Published in final edited form as:

Curr Top Microbiol Immunol. 2018 ; 419: 243–280. doi:10.1007/82_2017_31.

Roles of Non-coding RNAs During Herpesvirus Infection

Meaghan H. Hancock and

Vaccine and Gene Therapy Institute at Oregon Health and Science University, Beaverton, OR, USA

Rebecca L. Skalsky

Vaccine and Gene Therapy Institute at Oregon Health and Science University, Beaverton, OR, USA

Abstract

Non-coding RNAs (ncRNAs) play essential roles in multiple aspects of the life cycles of herpesviruses and contribute to lifelong persistence of herpesviruses within their respective hosts. In this chapter, we discuss the types of ncRNAs produced by the different herpesvirus families during infection, some of the cellular ncRNAs manipulated by these viruses, and the overall contributions of ncRNAs to the viral life cycle, influence on the host environment, and pathogenesis.

1 Introduction

Herpesviruses are large, enveloped, double-stranded DNA viruses that establish life-long, persistent infections in their hosts. Classified into three families (alpha, beta, and gamma) based on sequence homology and genome structure, all herpesviruses transition between two complex, distinct states during their life cycles: lytic and latent replication. The ability to successfully navigate between these phases all the while effectively combating host anti-viral innate and adaptive immune responses is facilitated in part by viral non-coding RNAs (ncRNAs). Viral genomes are compact in size, thus herpesviruses maximize use of their limited genetic space by encoding RNAs with multi-functional roles. Furthermore, many viral ncRNAs are non-immunogenic and can be expressed in times of host shut-off when protein translation is inhibited. These unique features of viral ncRNAs provide multiple methods for strategically manipulating gene expression within the internal and external cellular environment to effectively support the herpesvirus life cycle. While many herpesvirus ncRNAs continue to be defined and functionally characterized, numerous studies have highlighted the important and diverse roles that these molecules play in regulating viral replication, gene expression, and virus persistence.

2 Non-coding RNAs Made by Herpesviruses

Non-coding RNAs are defined as functional RNAs that lack protein-coding potential and do not associate with translation factors such as ribosomes. In humans, a surprisingly

significant portion of the genome (>75%) is transcribed, yet only ~2% of these transcripts are protein coding (Kapranov et al. 2007). The remaining transcripts represent ncRNAs which include highly abundant ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and regulatory RNAs such as microRNAs (miRNAs), piwiRNAs (piRNAs), small nucleolar RNAs (snoRNAs) and long ncRNAs. Many of these classes of ncRNAs have been observed in herpesviruses which are subject to the same control networks that regulate cellular gene expression, and produce viral transcripts that share structural features with cellular RNAs. For protein-coding viral mRNAs, this includes a 5' methylguanosine cap, 3' polyadenylated tail, and cis elements that regulate translation. The ncRNAs are more diverse in structure, size, and function. Based on size, the herpesvirus ncRNAs fall into two general classes: long ncRNAs (>150–200 nt) and small ncRNAs (~18–100 nt). Figure 1a shows the genomic locations of several ncRNAs encoded by human herpesviruses representative of the three families.

2.1 Herpesvirus-Encoded MiRNAs: The Short of It

The majority of viral small ncRNAs are ~22 nt cytoplasmic, single-stranded miRNAs that direct activity of the cellular RNA interference machinery and contribute to the silencing of target RNAs. Over 300 herpesvirus miRNAs have been identified within the past 12 years, and at least seven of the nine known human herpesviruses have been shown to encode miRNAs (Pfeffer et al. 2004, 2005; Samols et al. 2005; Cai et al. 2005; Grey et al. 2005; Umbach et al. 2008; Chen et al. 2010; Tuddenham et al. 2012; Tang et al. 2008); to date, no miRNAs have been identified for varicella zoster virus (VZV) (Umbach et al. 2009), the causative agent of chicken pox and shingles, or human herpesvirus 7 (HHV7) which, along with HHV6B, is associated with roseola in young children. miRNAs and other small ncRNAs may yet be identified for these viruses as additional RNA sequencing studies are performed. Both the alpha and gamma-herpesvirus miRNAs are organized in clusters (Fig. 1a) within genomic loci that are transcriptionally active during latent infection; many of these viral miRNAs are processed from stable introns of larger ncRNAs as described below. In contrast, beta-herpesvirus miRNAs, such as the human cytomegalovirus (HCMV) miRNAs, are dispersed throughout the viral genome and many are encoded within protein-coding regions that are transcriptionally active during acute stages of infection.

MiRNAs are potent post-transcriptional regulators of gene expression that are essential players in herpesvirus biology. miRNAs act by guiding the RNA induced silencing complex (RISC) to sequence complementary sites on target RNAs—primarily in the 3' UTRs of mRNAs, which subsequently interferes with protein expression and leads to destabilization of the target RNA (Bartel 2004, 2009). Over 1800 miRNAs are encoded within the human genome and are predicted to regulate upwards of 30–50% of protein-coding transcripts (Bartel 2009). Consequently, miRNAs regulate multiple biological processes, including cell differentiation, proliferation, apoptosis, stress responses, and the development of immunological responses. Aberrant miRNA activity, such as the loss of miRNA binding sites in 3' UTRs from alternative splicing or polyadenylation, has been observed in many diseases including cancer (Mayr and Bartel 2009; Sandberg et al. 2008).

Pfeffer et al. (2004) identified the first viral miRNAs in 2004 by sequencing size fractionated, small RNAs (18–24 nt) from B cells infected with the human gamma-herpesvirus, Epstein Barr virus (EBV). EBV is a highly successful pathogen and persistently infects >90% of adults worldwide. While primary infection is asymptomatic or induces infectious mononucleosis, in immunocompromised individuals, the virus is linked to malignancies such as Burkitt's lymphoma, non-Hodgkin's lymphoma, and nasopharyngeal carcinoma. EBV encodes a total of 25 precursor miRNAs (pre-miRNAs), three within the BHRF1 region and two large miRNA clusters within the BamHI rightward transcripts (BART) region (Cai et al. 2005; Chen et al. 2010; Grundhoff and Sullivan 2011; Pfeffer et al. 2004, 2005; Skalsky and Cullen 2015) (Fig. 1a). The BART miRNAs, transcribed from the BART P1 and P2 promoters, are detectable at varying levels throughout infection while the BHRF1 miRNAs are expressed during specific latency programs when the latent C or W promoters are active (Amoroso et al. 2011; Edwards et al. 2008; Xing and Kieff 2011). Homologs of the EBV miRNAs can be found in several Old World non-human primate (NHP) lymphocryptoviruses (LCV), including rhesus LCV and herpesvirus papio, which infects baboons (Cai et al. 2006; Riley et al. 2010; Skalsky et al. 2014; Walz et al. 2010). rLCV currently holds the record for most viral miRNAs; 70 potential miRNAs can be generated from the 35 pre-miRNAs (Riley et al. 2010; Skalsky et al. 2014; Walz et al. 2010).

