrator whereas MuHV-4 pre-miRNAs are generated by tRNAse Z. In both cases, additional unknown nucleases contribute to the pre-miRNA release. To date, BLV still remains the sole retrovirus-encoding miRNAs. BLV has developed a unique way to generate pre-miRNAs which are processed directly by Pol III into endogenous small hairpin miRNAs (shRNAs) (15). All viral pre-miRNAs described above follow subsequently the same downstream, mature miRNAs biogenesis, which merge with the canonical pathway.

The RISC-bound miRNA complex can guide the recognition of a sequence in the 3′-untranslated region (3′-UTR) of a target mRNA, which has either extensive or partial complementarity to the miRNA, to induce mRNA cleavage and degradation or mRNA translational inhibition, respectively (25).

Herpesviruses and miRNAs

The vast majority of viral miRNAs identified so far were reported to be expressed by members of the *Herpesviridae* family (8, 14, 16, 26) and at least one member of the *Alloherpesviridae* family (27). Viral miRNAs were characterized as the most recently discovered weapons in herpesvirus genomes. The majority of miRNAs have been studied in the *Herpesviridae* family that includes viruses infecting birds and mammals and classified into three subfamilies: *alpha*-, *beta*- and *gammaherpesvirinae*. The first viral miRNAs were found in the Epstein-Barr virus (EBV) genome, a gammaherpesvirus largely infecting humans. EBV miRNAs were identified to be clustered into two distinct regions of the genome: the *Bam*HI fragment H rightward open reading frame 1 (BHRF1) miRNA cluster containing three miRNA precursors and the *Bam*A rightward transcript (BART) miRNA cluster containing 22 miRNA precursors (8, 28). The distribution of viral miRNAs across the genome can vary from one herpesvirus species to the other. In most gammaherpesviruses, miRNAs are distributed across the genome as clusters (29) whereas miRNAs of cytomegaloviruses are spread across the viral genome (30).

Recent studies highlighted the increasing complexity in the understanding of viral miRNA functions. For example, miRNAs expressed in both Kaposi's sarcoma-associated herpesvirus (KSHV) and EBV-infected cells were shown to be transferred through exosomes to uninfected recipient cells where they were able to repress the expression of target genes (31, 32). In addition, a recent study showed that viral miRNAs could be found within EBV virions and might be involved in controlling early steps of infection (33). Also, a viral miRNA encoded by human cytomegalovirus (hCMV) was shown

to unusually target mRNAs 5′-untranslated transcript regions (5′-UTRs) (34).

In herpesviruses, the viral life cycle has both latent and lytic replication phases, and herpesvirus-encoded miRNAs may be differentially expressed over time. Interestingly, recent studies have shown that herpesviruses can exhibit a distinct pattern of viral miRNAs expression in lytic and latent infection (35, 36). Within the viral cycle, latent infection is a key feature in herpesvirus biology and is associated with a restricted viral gene expression program that allows viral persistence in infected cells while evading immune surveillance. Among these rare transcripts, non-coding RNAs (ncRNAs), such as miRNAs, were shown to be expressed in latently infected cells, suggesting that viral miRNAs could play an important role in herpesvirus latency (12, 37, 38). Despite this widespread observation, several studies have reported that herpesvirus-encoded miRNAs could be differentially expressed during latency. Indeed, EBV can exhibit different patterns of latent gene expression depending on the type of infected cells. Most of the time, EBV latency within B cells progresses from latency III to latency II to latency I in contrast to latently infected epithelial cells, such as nasopharyngeal carcinoma (NPC) cells, which can support only the latency II program (39, 40). Interestingly, the expression of BHRF1 cluster of EBV miRNAs is selectively expressed in B cells undergoing latency III, including lymphoblastoid cell lines (LCLs) and Burkitt's lymphoma (BL) cells (13). Indeed, the expression of the BHRF1 miRNA cluster was not detected in cells undergoing the EBV latency II or I infection programs. In contrast, the viral BART miRNA cluster is highly expressed in EBV infected cells supporting latency II infection such as NPCs and primary effusion lymphoma (PEL) cells, whereas this miRNA cluster was hardly detectable in LCLs and in most BL cell lines (13). These observations suggest that herpesvirus-encoded miRNAs could play distinct roles throughout the viral lifecycle in the different infected cell types. The suggestive role played by viral miRNA in herpesvirus latency is furthermore supported by the observation that miRNA genes encoded by alpha- and gammaherpesviruses were found to be clustered within regions of the genome associated with latent gene expression (14). In KSHV, the 12-identified miRNAs are expressed during viral latency from polycistronic transcripts which also encode the KSHV latent proteins (41). The accumulated evidence regarding the role played by herpesvirus-encoded miRNAs in regulation of viral latency, cell cycle, lymphomagenesis and immune evasion will be discussed in the present review (Figure 1 and Table 1).

Herpesvirus-encoded miRNAs target viral and cellular transcripts to promote latency

In order to switch from latent to lytic replication, herpesviruses express *trans*-activating proteins which are able to stimulate the expression of lytic genes (42–45). Viral miRNAs encoded by herpesviruses have been demonstrated to promote long-term persistence through restricting the lytic replication program. Indeed, some of these miRNAs are able to target several immediate-early transactivators to lytic replication. Examples include EBV miR BART20-5p (46), ovine herpesvirus 2 (OvHV-2) miR-5 (47), and KSHV miRNAs miR-K1 (48), miR-K9* (49), miR-K5 (50) and miR-K7-5p (51), which have been shown to target reactivation transactivator (RTA) transcripts and thus promote latency. Similarly, herpesvirus simplex 1 (HSV-1) miR-H2 has been shown to target the HSV-1 functional equivalent of RTA, called ICP0, thus preventing reactivation (52). Likewise gallid herpesvirus 2 (GaHV-2) miR-M7-5p targets the immediate-early genes *ICP4* and *ICP27* to promote latency in infected chickens (53). Additional studies have demonstrated that other viral miRNAs were able to target transactivator genes, such as in the case of HSV-2 (54, 55) and hCMV (30) that contribute to the maintenance of latency. However, the relative importance of the miRNA-mediated control of viral reactivation remains to be determined. Interestingly, the lack of viral miRNAs involved in targeting transactivator genes resulted in a small increase of viral reactivation (2- to 3-fold) in contrast to the function of inducers of transactivator transcription (10- to 40-fold). It was therefore suggested that viral miRNAs might act as a "safety mechanism" (49). Such a mechanism is thought to prevent random variation in the levels of basal transactivator transcripts to fine-tune the control of latency and prevent inappropriate reactivation.