A second lymphotropic human gamma-herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV), encodes 12 pre-miRNAs within the latency-associated region. KSHV is linked to KS and lymphoproliferative diseases such as primary effusion lymphoma (PEL). 10 of the KSHV miRNAs are expressed from an intron mapping between vFLIP (ORF71) and K12 while the other two are encoded within K12 (Cai et al. 2005; Pfeffer et al. 2005; Samols et al. 2005). Rhesus rhadinovirus (RRV) and other NHP rhadinoviruses encode positional homologs of the KSHV miRNAs and at least one of the NHP virus miRNAs has sequence homology to KSHV miR-K10a (Schafer et al. 2007; Umbach et al. 2009; Walz et al. 2010; Bruce et al. 2013; Skalsky et al. 2016). Small RNA sequencing studies on KSHV infected B cell lymphoma lines revealed that viral miRNAs account for a large portion (~30–70%) of the total miRNA population (Gottwein et al. 2011; Umbach et al. 2010), and are therefore expected to exert significant impacts on host gene expression.

Neurotropic alpha-herpesviruses such as Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), the rhesus macaque herpes B virus, and the highly infectious pseudorabies virus (PRV) of swine, encode a locus known as the latency associated transcript (LAT) which remains transcriptionally active during latency. No viral proteins are produced; rather, large, capped and polyadenylated transcripts accumulate to high levels and are each spliced to liberate a stable intron (Tycowski et al. 2015). The unstable spliced LAT exon is cleaved by the miRNA biogenesis machinery to liberate multiple miRNAs. HSV-1 and HSV-2 establish latent infection in the trigeminal or sacral dorsal root ganglia neurons, respectively. Disease can arise from viral reactivation and usually presents as lesions in the form of cold sores for HSV-1 or genital sores for HSV-2. During lytic HSV-1 or HSV-2 infection, miRNAs from LAT and other genomic loci can be detected (Jurak et al. 2014; Umbach et al. 2008; Tang et al. 2008). A total of 17 miRNAs have been reported for HSV-1; however, only 9 of these (namely, miR-H1 to miR-H8 and miR-H11) were demonstrated to be actively loaded into RISC following in vitro HSV-1 17syn + infection of neuronal cell lines (Flores et al. 2013).

Expression levels of HSV-1 miRNAs vary significantly, and inconsistencies in viral miRNA detection in many studies are likely due to the lack of tractable models for HSV-1 infection.

Human cytomegalovirus (HCMV), a ubiquitous virus that is generally asymptomatic in immunocompetent individuals but can cause severe disease in immunocompromised individuals such as neonates and transplant recipients (Britt 2008), encodes 11 pre-miRNAs scattered throughout its genome. All HCMV miRNAs are detected during lytic infection of fibroblasts (Dunn et al. 2005; Grey et al. 2005; Stark et al. 2012) however only a subset of the miRNAs are detectable in in vitro latency models (Fu et al. 2014; Lau et al. 2016; Meshesha et al. 2016; Shen et al. 2014). Mouse CMV (MCMV) and rat CMV (RCMV) encode 18 and 14 pre-miRNAs, respectively, which, like HCMV, are scattered throughout the genome but bear no homology to the primate CMV miRNAs (Buck et al. 2007; Meyer et al. 2011).

2.2 Biogenesis of Viral MicroRNAs

Most herpesvirus miRNAs are processed by the canonical host miRNA biogenesis pathway, whereby long pri-miRNAs are transcribed by RNA Pol II and cleaved by nuclear RNase III Drosha along with its co-factor DGCR8 (DiGeorge critical region 8) (Fig. 1b) (Ambros 2004; Bartel 2004, 2009; Ha and Kim 2014). Drosha cleavage releases a ~60 nt precursor miRNA (pre-miRNA) hairpin containing a 5' mono-phosphate and 2 nt 3' overhang. Pre-miRNAs are then exported to the cytoplasm through the nuclear pore complex via Exportin-5 (XPO5) and cleaved by a second RNase III enzyme, Dicer, to liberate ~22 nt miRNA:miRNA* duplexes. One duplex strand is bound by an Argonaute protein and incorporated into RISC as the mature miRNA. Viral miRNA biogenesis is dependent solely on the cellular machinery and to date, there are no viral factors known to be required for the generation of herpesvirus miRNAs. Single nucleotide polymorphisms (SNPs) in several herpesvirus pre-miRNA sequences, particularly for two KSHV miRNAs, miR-K12-5 and miR-K12-9, have been shown to alter miRNA processing events by interfering with Drosha and/or Dicer cleavage (Han et al. 2013; Gottwein et al. 2006). Additionally, post-transcriptional modifications of pri- or pre-miRNAs, such as adenosine to inosine (A-to-I) editing by the ADAR1 enzyme, can alter miRNA biogenesis. A-to-I editing of the EBV primary miR-BART6 transcript abrogates Drosha cleavage, release of the pre-miRNA, and thus alters total expression levels of the mature miR-BART6 (Iizasa et al. 2010). The clinical significance of SNPs and post-transcriptional modifications in human gamma-herpesvirus miRNAs has not been fully evaluated, although it is thought these may contribute to increased pathogenesis risk or disease states by altering viral miRNA expression levels (Iizasa et al. 2010; Han et al. 2013; Marshall et al. 2010).

Not all herpesviruses exploit Drosha for miRNA biogenesis. At least two gamma-herpesviruses have been shown to generate miRNAs in a Drosha- and DGCR8-independent manner. The 14 murine MHV68 pre-miRNAs are generated from eight RNA Pol III tRNA-pre-miRNA chimera transcripts to yield a total of 28 mature miRNAs. These MHV68 tRNA-miRNA-encoding RNAs (TMERs) are cleaved by cellular tRNaseZ (ELAC1) and other as yet unknown nucleases to separate the pre-miRNAs from the tRNA prior to export into the cytoplasm and canonical Dicer cleavage (Bogerd et al. 2010; Diebel et al. 2010; Tycowski et

al. 2015). Many MHV68 TMERs contain one tRNA and two pre-miRNAs, and mature miRNAs generated from the pre-miRNAs closest to the tRNAs are more abundant. The second pre-miRNA terminates in a 3' polyU tract, a remnant of RNA Pol III transcription.

Pre-miRNAs of the oncogenic New World monkey virus herpesvirus saimiri (HVS) are co-transcribed with the HVS Sm class U RNAs (HSURs) by RNA Pol II (Croen et al. 1987). The resulting multi-hairpin transcripts contain cis elements, including Sm binding regions and uridine-rich, small nuclear RNA (snRNA) 3' signal elements (3' box) that contribute to further processing. The host Integrator complex, consisting of ~12 proteins in humans that are responsible for snRNA biogenesis, recognizes the 3' box and cleaves the transcripts, thereby separating the 5' HSURs from the downstream viral pre-miRNAs (Croen et al. 1987). This cleavage event generates the 5' ends of the pre-miRNAs; another 3' box-like sequence element recognized by the Integrator complex is needed to generate the 3' ends of the pre-miRNAs (Xie et al. 2015). Released pre-miRNAs are subsequently exported into the cytoplasm to join the canonical miRNA biogenesis pathway (Fig. 1b). Elucidating these non-canonical pathways for MHV68 and HVS miRNA processing has contributed to our overall understanding of miRNA biogenesis in eukaryotes.

2.3 Other Herpesvirus Small ncRNAs

In addition to miRNAs, herpesviruses express a number of other small ncRNAs, although the regulatory functions for many are still unclear. Encoded 100 nt downstream of EBV miR-BART2 is a 65 nt viral snoRNA (v-snoRNA1) (Hutzinger et al. 2009) (Fig. 1a). V-snoRNA1 contains sequence and structural elements similar to canonical C/D box cellular snoRNAs that localize to the nucleolus, interact with Nop65, Nop58, and fibrillarin to form functional snoRNA protein (snoRNP) complexes, and chemically modify RNAs. A smaller 24-nt RNA with miRNA-like characteristics may be processed from v-snoRNA1 and can be detected by Northern blot in both EBV + B and epithelial cells (Hutzinger et al. 2009; Lung et al. 2013). The 24 nt v-snoRNA1-derived RNA was reported to bind to a region in the EBV BALF5 3' UTR and induce cleavage of the BALF5 mRNA (Hutzinger et al. 2009). How this interaction might occur is unclear since the v-snoRNA1 is not detectable in RISC immunoprecipitation experiments (Riley et al. 2012; Skalsky et al. 2012).

Identified in RNA-seq datasets from latently infected B cells, the EBV stable intronic sequence RNA 1 (sisRNA-1) is a highly abundant, 81 nt RNA derived from a stable intron that arises from transcription across the five to eight W repeats (Moss and Steitz 2013). These W repeats are included as the 5' ends of long transcripts encoding the EBV nuclear antigens (EBNAs); some of these EBNA transcripts might have additional ncRNA capabilities (Concha et al. 2012; Moss and Steitz 2013). Functions for EBV sisRNA-1 are unknown; however, the RNA contains a short U-rich hairpin loop and additional motifs that may interact with proteins (Moss and Steitz 2013). RNA-seq experiments have unveiled hundreds of other novel EBV transcripts and stable viral introns produced during lytic infection that appear to lack protein coding potential and may have regulatory functions (Cao et al. 2015; Concha et al. 2012; O'Grady et al. 2014); at least some of these novel EBV ncRNAs may sequester miRNAs and alter viral gene expression (Cao et al. 2015).

Two HSV-1 small ncRNAs [sRNA1 (65 nt) and sRNA2 (36 nt)] arise from within the first 1.5 kb of the stable LAT intron during productive HSV-1 infection of neuronal cultures (Perng et al. 1994) and in latently infected mouse trigeminal ganglia (Stevens et al. 1987). LAT sRNA2 inhibits the expression of ICP4 in transient co-transfection experiments, and both sRNAs contribute to blocking productive infection of a LAT-null virus. Additionally, both sRNAs cooperate to partially block cold-shock induced apoptosis in neuro-2A cells (Stevens et al. 1987). Thus, the functions of these sRNAs may contribute to the overall requirement of the LAT region in latency and reactivation of HSV-1.

Varying in size from 75–143 nt in length, the seven HSURs of HVS are abundantly expressed in latently infected marmoset T cells (10^3 – 10^4 copies/cell) [reviewed in (Tycowski et al. 2015)]. The HSURs share some sequence similarities to Sm-class small nuclear RNAs (snRNAs). These non-coding RNAs are dispensable for the T cell transformation phenotype of HVS, but remain highly conserved in other New World primate gamma-herpesviruses, suggesting other important functions for the virus. At least one of the HSURs can sequester a host miRNA (discussed below) that contributes to T cell development (Cazalla et al. 2010).

2.4 The Long(er) Non-coding RNAs

Some of the most abundant viral transcripts in herpesvirus-infected cells are lncRNAs. HCMV expresses four major ncRNAs (RNA2.7, RNA1.2, RNA4.9, and RNA5.0) (Fig. 1a) that account for greater than 65% of polyadenylated transcripts in infected human fibroblasts in vitro (Gatherer et al. 2011). RNA2.7 (also known as b2.7), the best-studied HCMV lncRNA, is expressed with early kinetics and accounts for more than 20% of polyadenylated lytic viral transcripts (McDonough and Spector 1983; McDonough et al. 1985). Additionally, RNA 2.7 has been detected both in in vitro latency models and in seropositive donors (Rossetto et al. 2013). RNA2.7 is encoded within the inverted repeats flanking the unique long (U_L) segment of the viral genome, has a cytoplasmic localization (Gawn and Greaves 2002), and may encode several short polypeptides (Stern-Ginossar et al. 2012). Another HCMV lncRNA, RNA4.9, has also been detected in seropositive donors and in in vitro models of HCMV latency (Rossetto et al. 2013). RNA4.9 initiates within oriLyt, terminates just downstream of UL69 (Gatherer et al. 2011) and may encode a polypeptide (Stern-Ginossar et al. 2012). RNA5.0 has been identified as an AT-rich stable intron encoded between the UL105 and UL111a loci. Mutation of the 5' splice donor site does not affect viral replication in fibroblasts (Kulesza and Shenk 2004). The MCMV homolog of RNA 5.0 is also dispensable for replication in fibroblasts but may be a virulence factor in vivo (Kulesza and Shenk 2006). MCMV may also encode other long non-coding RNAs that await characterization (Juranic Lisnic et al. 2013). Finally, HCMV RNA 1.2 is encoded within the terminal repeats, may also encode a polypeptide (Stern-Ginossar et al. 2012), but has not been studied extensively (Gatherer et al. 2011; Hutchinson and Tocci 1986).

EBV expresses two nuclear RNA pol III transcripts, EBER1 (167 nt) and EBER2 (172 nt), that accumulate to $\sim 10^5$ – 10^6 copies per cell and are the most abundant viral transcripts in EBV-infected cells (Lerner et al. 1981; Tycowski et al. 2015). The EBERs are detectable throughout EBV infection and therefore are often used for diagnostics. Originally identified

as interactors of the La protein, which binds the RNA pol III polyU tracts of the EBERs, both EBER1 and EBER2 form extensively base-paired secondary structures to scaffold RNA binding proteins. EBER1 relocates the ribosomal protein L22 to the nucleus (Fok et al. 2006) and also binds several cellular hnRNPs including AUF1 (Lee et al. 2012), which alters the stability of AU-rich RNAs. Recently identified, novel functions for EBER2 (Lee et al. 2015) are discussed below and illustrated in Fig. 2.

During lytic replication, the 1.1 kb Kaposi-sarcoma associated herpesvirus (KSHV) polyadenylated nuclear RNA (PAN) makes up >75% of the total polyadenylated transcripts in KSHV infected cells (Arias et al. 2014; Conrad 2016). Recent studies show that PAN may be virion associated and accumulates in the nucleus at high levels (>500,000 copies/cell) within a few hours after infection (Rossetto and Pari 2012; Arias et al. 2014; Conrad 2016). PAN contains a 79 nt triple helical expression and nuclear retention element (ENE) that contributes to its stable secondary structure (Fig. 2a) and facilitates nuclear localization (Devi-Rao et al. 1994; Conrad and Steitz 2005). Sequence analysis of other rhadinovirus genomes such as rhesus rhadinovirus (RRV), japanese macaque rhadinovirus (JMRV), and retroperitoneal fibromatosis-associated herpesvirus (RFHV), indicate the presence of PAN homologs (Estep et al. 2013; Tycowski et al. 2012).