In addition to regulating viral gene expression levels, herpesviruses must control cellular gene expression in order to allow the establishment of long-term latency. Therefore, it is logical to assume that viral miRNAs can target cellular transcripts initially involved in promoting acute replication. However, viral miRNAs may also be involved in modulating cellular expression to create an environment more conducive to reactivation. First, KSHV miR-K1 and miR-K3 both inhibit translation of two cellular nuclear factor kappa B (NF-κB) inhibitors (48, 56). In fact, the NF-κB signaling pathway was previously reported to play a key role in restricting KSHV acute replication through downregulating RTA expression (57). Therefore, by targeting NF-κB inhibitors, KSHV viral miRNAs are able to decrease RTA expression levels and promote latency. Furthermore, miR-K3 and miR-K11 were reported to target

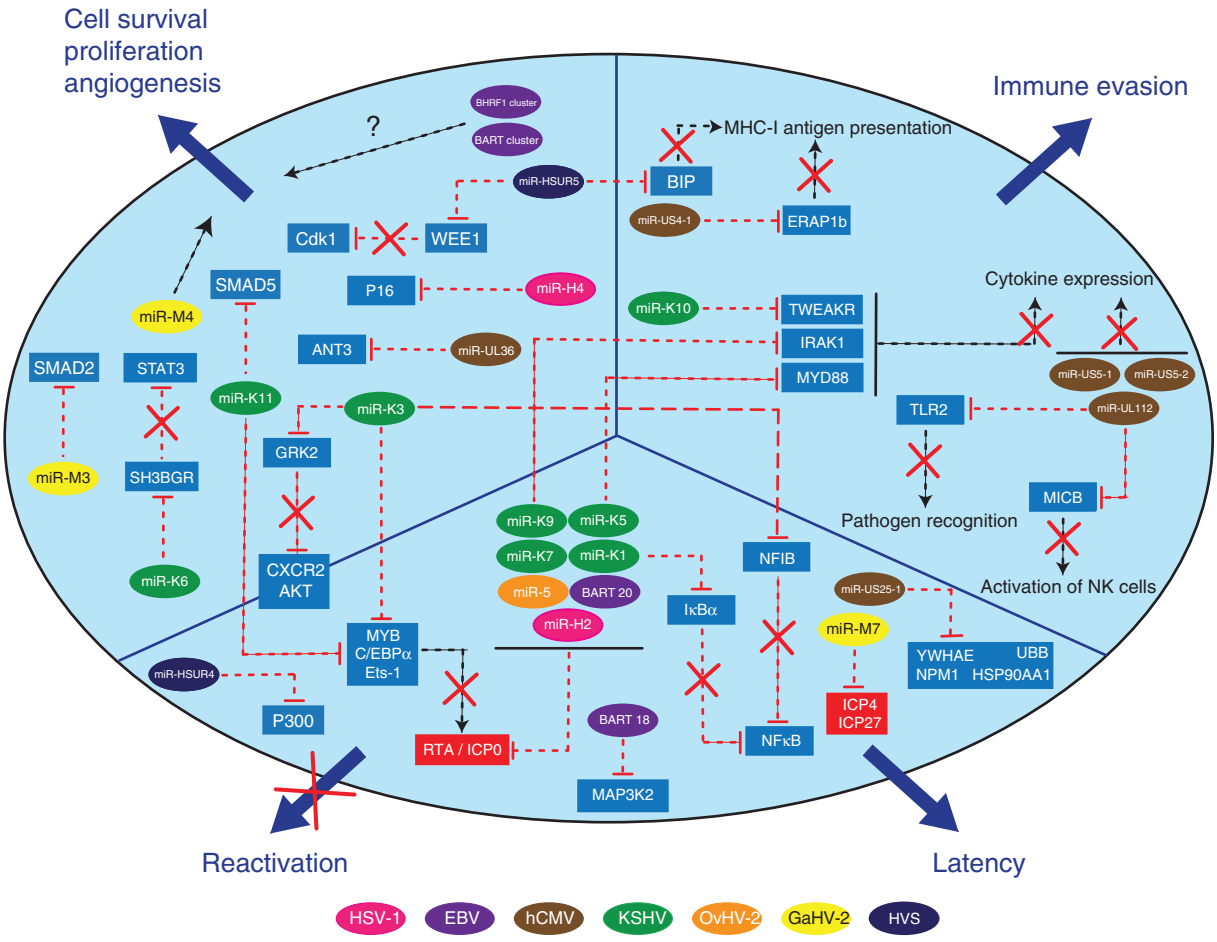

Figure 1: Regulation of herpesvirus infection by viral miRNAs.
Cellular pathways of herpesvirus miRNAs leading to the regulation of cell survival, proliferation and angiogenesis, evasion of the immune anti-viral response, and viral persistence. Blue boxes represent cellular transcripts and red boxes are viral transcripts. Viral miRNAs are presented as colored ovals with color-code specific to the herpesvirus species the miRNA belongs to. Blunted dotted red lines indicate negative regulation and black dotted arrows indicate positive regulation. MYB, C/EBPβ and Ets-1 are cellular transcription factors. P16 is a cellular tumor suppressor protein. WEE1 is a nuclear kinase. P300 is a transcription coactivator. ANT3, Adenine nucleotide translocase 3; STAT3, signal transducer and activator of transcription 3; SH3BGR, SH3 domain binding glutamate-rich protein. SMAD5, SMAD family member 5. Cdk1, cyclin-dependent kinase 1. BIP, binding immunoglobulin protein. GRK2, G protein-coupled receptor kinase 2; ERAP1b, endoplasmic reticulum aminopeptidase 1b; TWEAKR, TNF-like weak inducer of apoptosis receptor; MYD88, myeloid differentiation primary response 88; IRAK1, interleukin-1 receptor-associated kinase 1; TLR2, Toll-like receptor 2; MICB, MHC class I-related chain B; NFIB, nuclear factor I B; MAP3K2, mitogen-activated protein kinase kinase kinase 2; YWHAE, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon; UBB, polyubiquitin-B precursor; NPM1, nucleophosmin; HSP90AA1, heat-shock protein 90 kDa α family class A member 1; HSV-1, herpesvirus simplex 1 (human herpesvirus 1); EBV, Epstein-Barr virus (human herpesvirus 4); hCMV, human cytomegalovirus (human herpesvirus 5); KSHV, Kaposi sarcoma-associated herpesvirus (human herpesvirus 8); HVS, herpesvirus saimiri (saimiriine herpesvirus 2); OvHV-2, ovine herpesvirus 2; GaHV-2, gallid herpesvirus 2 (Marek's disease virus).

several cellular transcription factors, which are able to activate RTA, thus indirectly promoting KSHV latency (58). In addition, a recent study has shown that miR-K3 directly targets G protein-coupled receptor kinase 2 (GRK2) to negatively regulate viral lytic replication through activating C-X-C motif chemokine receptor 2 (CXCR2)/protein kinase B (AkT) signaling (59). Finally, another study has suggested that several additional KSHV miRNAs, including miR-K5, 9, 10a and 10b, could be involved in sensitizing cells to reactivation stimuli in order to induce KSHV exit of latency (60). In EBV, mitogen-activated protein kinase kinase kinase 2 (MAP3K2) was shown to be targeted by BART-miR-18-5p (61). As activation of the MAPK signaling pathway was reported to be required for efficient reactivation (62), BART-miR-18-5p contributes to limit lytic replication in infected cells. Similarly, miR-US25-1-5p encoded by hCMV was shown to target multiple cellular genes to inhibit viral replication (63). Further supporting the role of

Table 1: Herpesvirus-encoded microRNAs (miRNAs) and related functions and regulatory mechanisms.

Viral species	miRNA	Cellular/viral target	Function	References
KSHV	miR-K1	IκBα, RTA	Pro-latency	(48)
	miR-K3	NFIB, MYB, C/EBPα, Ets-1	Pro-latency	(56, 58)
		GRK2	Pro-latency, pro-angiogenesis	(59, 79)
	miR-K5	MYD88	Immune evasion (reduced cytokine expression)	(103)
		RTA	Pro-latency	(50)
	miR-K6-3p	SH3BGR	Pro-angiogenesis	(80)
	miR-K7-5p	RTA	Pro-latency	(51)
	miR-K9	RTA, IRAK1	Pro-latency, immune evasion (reduced cytokine expression)	(49, 103)
	miR-K10	TWEAKR	Immune-evasion (reduced cytokine expression)	(102)
	miR-K11	SMAD5	Inhibition of apoptosis	(78)
		MYB, C/EBPα, Ets-1	Pro-latency	(58)
EBV	BART18-5p	MAP3K2	Pro-latency	(61)
	BART20-5p	RTA	Pro-latency	(46)
	BART5	PUMA	Inhibition of apoptosis	(66)
HVS	miR-HSUR4-3p	P300	Pro-latency	(64)
	miR-HSUR5-3p	BIP	Immune evasion (reduced MHC class I maturation)	(64)
		WEE1	Inhibition of apoptosis	(64)
OvHV-2	miR-5	RTA	Pro-latency	(47)
HSV-1	miR-H2	RTA	Pro-latency	(52)
	miR-H4-5p	P16	Cell proliferation and invasion	(65)
GaHV-2	miR-M3	SMAD2	Inhibition of apoptosis	(94)
	miR-M7-5p	ICP4, ICP27	Pro-latency	(53)
hCMV	miR-UL36-5p	ANT3	Inhibition of apoptosis	(69)
	miR-UL112-3p	MICB	Immune evasion (reduced activation of NK cells)	(105)
		TLR2	Immune evasion (reduced pathogen recognition)	(104)
		Multiple components of the host secretory pathway	Immune evasion (reduced cytokine secretion)	(100)
	miR-US25-1-5p	YWHAE, NPM1, UBB, HSP90AA1	Pro-latency	(63)
	miR-US4-1	ERAP1b	Immune evasion (reduced MHC class I presentation)	(107)
	miR-US5-1	Multiple components of the host secretory pathway	Immune evasion (reduced cytokine secretion)	(100)
	miR-US5-2	Multiple components of the host secretory pathway	Immune evasion (reduced cytokine secretion)	(100)

herpesvirus miRNAs in restraining reactivation, a recent report has shown that miR-HSUR4-3p present in the herpesvirus saimiri (HVS) U-rich RNAs (HSURs) could repress the expression of the p300 transcriptional coactivator by binding the open reading frame of its mRNA (64).

Survival and proliferation of latently infected cells and herpesvirus-encoded miRNAs

In addition to restricting the lytic replication program, herpesvirus-encoded miRNAs can also contribute to the survival and proliferation of latently infected cells.

First, HSV-1 miR-H4-5p encoded by latency-associated transcripts (LATs) was shown to promote cell proliferation and invasion in nerve cells by directly targeting a cell cycle inhibitor (65). Interestingly, HVS miR-HSUR5-3p downregulated WEE1, a negative regulator of cell cycle progression, leading to reduced phosphorylation of its substrate, cyclin-dependent kinase (CDK1) (64). Furthermore, several studies have shown the potential role of some EBV and hCMV miRNAs in inhibiting cell apoptosis through restriction of expression of pro-apoptotic proteins (66–69). Recent *in vitro* studies highlighted the role of viral miRNAs in EBV-mediated oncogenesis. First, the lack of expression of the BHRF1 miRNA cluster led to

reduced transformation capacity and increased apoptosis of primary infected B cells (70, 71). Similarly, the BART miRNA cluster was shown to promote proliferation of primary infected B cells and inhibit apoptosis in BL cells through targeting caspase-3 (72). In NPC, EBV miR-BART1 was able to induce metastasis (73). However, two recent *in vivo* studies using a humanized mouse model led to contradictory results. First, it was observed that BHRF1 miRNAs contribute to viral growth but did not play a role in EBV-mediated oncogenesis (74). Furthermore, a second study showed that although the BART miRNA cluster of the EBV M81 strain did not affect the ability of the virus to induce tumors, these viral miRNAs were able to repress B-cell tumorigenesis (75).

Several KSHV miRNAs were also shown to inhibit cell death by targeting different proteins involved in the induction of apoptosis (48, 76–78). Moreover, two recent studies have shown that miR-K3 targets GRK2 to promote endothelial cell migration and angiogenesis and, therefore, could participate to tumor dissemination (59, 79). In addition, KSHV-encoded miR-K6-3p was lately reported to activate the signal transducer and activator of transcription 3 (*STAT3*) pathway through directly targeting its inhibitor, the SH3 domain binding glutamate-rich protein (SH3BGR) (80). This study revealed that miR-K6-3p facilitates endothelial cell migration and angiogenesis leading to the dissemination of KSHV-induced tumors.