Particularly for EBV and KSHV, recent transcriptome-wide approaches to characterize viral gene expression patterns have uncovered several novel lncRNAs present during lytic infection, and similar to HCMV lncRNAs, many initiate near lytic origins. EBV BHLF1 is an abundant 2.5 kb ori-LytL associated transcript that has been gaining recognition as a potential viral lncRNA since the transcript seems to lack protein coding potential due to several stop codons (Arvey et al. 2012; Krug 2013); BHLF1 may contribute to control of lytic DNA replication through regulation of the Cp and Wp promoters (Rennekamp and Lieberman 2011). Encoded within the BamHI locus adjacent to ori-LytR, the EBV LF3 transcript also contains multiple stop codons, suggesting function as a ncRNA (Lin et al. 2013). Other EBV ncRNAs include the alternatively spliced, polyadenylated nuclear BART lncRNAs that negatively regulate expression of several host genes (Marquitz et al. 2014), a second ~2.8 kb sisRNA (sisRNA-2) generated from the larger introns of W repeat transcripts that has no characterized function (Moss and Steitz 2013), and bi-directional oriP transcripts that remain nuclear, are subject to ADAR hyper-editing, and may fold into long hairpin structures (Cao et al. 2015). GapmeR knockdown of oriPtL activity in EBV + Burkitt's lymphoma cell lines indicates a role in enhancing lytic replication (Cao et al. 2015). KSHV similarly transcribes several non-coding regions of the viral genome during lytic reactivation that are also adjacent to an oriLyt, which may allow replication factors access to the viral DNA (Wang et al. 2008; Chandriani et al. 2010).

HSV LAT (latency-associated transcript) is the most abundantly transcribed gene in infected neurons and is encoded anti-sense to ICP0 (Kim et al. 2015). LAT encodes an 8.3 kb transcript that is spliced to yield a stable 2 kb intron that can be further processed into a stable 1.5 kb intron (Javier et al. 1988) that is found at $>10^4$ copies/cell. The role of the 1.5 kb stable intron in latency establishment, maintenance and reactivation has been extensively studied [reviewed in (Scarpini et al. 2001)] and is discussed below.

3 Functions of Viral lncRNAs

Much of what we know about lncRNA functions comes from studies to characterize the >60,000 cellular lncRNAs encoded within the human genome (Iyer et al. 2015). Many cellular lncRNAs are transcribed by RNA pol II and are subject to splicing and polyadenylation akin to cellular mRNAs (Derrien et al. 2012). More common to lncRNAs is their nuclear localization, unique secondary or tertiary structures, and interactions with other RNAs, DNA, or proteins for activity (Cech and Steitz 2014). Cellular lncRNAs have been shown to regulate gene expression through chromatin remodeling or interactions with splicing machinery; some act as molecular scaffolds to bring effector components into close proximity while others contribute to RNA stability or post-transcriptional or post-translational modifications [reviewed in (Cech and Steitz 2014)]. Together, these studies have revealed the diversity of lncRNA functions, and thus, we would expect viral lncRNAs to harbor comparable or perhaps, even more unique roles.

3.1 Roles in Metabolic Processes and Apoptosis

Functions for the HCMV lncRNAs during viral infection have not been extensively studied. Reeves et al. (2007) examined the interacting partners of HCMV RNA2.7 and identified the mitochondrial Complex I subunit NADH Ubiquinone Oxidoreductase. RNA2.7 blocked the relocalization of cellular GRIM-19, a subunit of Complex I required for its assembly and function, in response to the Complex I inhibitor rotenone. The maintenance of Complex I function allows for the stabilization of mitochondrial membrane potential and continued production of ATP. Although deletion of RNA 2.7 from the viral genome does not greatly affect viral growth in fibroblasts (McSharry et al. 2003; Reeves et al. 2007), there is an observed growth disadvantage in the presence of rotenone or low glucose growth medium (Reeves et al. 2007). Continued ATP production is likely critical for efficient lytic replication and HCMV mediates this, at least in part, through the function of its lncRNA.

The HSV stable intron LAT plays an important role in blocking apoptosis in infected neurons, although the exact mechanism remains unknown (Marshall et al. 2010; Ray et al. 2012). Caspase 8 and 9-induced apoptosis is blocked by LAT (Hansen et al. 2010a) and caspase 3 activation in response to Granzyme B can also be inhibited by LAT (Jiang et al. 2011). Two sRNAs encoded within the first 1.5 kb of the LAT region may contribute to this phenotype (Shen et al. 2014). Given that HSV establishes latency in long-lived neuronal cells, blocking apoptosis is likely a critical function of LAT during the lifespan of the host.

3.2 Viral Long ncRNAs Modify Viral Gene Expression

Several cellular and viral lncRNAs have been found associated with chromatin remodeling factors (Khalil et al. 2009; Rossetto and Pari 2012) and are thought to act as molecular scaffolds to allow for histone modifications in the epigenetic control of viral or cellular gene expression (Chinen and Tani 2012; Saxena and Carninci 2011). Following trafficking of the viral DNA into the nucleus, herpesvirus genomes become nucleosome-associated and take on highly structured, organized chromatin architecture (Toth et al. 2010). Histone modifications compact the chromatin to repress transcription, which is a key step in silencing viral gene expression for latency.

There are several examples of herpesvirus lncRNAs that directly contribute to transcriptional control of viral immediate early promoters. In latently infected CD14 + monocytes, HCMV RNA4.9 interacts with components of the polycomb repressor complex PRC2 (Fig. 2d) and may be involved in the enrichment of H3K27me3 on histone tails at the site of the HCMV major immediate early promoter (MIEP) (Rossetto et al. 2013). PRC complexes are also involved in the formation of heterochromatin and histone marks on KSHV genomes, and may be titrated away from the viral genome by KSHV PAN to enhance lytic replication [reviewed in (Campbell et al. 2014)]. Recent ChIRP (chromatin isolation by RNA purification) experiments on KSHV infected B cells indicate that KSHV PAN lncRNA interacts with PRC2 components such as the lysine methylase Ezh2 (Rossetto et al. 2013). PAN also interacts with demethylases JMJD3 and UTX that act on the ORF50 promoter to drive expression of the viral transactivator RTA (Fig. 2b) (Rossetto and Pari 2012). It was originally determined that HSV LAT contributes to the heterochromatinization of lytic gene promoters during latency through the induction of lysine 9 methylation on histone H3 (Wang et al. 2005). Additionally, facultative heterochromatin (H3K9 and H3K27 trimethylations) on lytic gene promoters during latency is reduced in small animal models of HSV-1 using LAT-deficient viruses (Amelio et al. 2006; Cliffe et al. 2009; Kwiatkowski et al. 2009). The mechanism of LAT-induced heterochromatinization of the lytic viral gene promoters is unclear; however, LAT may be involved in recruitment of chromatin remodeling complexes (Kwiatkowski et al. 2009; Cliffe et al. 2013).

In vivo studies suggest that PAN can also interact with the KSHV latency associated nuclear antigen (LANA). LANA has many functions in the maintenance of latent viral genomes, and has been shown to repress transcription from lytic viral promoters including the ORF50 promoter (Lan et al. 2004). In vitro, the PAN/LANA interaction has been mapped to LANA's DNA binding domain which inhibits interactions with histone H3 (Campbell et al. 2014). One current model is that PAN sequesters LANA from binding lytic viral promoters (Fig. 2a).

Recent work from the Steitz group demonstrated that the EBV nuclear EBER2 physically interacts with paired box protein 5 (PAX5), a transcription factor involved in B cell differentiation (Li et al. 2011). Capture hybridization analysis of RNA targets (CHART) was used to identify areas of DNA where EBER2 localized. This analysis showed that EBER2 can bind to the EBV terminal repeats at sites that overlapped with the previously reported PAX5 binding sites (Arvey et al. 2012). Subsequent experiments demonstrated that EBER2 and PAX5 interact to form a complex that includes paraspeckle components SFPQ, NONO, and RBM14 to help regulate LMP1 and LMP2A RNA expression (Lee et al. 2015, 2016) (Fig. 2c). Consistent with this model, deletion of EBER2 from the virus leads to a ~5-fold increase in LMP2A transcript levels in lymphoblastoid cell lines (LCLs); surprisingly, no significant changes were observed at the protein level (Gregorovic et al. 2015).