The lack of any reliable model for transformation of human cells severely limits the research on KSHV-induced oncogenesis. Nonetheless, a potential role of KSHV miRNAs in promoting cellular transformation using a KSHV-infected rat mesenchymal cell model was recently highlighted (81). Interestingly, KSHV miR-K11 was reported to share sequence homology with cellular miR-155 (82). Overexpression of miR-155 is associated with abnormal proliferation of B cells (83–85). Thus, miR-K11 could contribute to KSHV-induced cell proliferation by mimicking miR-155 function. Two studies conducted in a humanized mouse model confirmed miR-K11 as a functional ortholog of miR-155, which indicates that miR-K11 might play a key role in the proliferation of KSHV-infected cells (86–88). Interesting data on the role of miR-155 orthologs encoded by herpesviruses were reported in chickens infected with GaHV-2, an alphaherpesvirus causing Marek's disease. GaHV-2 encodes two main clusters of viral miRNAs, among which miR-M4 is a functional ortholog of miR-155 that contributes to Marek's disease oncogenesis (89–91). Deletion of the miR-155 ortholog miR-M4 of GaHV-2 was sufficient to inhibit the induction of lymphomas in chicken (92). Importantly, this was the first observation of a direct role of a virus-encoded miRNA in oncogenesis in the context of a natural infection model *in vivo*. Besides miR-M4, GaHV-2 express another 13 miRNAs, and a recent report by Teng et al. highlighted important roles of miRNAs clustered in the *meq* oncogene cluster (93). As such, individual mutants of GaHV-2 miR-M1, -M2, -M3, -M5, or -M9 showed significant attenuation with reduced induction of tumors in infected chicken compared to the virulent strain. Interestingly, GaHV-2 miR-M3 was shown to suppress cisplatin-induced apoptosis through targeting mothers against decapentaplegic homolog 2 (SMAD2) of the transforming growth factor β pathway (94).

Besides the striking effects of miR-155 orthologs, direct involvement of viral miRNAs has been difficult to demonstrate *in vivo*. A recent study performed in pigs revealed that deletion of nine of the 11 miRNAs encoded in the large latency transcript of pseudorabies virus (PRV, suid herpesvirus 1) had little effect on latency establishment in the trigeminal ganglia (95). Recent studies have investigated the potential function of MuHV-4-encoded miRNAs in mice (96–98). Using MuHV-4 recombinant strains specifically deficient for the expression of viral miRNAs, the efficiency of B cell infection *in vivo* was only slightly affected. Moreover, a recent report has highlighted the role of MuHV-4 TMER4 stem-loops in virus dissemination to peripheral sites after intranasal infection, but such reduced dissemination to the spleen was independent of viral miRNAs encoded in TMER4 (98). These results suggest that ncRNAs, not only miRNAs, play major roles during infection *in vivo* and highlight the major importance of using genetically engineered recombinant viruses in animal models to address their roles in specific settings. Indeed, while the absence of MuHV-4 miRNAs did not significantly affect the colonization and latency in immunocompetent mice, impairment of MuHV-4 miRNA expression reduced the virulence of MuHV-4 infection in immunocompromised IFN-γ-deficient mice, suggesting a role in promoting gammaherpesvirus-associated disease (96, 97). In addition, research conducted on malignant catarrhal fever (MCF), a T-cell lymphoproliferative cattle disease induced by OvHV-2 or alcelaphine herpesvirus 1 (AlHV-1) revealed the expression of a very high number of miRNAs after propagation of LCLs and *in vivo* (73, 74). However, the deletion from the AlHV-1 genome of 28 clustered-viral miRNAs did not alter the induction of MCF in the rabbit model (99). These results suggested that AlHV-1 miRNAs are dispensable for lymphoproliferation and MCF lesions. Nonetheless, we could consider their roles in the adaptation of the virus to its natural host, the wildebeest.

Viral miRNAs targeting cellular transcripts to contribute to immune evasion

In order to allow efficient persistent infection, herpesviruses have developed multiple strategies to evade the host immune response, including mechanisms that involve viral miRNAs as effectors.

Several viral miRNAs are able to reduce immune cell recruitment through inhibiting secretion of pro-inflammatory cytokines. Indeed, hCMV miR-UL112, miR-US5-1 and miR-US5-2 target components of the secretory pathway resulting in decreased release of several pro-inflammatory cytokines such as tumor necrosis factor (TNF-)α and interleukin (IL-)6 (100). Similarly, KSHV encodes miRNAs that also target genes involved in cytokine secretion. TNF-like weak inducer of apoptosis (TWEAK) is a pro-inflammatory cytokine (101) and its receptor, TWEAKR, was identified as a target of KSHV miR-K10 leading to reduced expression of several pro-inflammatory cytokines (102). More recent studies have demonstrated that KSHV miR-K5 and -K9 can also reduce expression of inflammatory cytokines through targeting proteins of the NFκB signaling pathway (48, 56, 103). Finally, hCMV miR-UL-112-3p was recently shown to downregulate Toll-like receptor 2 (TLR2), thus affecting pathogen recognition (104).

Viral miRNAs were also shown to counteract cytotoxic T cell and natural killer (NK) cell activation. Indeed, hCMV miR-UL112 has been shown to target MHC class I-related chain B (MICB) which is a stress-induced ligand capable of activating the cytotoxic response of NK cells (105). Notably, miR-UL112 is able to inhibit MICB expression resulting in a reduced activation of NK cells. As EBV and KSHV were shown to encode miRNAs targeting MICB (106), this mechanism might be conserved in the *Herpesviridae* family. In addition, HVS miR-HSUR5-3p could regulate BiP, a chaperone facilitating maturation of MHC class I molecules in the endoplasmic reticulum (64), and hCMV miR-US4-1 was reported to downregulate antigen loading on MHC class I in infected cells through targeting the endoplasmic reticulum aminopeptidase ERAP1b (107).

Herpesviruses exploit host regulation of viral transcripts and miRNAs to control latency and lytic reactivation

We have focused in this review on herpesvirus-encoded miRNAs and their important functions in the course of viral infection and pathogenesis. However, an increasing body of evidence also highlighted the importance of host miRNAs regulating viral gene transcription and regulation of viral miRNA expression by cellular proteins. Herpesviruses have evolved strategies to turn cellular miRNAs to their advantage to regulate cellular pathways that are critical for virus latency. Examples of such regulatory mechanisms are as follows: In hCMV infected cells, a cellular miRNA family named miR-200 was shown to be involved in maintenance of viral latency through targeting a viral transactivator protein (108). Moreover, hCMV-infected cells showed a reduction in cellular miR-92a levels leading to an increase of interleukin-10 (IL-10) expression (109), which was associated with protection from apoptosis and hCMV maintenance of latency. In the case of HSV-1 infected cells, the neuronal-specific miR-138 miRNA was reported to target ICP0, thus promoting latency (110). In addition, it has been demonstrated that the tumor suppressor protein p53 could control the expression of viral miRNAs from the latency-associated transcript during viral latency and lymphomagenesis (111), suggesting that virulent viruses also exploit the cellular p53 regulatory network.

Conclusion

The biology of ncRNAs and, in particular viral miRNAs, has gained an increasing interest over the last decade and our knowledge in this field is constantly expanding. Here, we have presented an overview focusing on herpesvirus-encoded miRNAs. To date, only few functions have been uncovered for viral miRNAs in the pathogenesis of herpesvirus-associated diseases. However, a number of recent studies have given much momentum on addressing the functions of viral miRNAs *in vivo* and have highlighted the importance of complementary genetic recombineering approaches to tackle miRNA functions in a viral context during infection *in vivo*. Following these tracks, future studies will likely uncover unprecedented functions for viral miRNAs. Whereas cellular miRNAs are highly conserved between species, viral miRNAs seem to be very virus-specific and well-adapted to their natural host. As a result, herpesvirus-encoded miRNAs and associated functions are much likely derived from this co-evolution, which renders the use of natural hosts as *in vivo* models an inevitable venue for highlighting potential roles in, for example, viral persistence, tumorigenesis and immune evasion.