4 Small Silencers of Viral Gene Expression

Viral miRNAs are considered key contributors in mediating the balance between lytic and latent replication [reviewed in (Grey 2015)]. One way in which viral miRNAs can modulate this balance is through the direct silencing of lytic transcripts, which is important for

establishing and/or maintaining latency. EBV miR-BART2-5p, which is encoded anti-sense to the viral DNA polymerase catalytic subunit BALF5, has perfect complementarity to the BALF5 3' UTR and induces site-specific endonucleolytic cleavage of the BALF5 transcript during lytic replication (Barth et al. 2008; Pfeffer et al. 2004). HCMV miR-UL112, miR-US33, miR-US5-1, and miR-US5-2 are each encoded on strands opposite of viral protein coding genes, and therefore postulated to inhibit viral gene expression. HCMV miR-UL112 is anti-sense to UL114 (Pfeffer et al. 2005), which encodes a uracil DNA glycosylase involved in DNA replication, and inhibits UL114 protein expression by direct binding to the UL114 transcript (Stern-Ginossar et al. 2009). Surprisingly, despite full complementarity between miR-UL112 and the UL114 mRNA, there is no evidence that miR-UL112 acts like a siRNA to induce cleavage of the UL114 transcript. miR-US5-1 and miR-US5-2 are both anti-sense to US7 and act cooperatively through two perfect binding sites and one imperfect binding site to inhibit US7 protein expression (Tirabassi et al. 2011). While US7 functions are uncharacterized, rhesus CMV encodes homologs of US7 and miR-US5-2 (Tirabassi et al. 2011; Hancock et al. 2012), indicating that these interactions are important to the CMV life cycle.

Herpesvirus miRNAs further contribute to the lytic/latent balance by restricting immediate early viral transactivators (Grey et al. 2007; Umbach et al. 2008). HCMV miR-UL112 negatively regulates the major immediate early transcript IE72 (UL123, IE1) as well as UL112/113, which is involved in viral DNA replication, and UL120/121, which presumably encodes a membrane protein (Grey et al. 2007). Mutation of miR-UL112 within the context of full-length virus or the miR-UL112 binding site within the UL123 3' UTR leads to an increase in IE1 expression (Murphy et al. 2008). Additional studies demonstrate that post-transcriptional regulation of major viral lytic transactivators may be a conserved function of herpesvirus miRNAs. miR-H2-3p of HSV-1 is encoded antisense to ICP0 and thus silences protein expression from the transcript (Umbach et al. 2008). HSV-2 encodes a positional homolog, miR-III, that likewise targets the HSV-2 ICP0 mRNA (Umbach et al. 2010; Tang et al. 2008). Both miR-H2-3p and miR-III have been shown to decrease ICP0 protein expression in latency models (Umbach et al. 2008; Flores et al. 2013). Additionally, miR-H6 of HSV-1 targets the IE transcriptional activator ICP4 (Umbach et al. 2008; Tang et al. 2008, 2011). HSV-1 miR-H4 (Flores et al. 2013) and HSV-2 miR-I and miR-II (Tang et al. 2008, 2011) overlap with the coding sequence and 5' UTR of the virulence factor ICP34.5 and have been shown to decrease protein expression. There is no experimental evidence for viral targets of the PRV miRNAs and no miRNAs are directly complementary to PRV transcripts. However, in silico analysis suggests that the ICP0 and ICP4 homologs in PRV may also be PRV miRNA targets.

For human gamma-herpesviruses, studies of direct viral miRNA control over viral transactivators have not been so straightforward. KSHV miR-K9* was reported to target the ORF50 RNA, thus inhibiting expression of the immediate-early transactivator RTA and preventing lytic cycle entry during KSHV latent infection (Bellare and Ganem 2009); however, these observations have not been confirmed and other studies indicate the interaction may be indirect (Lei et al. 2010; Lu et al. 2010). EBV miR-BART20-5p has been reported to target transcripts of two immediate early transactivators, EBV BZLF1 and BRLF1, which share a 3' UTR (Jung et al. 2014). These interactions may also be indirect,

as RISC-capture experiments on EBV + cells have failed to confirm miR-BART20-5p binding sites within the BZLF1 or BHRF1 RNAs (Kang et al. 2015; Riley et al. 2012; Skalsky et al. 2014). Analysis of RISC-associated sites in wild-type EBV-infected LCLs indicate that EBV BALF2, BNRF1, BHRF1 and LMP1 transcripts are regulated by viral miRNAs (Skalsky et al. 2014). Viral miRNA targeting of LMP1 and BHRF1 3' UTRs have been confirmed by several groups, and functional consequences of LMP1 regulation have been examined with regard to the activation of NF- κ B signaling pathways (Lo et al. 2007; Riley et al. 2012; Skalsky et al. 2012, 2014). EBV LMP2A and EBNA2 are also subject to viral miRNA regulation (Riley et al. 2012; Skalsky et al. 2012, 2014; Lung et al. 2009; Kang et al. 2015), but like LMP1 and BHRF1, do not act as transactivators of the lytic cascade.

4.1 Host Silencers of Viral Gene Expression

Not only do herpesviruses utilize their own miRNAs to aid in latency establishment, but several examples exist whereby cellular miRNAs can directly limit herpesvirus gene expression in specific cell types. This was first demonstrated for EBV latent membrane protein 1 (LMP1) which is negatively regulated by the myc-regulated miR-17 seed family (includes miR-17, miR-20a, miR-106a/b) through a conserved binding site within the LMP1 3' UTR (Riley et al. 2012; Skalsky et al. 2012, 2014). The functional consequences of this interaction have not been fully evaluated; however, an attractive hypothesis is that the timing and levels of LMP1 transcripts together with kinetics of miR-17 expression following de novo B cell infection may play an important part in transitioning through EBV-mediated B cell growth and differentiation programs (Forte and Luftig 2011; Skalsky and Cullen 2015).

Use of cell-type specific host miRNAs to silence lytic transcripts is an emerging theme for alpha- and beta-herpesviruses. The neuronal-specific miRNA miR-138 was shown to target the ICP0 transcript of HSV-1 (Pan et al. 2014). When the miR-138 target site within the ICP0 3' UTR was mutated, the resultant virus displayed enhanced expression of lytic transcripts both in neuronal cultures and following corneal inoculation in mice resulting in increased mortality. Likewise, HCMV utilizes members of the miR-200 family to target the 3' UTR of UL122 (IE2) (O'Connor et al. 2014). miR-200 family members are expressed highest in undifferentiated cells of the myeloid lineage, where HCMV establishes latency. Using in vitro systems of HCMV latency, the authors show that a mutant lacking the miR-200 binding site in the 3' UTR of UL122 does not efficiently establish latency as measured by IE1 expression and production of extracellular virions. Thus, both HSV-1 and HCMV may have evolved to co-opt cellular miRNAs for targeting their major viral transactivators in order to aid in latency establishment.

5 Viral MiRNA Regulation of Transcription Factors

A broader way that viral miRNAs indirectly control the timing and/or level of viral gene expression is through the targeting of host transcription factors (TFs). For example, one way KSHV miR-K3 may inhibit lytic replication is through the targeting of cellular CAAT box TF nuclear factor I/B (NFIB) which binds the KSHV ORF50 promoter region and activates RTA expression (Lu et al. 2010). KSHV miRNAs miR-K3 and miR-K11 also target the B cell TFs MYB, Ets-1, and C/EBP α , which activate the ORF50/RTA promoter (Gottwein et

al. 2011; Haecker et al. 2012; Plaisance-Bonstaff et al. 2014). Similarly, EBV miR-BHRF1-2 targets PRDM1/BLIMP1, a master regulator of the B cell transcriptional program during B cell differentiation (Skalsky et al. 2012; Ma et al. 2016). BLIMP1 can activate the EBV immediate-early BZLF1 and BRLF1 promoters via multiple BLIMP1-responsive elements (Reusch et al. 2015). While a BHRF1-2 miRNA knockout virus does not spontaneously reactivate in vitro (Feederle et al. 2011b), regulation of BLIMP1 by miR-BHRF1-2 at a key stage of infection in vivo may help promote latency.

Herpesvirus miRNAs regulate many other TFs that drive transcriptional reprogramming of host cells and consequently, can impact viral gene expression patterns through more complicated layers of transcriptional control. A clear example of this are the KSHV miRNAs, in particular, miR-K11 and miR-K6, that can induce endothelial cell reprogramming by targeting the bZIP transcription factor MAF which mediates terminal differentiation of several cell types including lymphatic endothelial cells (Hansen 2010b). Additionally, NF- κ B, AP-1, and other signaling pathways that result in transcriptional activation are regulated at multiple steps by KSHV, EBV, and other gamma-herpesvirus miRNAs (Gottwein et al. 2007; Skalsky et al. 2014, 2016; Puig-Barbera et al. 2016; Abend et al. 2012). Both NF- κ B and AP-1 are activated upon gamma-herpesvirus infection and control multiple cellular processes including proliferation and differentiation as well as cytokine responses and inflammation. These and other TFs are also involved in the latent/lytic switch since the TFs bind response elements within promoter regions of several herpesvirus immediate early genes (Caposio et al. 2007; Kenney and Mertz 2014).

Individual viral miRNAs often exert subtle effects on protein expression levels. These subtle effects of single miRNAs can be amplified through TF targeting (Sun et al. 2014), and may be further amplified through the additive or combinatorial effects of multiple viral miRNAs acting in concert with cellular miRNAs coopted by the virus. Subtle inhibition of specific host TFs may also lead to dramatic changes in TF site occupancy- particularly in cases where an array of multiple TFs are needed to govern gene expression. EBV EBNA2, for example, forms super-enhancers with multiple B cell master TFs that contribute to B cell proliferation; transcripts for several of these super-enhancer TFs are RISC-associated and contain binding sites for multiple EBV miRNAs as well as host miRNAs such as miR-155, miR-146a, and miR-17 that are upregulated during EBV infection (Zhou et al. 2015; Gottwein et al. 2011; Riley et al. 2012; Skalsky et al. 2012).

6 Mimicry of Host MiRNA Function

While most herpesvirus miRNAs exhibit little or no sequence conservation to other viral miRNAs (exceptions being closely related primate gamma-herpesviruses) or their cellular counterparts, there are interesting examples of homology to host miRNA seed sequences (nt 2–7 or 2–8). Mimicking of this effector seed region may allow viral miRNAs to act in part as functional homologs and tie into host miRNA regulatory networks through the binding of cognate sites on cellular transcripts [reviewed in (Kincaid and Sullivan 2012)]. Such mimicry was first demonstrated for KSHV miR-K11 which bears seed homology to the miR-155 oncomiR. miR-155 is a key regulator of lymphocyte differentiation and is dysregulated in many hematopoietic cancers. Notably, 5' nucleotides 1–8 of KSHV miR-

K11 and miR-155 are identical, and initial gene expression studies demonstrated that miR-K11 and miR-155 could regulate an overlapping set of target cellular transcripts (Skalsky et al. 2007; Gottwein et al. 2007). Further in vivo experiments in humanized NOD/LtSz-scid IL2R γ (null) mice demonstrated that KSHV miR-K11 and miR-155 could both induce expansion of splenic B lymphocytes, in part through inhibition of the transcriptional regulator C/EBP β (Boss et al. 2011). Intriguingly, the avian lymphotropic alpha-herpesvirus MDV-1 also encodes a miR-155 homolog, miR-M4. The presence of this miRNA was shown to contribute to T cell lymphoma formation in chickens (Zhao et al. 2011) and recent RISC-capture studies revealed many conserved miR-M4 and miR-155 targets that may play a role in lymphomagenesis (Parnas et al. 2014).

KSHV encodes additional cellular miRNA mimics including a mimic of miR-23a/b (KSHV miR-K3) and a mimic of miR-142-3p (miR-K10a) (Manzano et al. 2013, 2015; Gottwein et al. 2011). miRNA targetome studies demonstrate that at least some targets of miR-142-3p and miR-K10a overlap (Gottwein et al. 2011), and functional assays have related these back to the regulation of transforming growth factor (TGF) β signaling (Lei et al. 2012). miR-23 family members are highly expressed in endothelial cells and tightly linked to hematopoietic lineage commitment; recent reports demonstrate opposing functions of miR-23a and miR-23b related to vascular angiogenesis and endothelial cell permeability (Li et al. 2016). For B cells, miR-23a is an inhibitor of B cell lymphopoiesis and blocks expression of B cell TFs such as EBF1 and PAX5 (Kurkewich et al. 2016). KSHV infects both endothelial and B cells, and at present, it is unclear in which of these cell types that miR-K3 activity has the most impact (Manzano et al. 2013). Manzano and colleagues hypothesized that miR-K3 might substitute for miR-23 function in B cells where the miRNA is absent or at very low levels; this may also be the case for miR-K11 which is detected in KSHV-infected B cell lymphoma types that do not express high levels of miR-155 (Manzano et al. 2013; Gottwein et al. 2007; Skalsky et al. 2007). Recent studies demonstrate that closely related non-human primate rhadinoviruses, RRV and JMRV, encode mimics of the miR-17/20/106 seed family (Skalsky et al. 2016). Functional analysis demonstrated that JMRV miR-J8, specifically, can block cytokine-mediated NF- κ B activation as well as target 3' UTRs bearing miR-17 cognate target sites. While this activity can dampen anti-viral inflammatory responses, miR-17 is also associated with B cell cancers and thus, the mimicking of miR-17 family members may contribute to the oncogenicity of these NHP rhadinoviruses.

7 Cataloging Targets Reveals Diversity in Networks Regulated by Viral MiRNAs

While sequence homology to host miRNAs or perfect complementarity to viral protein coding transcripts provides relatively easy insight into a handful of targets for herpesvirus miRNAs, comprehensively identifying targets for viral miRNAs has been a daunting challenge. Target recognition requires only limited sequence complementarity (generally, ~7–8 nt in the miRNA seed) between a miRNA and target site, thus a single miRNA can bind an estimated 150–200 unique sites (Bartel 2009). This is further complicated by the fact that viral miRNA effector seed sequences are not nearly as conserved as the cellular

miRNAs; thus, miRNA target prediction programs that rely on evolutionary scoring measures do not necessarily work for predicting viral miRNA targets.