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References

1. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843–54.
2. Cullen BR. Viruses and microRNAs. Nat Genet 2006; 38(Suppl): S25–30.
3. Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. Nature 2005; 435: 682–6.
4. Hussain M, Taft RJ, Asgari S. An insect virus-encoded microRNA regulates viral replication. J Virol 2008; 82: 9164–70.
5. Singh C, Singh J, Nagaraju J. A baculovirus-encoded microRNA (miRNA) suppresses its host miRNA biogenesis by regulating the exportin-5 cofactor Ran. J Virol 2012; 86: 7867–79.
6. Yan Y, Cui H, Jiang S, Huang Y, Huang X, Wei S, Xu W, Qin Q. Identification of a novel marine fish virus, Singapore grouper iridovirus-encoded microRNAs expressed in grouper cells by solexa sequencing. PLoS One 2011; 6: e19148.
7. Aparicio O, Razquin N, Zaratiegui M, Narvaiza I, Fortes P. Adenovirus virus-associated RNA is processed to functional interfering RNAs involved in virus production. J Virol 2006; 80: 1376–84.
8. Pfeffer S, Zavolan M, Grasser FA, Chien M, Russo JJ, Ju J, John B, Enright AJ, Marks D, Sander C, Tuschl T. Identification of virus-encoded microRNAs. Science 2004; 304: 734–6.
9. Rosewick N, Momont M, Durkin K, Takeda H, Caiment F, Cleuter Y, Vernin C, Mortreux F, Wattel E, Burny A, Georges M, Van den Broeke A. Deep sequencing reveals abundant noncanonical retroviral microRNAs in B-cell leukemia/lymphoma. Proc Natl Acad Sci USA 2013; 110: 2306–11.
10. Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009; 19: 92–105.
11. Walz N, Christalla T, Tessmer U, Grundhoff A. A global analysis of evolutionary conservation among known and predicted gammaherpesvirus microRNAs. J Virol 2010; 84: 716–28.
12. Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR. Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. Proc Natl Acad Sci USA 2005; 102: 5570–5.
13. Cai X, Schäfer A, Lu S, Bilello JP, Desrosiers RC, Edwards R, Raab-Traub N, Cullen BR. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. PLoS Pathog 2006; 2: e23.
14. Grundhoff A, Sullivan CS. Virus-encoded microRNAs. Virology 2011; 411: 325–43.
15. Kincaid RP, Burke JM, Sullivan CS. RNA virus microRNA that mimics a B-cell oncomiR. Proc Natl Acad Sci USA 2012; 109: 3077–82.
16. Kincaid RP, Sullivan CS. Virus-encoded microRNAs: an overview and a look to the future. PLoS Pathog 2012; 8: e1003018.
17. Zhu Y, Haecker I, Yang Y, Gao SJ, Renne R. Gamma-Herpesvirus-encoded miRNAs and their roles in viral biology and pathogenesis. Curr Opin Virol 2013; 3: 266–75.
18. Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 2005; 6: 376–85.
19. Mah SM, Buske C, Humphries RK, Kuchenbauer F. MiRNA*: a passenger stranded in RNA-induced silencing complex? Crit Rev Eukaryot Gene Expr 2010; 20: 141–8.
20. Xie M, Steitz JA. Versatile microRNA biogenesis in animals and their viruses. RNA Biology 2014; 11: 673–81.
21. Yang JS, Lai EC. Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. Mol Cell 2011; 43: 892–903.
22. Cazalla D, Xie M, Steitz JA. A primate herpesvirus uses the integrator complex to generate viral microRNAs. Mol Cell 2011; 43: 982–92.
23. Bogerd HP, Karnowski HW, Cai X, Shin J, Pohlers M, Cullen BR. A mammalian herpesvirus uses noncanonical expression and processing mechanisms to generate viral MicroRNAs. Mol Cell 2010; 37: 135–42.
24. Diebel KW, Smith AL, van Dyk LF. Mature and functional viral miRNAs transcribed from novel RNA polymerase III promoters. RNA 2010; 16: 170–85.
25. Cullen BR. Herpesvirus microRNAs: phenotypes and functions. Curr Opin Virol 2011; 1: 211–5.
26. Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. Annu Rev Microbiol 2010; 64: 123–41.
27. Donohoe OH, Henshilwood K, Way K, Hakimjavadi R, Stone DM, Walls D. Identification and characterization of cyprinid herpesvirus-3 (CyHV-3) encoded microRNAs. PLoS One 2015; 10: e0125434.
28. Zhu JY, Pfuhl T, Motsch N, Barth S, Nicholls J, Grasser F, Meister G. Identification of novel Epstein-Barr virus microRNA genes from nasopharyngeal carcinomas. J Virol 2009; 83: 3333–41.
29. Nair V, Zavolan M. Virus-encoded microRNAs: novel regulators of gene expression. Trends Microbiol 2006; 14: 169–75.
30. Grey F, Meyers H, White EA, Spector DH, Nelson J. A human cytomegalovirus-encoded microRNA regulates expression of multiple viral genes involved in replication. PLoS Pathog 2007; 3: e163.
31. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, de Gruijl TD, Würdinger T, Middeldorp JM. Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci USA 2010; 107: 6328–33.
32. Rainy N, Zayoud M, Kloog Y, Rechavi O, Goldstein I. Viral oncomiR spreading between B and T cells is employed by Kaposi's sarcoma herpesvirus to induce non-cell-autonomous target gene regulation. Oncotarget 2016; 7: 41870–84.
33. Jochum S, Ruiss R, Moosmann A, Hammerschmidt W, Zeidler R. RNAs in Epstein-Barr virions control early steps of infection. Proc Natl Acad Sci USA 2012; 109: E1396–404.
34. Grey F, Tirabassi R, Meyers H, Wu G, McWeeney S, Hook L, Nelson JA. A viral microRNA down-regulates multiple cell cycle genes through mRNA 5′ UTRs. PLoS Pathog 2010; 6: e1000967.
35. Meshesha MK, Bentwich Z, Solomon SA, Avni YS. In vivo expression of human cytomegalovirus (HCMV) microRNAs during latency. Gene 2016; 575: 101–7.
36. Zhao P, Li X-J, Teng M, Dang L, Yu Z-H, Chi J-Q, Su JW, Zhang GP, Luo J. *In vivo* expression patterns of microRNAs of Gallid herpesvirus 2 (GaHV-2) during the virus life cycle and development of Marek's disease lymphomas. Virus Genes 2015; 50: 245–52.
37. Cosmopoulos K, Pegtel M, Hawkins J, Moffett H, Novina C, Middeldorp J, Thorley-Lawson DA. Comprehensive profiling of Epstein-Barr virus microRNAs in nasopharyngeal carcinoma. J Virol 2009; 83: 2357–67.
38. Umbach JL, Nagel MA, Cohrs RJ, Gilden DH, Cullen BR. Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. J Virol 2009; 83: 10677–83.
39. Salamon D, Adori M, Ujvari D, Wu L, Kis LL, Madapura HS, Nagy N, Klein G, Klein E. Latency type-dependent modulation

of Epstein-Barr virus-encoded latent membrane protein 1 expression by type I interferons in B cells. J Virol 2012; 86: 4701–7.
40. Amon W, Farrell PJ. Reactivation of Epstein-Barr virus from latency. Rev Med Virol 2005; 15: 149–56.
41. Cai X, Cullen BR. Transcriptional origin of Kaposi's sarcoma-associated herpesvirus microRNAs. J Virol 2006; 80: 2234–42.
42. Nicoll MP, Proenca JT, Efstathiou S. The molecular basis of herpes simplex virus latency. FEMS Microbiol Rev 2012; 36: 684–705.
43. Kumar A, Herbein G. Epigenetic regulation of human cytomegalovirus latency: an update. Epigenomics 2014; 6: 533–46.
44. Gennart I, Coupeau D, Pejakovic S, Laurent S, Rasschaert D, Muylkens B. Marek's disease: genetic regulation of gallid herpesvirus 2 infection and latency. Vet J 2015; 205: 339–48.
45. Miller G, El-Guindy A, Countryman J, Ye J, Gradoville L. Lytic cycle switches of oncogenic human gammaherpesviruses. Adv Cancer Res 2007; 97: 81–109.
46. Jung YJ, Choi H, Kim H, Lee SK. MicroRNA miR-BART20-5p stabilizes Epstein-Barr virus latency by directly targeting BZLF1 and BRLF1. J Virol 2014; 88: 9027–37.
47. Riaz A, Dry I, Levy CS, Hopkins J, Grey F, Shaw DJ, Dalziel RG. Ovine herpesvirus-2-encoded microRNAs target virus genes involved in virus latency. J Gen Virol 2014; 95: 472–80.
48. Lei X, Bai Z, Ye F, Xie J, Kim CG, Huang Y, Gao SJ. Regulation of NF-kappaB inhibitor IkappaBalpha and viral replication by a KSHV microRNA. Nat Cell Biol 2010; 12: 193–9.
49. Bellare P, Ganem D. Regulation of KSHV lytic switch protein expression by a virus-encoded microRNA: an evolutionary adaptation that fine-tunes lytic reactivation. Cell Host Microbe 2009; 6: 570–5.
50. Lu F, Stedman W, Yousef M, Renne R, Lieberman PM. Epigenetic regulation of Kaposi's sarcoma-associated herpesvirus latency by virus-encoded microRNAs that target Rta and the cellular Rbl2-DNMT pathway. J Virol 2010; 84: 2697–706.
51. Lin X, Liang D, He Z, Deng Q, Robertson ES, Lan K. miR-K12-7-5p encoded by Kaposi's sarcoma-associated herpesvirus stabilizes the latent state by targeting viral ORF50/RTA. PLoS One 2011; 6: e16224.
52. Jiang X, Brown D, Osorio N, Hsiang C, Li L, Chan L, BenMohamed L, Wechsler SL. A herpes simplex virus type 1 mutant disrupted for microRNA H2 with increased neurovirulence and rate of reactivation. J Neurovirol 2015; 21: 199–209.
53. Strassheim S, Stik G, Rasschaert D, Laurent S. mdv1-miR-M7-5p, located in the newly identified first intron of the latency-associated transcript of Marek's disease virus, targets the immediate-early genes ICP4 and ICP27. J Gen Virol 2012; 93(Pt 8): 1731–42.
54. Tang S, Bertke AS, Patel A, Wang K, Cohen JI, Krause PR. An acutely and latently expressed herpes simplex virus 2 viral microRNA inhibits expression of ICP34.5, a viral neurovirulence factor. Proc Natl Acad Sci USA 2008; 105: 10931–6.
55. Tang S, Patel A, Krause PR. Novel less-abundant viral microRNAs encoded by herpes simplex virus 2 latency-associated transcript and their roles in regulating ICP34.5 and ICP0 mRNAs. J Virol 2009; 83: 1433–42.
56. Lu CC, Li Z, Chu CY, Feng J, Feng J, Sun R, Rana TM. MicroRNAs encoded by Kaposi's sarcoma-associated herpesvirus regulate viral life cycle. EMBO Rep 2010; 11: 784–90.
57. Ye FC, Zhou FC, Xie JP, Kang T, Greene W, Kuhne K, Lei XF, Li QH, Gao SJ. Kaposi's sarcoma-associated herpesvirus latent gene vFLIP inhibits viral lytic replication through NF-κB-mediated suppression of the AP-1 pathway: a novel mechanism of virus control of latency. J Virol 2008; 82: 4235–49.
58. Plaisance-Bonstaff K, Choi HS, Beals T, Krueger BJ, Boss IW, Gay LA, Haecker I, Hu J, Renne R. KSHV miRNAs decrease expression of lytic genes in latently infected PEL and endothelial cells by targeting host transcription factors. Viruses 2014; 6: 4005–23.
59. Li W, Jia X, Shen C, Zhang M, Xu J, Shang Y, Zhu K, Hu M, Yan Q, Qin D, Lee MS, Zhu J, Lu H, Krueger BJ, Renne R, Gao SJ, Lu C. A KSHV microRNA enhances viral latency and induces angiogenesis by targeting GRK2 to activate the CXCR2/AKT pathway. Oncotarget 2016; 7: 32286–305.
60. Ziegelbauer JM, Sullivan CS, Ganem D. Tandem array-based expression screens identify host mRNA targets of virus-encoded microRNAs. Nat Genet 2009; 41: 130–4.
61. Qiu J, Thorley-Lawson DA. EBV microRNA BART 18-5p targets MAP3K2 to facilitate persistence *in vivo* by inhibiting viral replication in B cells. Proc Natl Acad Sci USA 2014; 111: 11157–62.
62. Adamson AL, Darr D, Holley-Guthrie E, Johnson RA, Mauser A, Swenson J, Kenney S. Epstein-Barr virus immediate-early proteins BZLF1 and BRLF1 activate the ATF2 transcription factor by increasing the levels of phosphorylated p38 and c-Jun N-terminal kinases. J Virol 2000; 74: 1224–33.
63. Jiang S, Qi Y, He R, Huang Y, Liu Z, Ma Y, Guo X, Shao Y, Sun Z, Ruan Q. Human cytomegalovirus microRNA miR-US25-1-5p inhibits its viral replication by targeting multiple cellular genes during infection. Gene 2015; 570: 108–14.
64. Guo YE, Oei T, Steitz JA. Herpesvirus saimiri microRNAS preferentially target host cell cycle regulators. J Virol 2015; 89: 10901–11.
65. Zhao H, Zhang C, Hou G, Song J. MicroRNA-H4-5p encoded by HSV-1 latency-associated transcript promotes cell proliferation, invasion and cell cycle progression via p16-mediated PI3K-Akt signaling pathway in SHSY5Y cells. Int J Clin Exp Med 2015; 8: 7526.
66. Choy EY, Siu KL, Kok KH, Lung RW, Tsang CM, To KF, Kwong DL, Tsao SW, Jin DY. An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. J Exp Med 2008; 205: 2551–60.
67. Kang D, Skalsky RL, Cullen BR. EBV BART microRNAs target multiple pro-apoptotic cellular genes to promote epithelial cell survival. PLoS Pathog 2015; 11: e1004979.
68. Marquitz AR, Mathur A, Nam CS, Raab-Traub N. The Epstein-Barr virus BART microRNAs target the pro-apoptotic protein bim. Virology 2011; 412: 392–400.
69. Guo X, Huang Y, Qi Y, Liu Z, Ma Y, Shao Y, Jiang S, Sun Z, Ruan Q. Human cytomegalovirus miR-UL36-5p inhibits apoptosis via downregulation of adenine nucleotide translocator 3 in cultured cells. Arch Virol 2015; 160: 2483–90.
70. Feederle R, Linnstaedt SD, Bannert H, Lips H, Bencun M, Cullen BR, Delecluse HJ. A viral microRNA cluster strongly potentiates the transforming properties of a human herpesvirus. PLoS Pathog 2011; 7: e1001294.
71. Seto E, Moosmann A, Gromminger S, Walz N, Grundhoff A, Hammerschmidt W. Micro RNAs of Epstein-Barr virus promote cell cycle progression and prevent apoptosis of primary human B cells. PLoS Pathog 2010; 6: e1001063.
72. Vereide DT, Seto E, Chiu YF, Hayes M, Tagawa T, Grundhoff A, Hammerschmidt W, Sugden B. Epstein-Barr virus maintains lymphomas via its miRNAs. Oncogene 2014; 33: 1258–64.
73. Cai L, Ye Y, Jiang Q, Chen Y, Lyu X, Li J, Wang S, Liu T, Cai H, Yao K, Li JL, Li X. Epstein-Barr virus-encoded microRNA BART1 induces