Significant advancements have been made over the last five to seven years in the ability to globally capture and catalog miRNA targets in a high-throughput manner. Cross-linking and immunoprecipitation (CLIP) approaches, such as RIP-Chip, PAR-CLIP, or HITS-CLIP, whereby RISC-associated RNAs are immunopurified directly, analyzed by either microarray or RNA sequencing, and analyzed further by bioinformatics were first applied to human B cells infected with KSHV and/or EBV (Dolken et al. 2010b; Gottwein et al. 2011; Skalsky et al. 2012, 2014; Riley et al. 2012). Large-scale proteomic approaches, such as SILAC (stable isotope labeling by amino acids in cell culture), have yielded additional insight into viral miRNA targets. To identify KSHV miRNA targets by SILAC, miRNA mimics were transfected into human umbilical vein endothelial cells (HUVECs), cells were pulse-labeled and analyzed by mass spectrometry alongside control cells to determine changes in the proteome (Gallaher et al. 2013). Collectively, these CLIP and proteomics studies have revealed that at least 15% of host protein coding transcripts are subject to viral miRNA regulation during latent infection. Not surprisingly, the viral miRNA targets identified in these studies are related to innate anti-viral immunity, cell survival, and multiple signaling pathways such as NF- κ B, Wnt, and MAPK signaling that control cell proliferation and differentiation through distinct transcriptional programs (Fig. 3). Recent temporal analysis of the HCMV miRNA targetome during productive infection identified enriched networks such as metabolic and immune processes, trafficking and transport, and DNA replication that are subject to miRNA regulation during early, intermediate, and late times post-infection, respectively (Kim et al. 2015). Similar to the gamma-herpesviruses, apoptosis and intracellular signal pathways were influenced by HCMV miRNAs throughout all time points, reinforcing common networks that are critical to the herpesvirus life cycle in general.

8 Combinatorial Targeting and MiRNA Synergy

miRNAs are often thought to have relatively minor effects on protein expression levels, (Bartel 2004, 2009), and while in many cases this can have a profound effect on cellular processes, redundancies and overlapping functions may be capable of overcoming the effect of a single miRNA on a single target protein. One means to overcome this issue is for a viral miRNA to target several components within a cellular process or signaling pathway. There are several examples of HCMV, in particular, utilizing this approach to block innate immune signaling, cell cycle regulation and secretory pathways.

Grey et al. (2010) were the first to suggest that viral miRNAs could cooperatively target multiple components within a cellular pathway, and identified several HCMV miR-US25-1 targets related to cell cycle control including cyclin E2, BRCC3, EID1, MAPRE2 and CD147. Interestingly, miR-US25-1 target sites were identified within 5' UTRs, rather than the 3' UTRs of these targets. In addition, HCMV encodes at least three miRNAs (miRs-US5-1, US5-2 and UL112-1) that target multiple components of the cellular secretory pathway, including RAB5C, RAB11A, SNAP23 and CDC42 (Hook et al. 2014). Hook and colleagues showed that infection of cells with viral mutants lacking these miRNAs results in significantly increased secretion of IL6 and TNF α as well as aberrant VAC formation and

increased secretion of non-infectious virus particles (Hook et al. 2014). Importantly, the full effect was only evident when all three miRNAs were mutated, indicating that targeting multiple components of the secretory pathway is essential for productive viral infection.

Another method used by herpesviruses to maximally downregulate protein expression is to utilize multiple viral miRNAs to target the same transcript. This was first demonstrated for the THBS1 (Thrombospondin 1) 3' UTR which is co-targeted by at least four KSHV miRNAs; inhibition of this cell-cell adhesion protein by multiple KSHV miRNAs may contribute to KS pathogenesis as this results in decreased TGF β activity (Samols et al. 2007). HCMV utilizes both miR-US5-1 and miR-US5-2 to synergistically downregulate the expression of US7 (Tirabassi et al. 2011). Some evidence exists that viral miRNAs may also work synergistically with cellular miRNAs in order to maximize protein downregulation. For example, cellular miRNA miR-376a acts in concert with HCMV miR-UL112-1 to downregulate the stress-induced ligand MICB (Nachmani et al. 2010). CLIP studies have demonstrated that in addition to host miR-17, the EBV LMP1 3' UTR is targeted by multiple EBV BART miRNAs (Riley et al. 2012; Skalsky et al. 2012; 2014; Lo et al. 2007). EBV BHRF1 transcripts may also be synergistically regulated since binding sites within the 3' UTR for EBV miR-BART10 as well as the miR-17 family and miR-142-3p have been captured by PAR-CLIP (Skalsky et al. 2012, 2014).

Co-targeting and cooperativity between HCMV miRNAs and human miRNAs (i.e. particularly, those induced upon HCMV infection) has recently been observed on a more widespread level; following de novo infection of human fibroblasts, these miRNAs collectively regulate genes involved in cell cycle networks and RNA/protein processing which may enhance virus replication and virion production (Kim et al. 2015). HITS-CLIP studies on EBV + Burkitt's lymphoma cell lines revealed that members of the miR-17/92 cluster and EBV miRNAs may also act in concert to co-target several host genes related to apoptosis or involved in B cell lymphomagenesis (Riley et al. 2012). Advancements in network analysis and system-wide approaches to capture miRNA targets at different infection stages will facilitate the realization of additional cooperativity between host and viral miRNAs.

9 Unique Interplay Between Herpesviruses and Cellular MiRNAs

Cellular miRNAs can have anti-viral functions (Seo et al. 2013) and it is clear that herpesviruses have evolved to modulate the overall levels of cellular miRNAs by transcriptional and post-transcriptional methods (Santhakumar et al. 2010). In an interesting twist, Buck et al. showed that herpesvirus transcripts could act as decoys for cellular miRNAs, functionally decreasing the miRNA levels in infected cells in a post-transcriptional manner (Buck et al. 2010). MCMV infection of mouse fibroblasts mediates the rapid downregulation of miR-27, a miRNA capable of limiting MCMV replication when overexpressed (Buck et al. 2010). A canonical miR-27 binding site was discovered that maps to the 3' UTR of m169, an ORF of unknown function (Buck et al. 2010). Mutation of the miR-27 binding site is capable of retargeting the transcript to other cellular miRNAs (Marcinowski et al. 2012). Importantly, Marcinowski et al. (2012) showed a growth defect in multiple organs in vivo using viral mutants incapable of binding miR-27. Similar results

with infection of SCID BALB/c mice indicate the defect is independent of the adaptive immune response. Why MCMV targets miR-27 for degradation is unclear, although the miRNA has roles in cell cycle progression, a process highly manipulated by CMVs. Interestingly, the miR-27 binding site is highly conserved in MCMV species, but not the closely related RCMV (McCaskill et al. 2015).

Lee et al. (2013) discovered an HCMV intergenic RNA sequence between UL144 and UL145 that functions to downregulate members of the miR-17–92 cluster termed the miRNA decay element (miRDE). HCMV IE1 and IE2 induce the transcription of pri-miR-17–92, however of the six miRNAs encoded within this cluster (Mogilyansky and Rigoutsos 2013) only mature miR-17 and miR-20a are selectively downregulated by the miRDE. In fact, levels of pre-miRNA, as well as the passenger miR-17* and miR-20a* strands, increase during infection. Unlike what is observed with MCMV and miR-27, binding of the miRNAs to the miRDE is non-canonical and does not affect miRDE stability. Like the m169 ORF of MCMV, the miRDE sequence can be re-engineered to target other cellular miRNAs. The miR-17 binding site within the intergenic region between UL144 and UL145 is highly conserved in clinical strains of HCMV and other Old World primates (McCaskill et al. 2015), however the consequence of downregulating miR-17 and miR-20a during HCMV infection is unclear. Peak viral titers are slightly decreased when the miRDE is mutated, a defect that can be rescued using antisense oligomers to miR-17 and miR-20a. The authors suggest that since miR-17 and miR-20a target cell cycle regulators, the virus may prevent cell cycle arrest, although this awaits experimental confirmation.