tumour metastasis by regulating PTEN-dependent pathways in nasopharyngeal carcinoma. Nat Commun 2015; 6: 7353.
74. Wahl A, Linnstaedt SD, Esoda C, Krisko JF, Martinez-Torres F, Delecluse HJ, Cullen BR, Garcia JV. A cluster of virus-encoded microRNAs accelerates acute systemic Epstein-Barr virus infection but does not significantly enhance virus-induced oncogenesis *in vivo*. J Virol 2013; 87: 5437–46.
75. Lin X, Tsai M-H, Shumilov A, Poirey R, Bannert H, Middeldorp JM, Feederle R, Delecluse HJ. The Epstein-Barr virus BART miRNA cluster of the M81 strain modulates multiple functions in primary B cells. PLoS Pathog 2015; 11: e1005344.
76. Suffert G, Malterer G, Hausser J, Viiliainen J, Fender A, Contrant M, Ivacevic T, Benes V, Gros F, Voinnet O, Zavolan M, Ojala PM, Haas JG, Pfeffer S. Kaposi's sarcoma herpesvirus microRNAs target caspase 3 and regulate apoptosis. PLoS Pathog 2011; 7: e1002405.
77. Gottwein E, Cullen BR. A human herpesvirus microRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest. J Virol 2010; 84: 5229–37.
78. Liu Y, Sun R, Lin X, Liang D, Deng Q, Lan K. Kaposi's sarcoma-associated herpesvirus-encoded microRNA miR-K12-11 attenuates transforming growth factor beta signaling through suppression of SMAD5. J Virol 2012; 86: 1372–81.
79. Hu M, Wang C, Li W, Lu W, Bai Z, Qin D, Yan Q, Zhu J, Krueger BJ, Renne R, Gao SJ, Lu C. A KSHV microRNA directly targets G protein-coupled receptor kinase 2 to promote the migration and invasion of endothelial cells by inducing CXCR2 and activating AKT signaling. PLoS Pathog 2015; 11: e1005171.
80. Li W, Yan Q, Ding X, Shen C, Hu M, Zhu Y, Qin D, Lu H, Krueger BJ, Renne R, Gao SJ, Lu C. The SH3BGR/STAT3 pathway regulates cell migration and angiogenesis induced by a gammaherpesvirus microRNA. PLoS Pathog 2016; 12: e1005605.
81. Moody R, Zhu Y, Huang Y, Cui X, Jones T, Bedolla R, Lei X, Bai Z, Gao SJ. KSHV microRNAs mediate cellular transformation and tumorigenesis by redundantly targeting cell growth and survival pathways. PLoS Pathog 2013; 9: e1003857.
82. Gottwein E, Mukherjee N, Sachse C, Frenzel C, Majoros WH, Chi JT, Braich R, Manoharan M, Soutschek J, Ohler U, Cullen BR. A viral microRNA functions as an orthologue of cellular miR-155. Nature 2007; 450: 1096–9.
83. Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc Natl Acad Sci USA 2005; 102: 3627–32.
84. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. Requirement of bic/microRNA-155 for normal immune function. Science 2007; 316: 608–11.
85. Costinean S, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci USA 2006; 103: 7024–9.
86. Schäfer A, Cai X, Bilello JP, Desrosiers RC, Cullen BR. Cloning and analysis of microRNAs encoded by the primate γ-herpesvirus rhesus monkey rhadinovirus. Virology 2007; 364: 21–7.
87. Boss IW, Nadeau PE, Abbott JR, Yang Y, Mergia A, Renne R. A Kaposi's sarcoma-associated herpesvirus-encoded ortholog of microRNA miR-155 induces human splenic B-cell expansion in NOD/LtSz-scid IL2Rgammanull mice. J Virol 2011; 85: 9877–86.
88. Sin SH, Kim YB, Dittmer DP. Latency locus complements microRNA 155 deficiency *in vivo*. J Virol 2013; 87: 11908–11.
89. Yu ZH, Teng M, Sun AJ, Yu LL, Hu B, Qu LH, Ding K, Cheng XC, Liu JX, Cui ZZ, Zhang GP, Luo J. Virus-encoded miR-155 ortholog is an important potential regulator but not essential for the development of lymphomas induced by very virulent Marek's disease virus. Virology 2014; 448: 55–64.
90. Zhao Y, Yao Y, Xu H, Lambeth L, Smith LP, Kgosana L, Wang X, Nair V. A functional microRNA-155 ortholog encoded by the oncogenic Marek's disease virus. J Virol 2009; 83: 489–92.
91. Chi JQ, Teng M, Yu ZH, Xu H, Su JW, Zhao P, Xing GX, Liang HD, Deng RG, Qu LH, Zhang GP, Luo J. Marek's disease virus-encoded analog of microRNA-155 activates the oncogene c-Myc by targeting LTBP1 and suppressing the TGF-β signaling pathway. Virology 2015; 476: 72–84.
92. Zhao Y, Xu H, Yao Y, Smith LP, Kgosana L, Green J, Petherbridge L, Baigent SJ, Nair V. Critical role of the virus-encoded microRNA-155 ortholog in the induction of Marek's disease lymphomas. PLoS Pathog 2011; 7: e1001305.
93. Teng M, Yu ZH, Sun AJ, Min YJ, Chi JQ, Zhao P, Su JW, Cui ZZ, Zhang GP, Luo J. The significance of the individual Meq-clustered miRNAs of Marek's disease virus in oncogenesis. J Gen Virol 2015; 96(Pt 3): 637–49.
94. Xu S, Xue C, Li J, Bi Y, Cao Y. Marek's disease virus type 1 microRNA miR-M3 suppresses cisplatin-induced apoptosis by targeting Smad2 of the transforming growth factor β signal pathway. J Virol 2011; 85: 276–85.
95. Mahjoub N, Dhorne-Pollet S, Fuchs W, Endale Ahanda ML, Lange E, Klupp B, Arya A, Loveland JE, Lefevre F, Mettenleiter TC, Giuffra E. A 2.5-kilobase deletion containing a cluster of nine microRNAs in the latency-associated-transcript locus of the pseudorabies virus affects the host response of porcine trigeminal ganglia during established latency. J Virol 2015; 89: 428–42.
96. Feldman ER, Kara M, Coleman CB, Grau KR, Oko LM, Krueger BJ, Renne R, van Dyk LF, Tibbetts SA. Virus-encoded microRNAs facilitate gammaherpesvirus latency and pathogenesis *in vivo*. mBio 2014; 5: e00981–14.
97. Diebel KW, Oko LM, Medina EM, Niemeyer BF, Warren CJ, Claypool DJ, Tibbetts SA, Cool CD, Clambey ET, van Dyk LF. Gammaherpesvirus small noncoding RNAs are bifunctional elements that regulate infection and contribute to virulence *in vivo*. mBio 2015; 6: e01670–14.
98. Feldman ER, Kara M, Oko LM, Grau KR, Krueger BJ, Zhang J, Feng P, van Dyk LF, Renne R, Tibbetts SA. A gammaherpesvirus noncoding RNA is essential for hematogenous dissemination and establishment of peripheral latency. mSphere 2016; 1.
99. Sorel O, Tuddenham L, Myster F, Palmeira L, Kerkhofs P, Pfeffer S, Vanderplasschen A, Dewals BG. Small RNA deep sequencing identifies viral microRNAs during malignant catarrhal fever induced by alcelaphine herpesvirus 1. J Gen Virol 2015; 96: 3360–72.
100. Hook LM, Grey F, Grabski R, Tirabassi R, Doyle T, Hancock M, Landais I, Jeng S, McWeeney S, Britt W, Nelson JA. Cytomegalovirus miRNAs target secretory pathway genes to facilitate formation of the virion assembly compartment and reduce cytokine secretion. Cell Host Microbe 2014; 15: 363–73.
101. Wiley SR, Winkles JA. TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. Cytokine Growth Factor Rev 2003; 14: 241–9.

102. Abend JR, Uldrick T, Ziegelbauer JM. Regulation of tumor necrosis factor-like weak inducer of apoptosis receptor protein (TWEAKR) expression by Kaposi's sarcoma-associated herpesvirus microRNA prevents TWEAK-induced apoptosis and inflammatory cytokine expression. J Virol 2010; 84: 12139–51.
103. Abend JR, Ramalingam D, Kieffer-Kwon P, Uldrick TS, Yarchoan R, Ziegelbauer JM. Kaposi's sarcoma-associated herpesvirus microRNAs target IRAK1 and MYD88, two components of the toll-like receptor/interleukin-1R signaling cascade, to reduce inflammatory-cytokine expression. J Virol 2012; 86: 11663–74.
104. Landais I, Pelton C, Streblow D, DeFilippis V, McWeeney S, Nelson JA. Human cytomegalovirus miR-UL112-3p targets TLR2 and modulates the TLR2/IRAK1/NF-κB signaling pathway. PLoS Pathog 2015; 11: e1004881.
105. Stern-Ginossar N, Elefant N, Zimmermann A, Wolf DG, Saleh N, Biton M, Horwitz E, Prokocimer Z, Prichard M, Hahn G, Goldman-Wohl D, Greenfield C, Yagel S, Hengel H, Altuvia Y, Margalit H, Mandelboim O. Host immune system gene targeting by a viral miRNA. Science 2007; 317: 376–81.
106. Nachmani D, Stern-Ginossar N, Sarid R, Mandelboim O. Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells. Cell Host Microbe 2009; 5: 376–85.
107. Kim S, Lee S, Shin J, Kim Y, Evnouchidou I, Kim D, Kim YK, Kim YE, Ahn JH, Riddell SR, Stratikos E, Kim VN, Ahn K. Human cytomegalovirus microRNA miR-US4-1 inhibits $CD8^+$ T cell responses by targeting the aminopeptidase ERAP1. Nat Immunol 2011; 12: 984–91.
108. O'Connor CM, Vanicek J, Murphy EA. Host microRNA regulation of human cytomegalovirus immediate early protein translation promotes viral latency. J Virol 2014; 88: 5524–32.
109. Poole E, McGregor Dallas SR, Colston J, Joseph RS, Sinclair J. Virally induced changes in cellular microRNAs maintain latency of human cytomegalovirus in $CD34^+$ progenitors. J Gen Virol 2011; 92: 1539–49.
110. 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.
111. Stik G, Laurent S, Coupeau D, Coutaud B, Dambrine G, Rasschaert D, Muylkens B. A p53-dependent promoter associated with polymorphic tandem repeats controls the expression of a viral transcript encoding clustered microRNAs. RNA 2010; 16: 2263–76.