As another example of miRNA sponging by herpesviruses, Cazalla et al. (2010) determined that HSURs 1 and 2 of HVS contain binding sites for three cellular miRNAs: miR-16, miR-27 and miR-142-3p. Co-immunoprecipitation experiments identified a direct association between HSUR snRNPs and the cellular miRNAs in HVS-transformed cells. Using plasmids expressing individual HSURs, a direct interaction between miR-27 and HSUR1 was determined. Analysis of T cell lines transformed with WT or HSUR-deleted viruses showed that miR-27 levels inversely correlate with HSUR1 expression. HSUR1 contains an AU-rich RNA decay element (ARE), which is involved in turnover of HSUR1. Mutation of the ARE sequence results in increased levels of HSUR1 and further decreased levels of miR-27, indicating that miR-27 turnover may occur through the ARE pathway. Increased levels of FOXO1, a confirmed target of miR-27 (Guttilla and White 2009), was observed when HSURs 1 and 2 were expressed. Further investigation into miR-27 targets identified GRB2 and other proteins involved in T cell receptor signaling that are modulated by HSUR1 expression, suggesting that HVS manipulation of miR-27 levels may contribute to the T cell transformation phenotype (Guo et al. 2014). The importance of HSUR binding of miR-16, a miRNA involved in cell cycle control (Liu et al. 2008) and miR-142-3p, a miRNA involved in hematopoietic differentiation (Lu et al. 2013) awaits investigation. miRNA decoys have not yet been demonstrated for human gamma-herpesviruses, although this has been alluded to for EBV (Cao et al. 2015).

10 In Vivo Phenotypes of Herpesvirus NcRNAs

Initial observations of EBV miRNAs provided some hints that herpesvirus miRNAs are not essential for basic aspects of the viral replication cycle and rather function as virulence factors to enhance viral persistence within a host. 17 of the 22 EBV BART miRNAs are absent from the prototypical EBV B95-8 laboratory strain and were in fact identified only after sequencing small RNAs from cells infected with patient-derived EBV strains (Pfeffer et al. 2004; Grundhoff et al. 2006; Chen et al. 2010). EBV B95-8 effectively immortalizes primary B cells and establishes latent infection comparable to full-length, wildtype virus in vitro. Deletion of all the BHRF1 miRNAs from EBV B95-8 has only a moderate effect on the outgrowth of LCLs in vitro (Feederle et al. 2011a). While difficult to test in vivo, in humanized mice, this BHRF1 miRNA mutant virus exhibits a significant delay in systemic infection compared to wild-type, with lower levels of mutant virus appearing in circulation following injection of the spleen (Wahl et al. 2013). Interestingly, EBV + tumors develop in these mice several weeks after primary infection with no measurable differences between wild-type B95-8 and the BHRF1 miRNA mutant (Wahl et al. 2013). Recent in vitro studies suggest that the BHRF1 miRNAs may play a more pertinent role during acute stages of infection during the transition to latency when robust T cell responses must be counteracted for effective long-term persistence (Albanese et al. 2016; Tagawa et al. 2016).

Overexpressing viral miRNAs in cells and injecting these cells into mice have tested phenotypes for EBV or KSHV miRNAs in the absence of infection. EBV-negative nasopharyngeal carcinoma (NPC) cells were engineered to express EBV miR-BART7-3p and injected into irradiated mice (Cai et al. 2015). Three weeks later, lymph nodes were examined for metastases; ~83% of mice injected with miR-BART7-3p expressing cells developed metastases compared to only ~17% of mice injected with control NPC cells (Cai et al. 2015). EBV miR-BART7-3p was subsequently shown to target PTEN (phosphatase and tensin homolog) and alter PI3 K/Akt signaling events that may contribute to epithelial-to-mesenchymal transition and enhance the metastatic potential of NPC cells (Cai et al. 2015). Ectopic EBV BART miRNA expression in AGS gastric carcinoma cells produced slightly different results when injected into the nasopharyngeal epithelial tissue of immunodeficient NSG mice (Qiu et al. 2015). In this xenograft mouse model, 5/5 mice injected with BART miRNA expressing cells developed large, aggressive tumors that required sacrifice within 74 days compared to 2/5 control mice that developed small tumors and remained healthy. In contrast to Cai et al. (2015), no significant differences in invasion or metastases were observed, which could be due to the overall expression levels of the individual EBV BART miRNAs within each model. As mentioned earlier, injection of human hematopoietic progenitor cells (HPCs) ectopically expressing KSHV miR-K11 into NOD/LtSz-scid IL2R γ (null) mice induced expansion of human CD19 + B lymphocytes in the spleen (Boss et al. 2011). Confirming these observations, expression of miR-K11 in HPCs in a different mouse model resulted in expansion of peripheral mature B cells as well as increased numbers of immature pre-B cells in bone marrow (Dahlke et al. 2012). Thus, in the absence of virus, these studies demonstrate that individual gamma-herpesvirus miRNAs can potentiate oncogenesis. One outstanding question is what functions these viral miRNAs

and other ncRNAs have with respect to pathogenesis during the course of natural infection or at least within the genetic background of a natural host.

10.1 Viral Small NcRNA Phenotypes in Natural Hosts

Loss of function studies using miRNA mutant herpesviruses in small animal models that are natural hosts have demonstrated that many viral miRNAs are dispensable for acute replication in vivo. Similar to HSV-1, swine PRV encodes multiple miRNAs within the LAT region. PRV is neurotropic, establishes latency in the trigeminal ganglia, and expresses an 8.4 kb polyadenylated large latency transcript (LLT) which is spliced to yield a 4.6 kb stable LLT intron encoding the PRV miRNAs. German Landrace pigs were inoculated intra-nasally with a LLT-deletion virus (spanning 9 of 11 PRV pre-miRNAs) (Mahjoub et al. 2015). Compared to wildtype virus, no differences in viral replication were observed during acute infection and surprisingly, the mutant virus established latency with no evidence of promiscuous lytic reactivation (Mahjoub et al. 2015). Recently, two groups investigated MHV68 small ncRNA (miRNA and TMER) knockout viruses compared to wild-type or revertant viruses (Feldman et al. 2014, 2016; Diebel et al. 2015). In vitro, viruses lacking the MHV68 small ncRNAs replicate with the same efficiency as wild-type virus. During acute infection in immunocompetent mice, there were no discernable differences in viral replication. Analysis of a panel of TMER-mutant viruses in vivo revealed that MHV68 TMER4 can contribute to viral dissemination independent of the viral miRNAs, which is important for establishment of latency in the periphery (Feldman et al. 2016).

The first in vivo phenotype for a beta-herpesvirus miRNA mutant was demonstrated during MCMV infection in mice (Dolken et al. 2010a). When two of the most highly expressed MCMV miRNAs [miR-M23-2 and miR-m21-1 (Dolken et al. 2007)] were mutated, the resultant virus showed a ~100-fold reduction in replication in the salivary gland at 14 days post-infection. This defect was dependent on mouse strain and viral dose and was not observed in other tissues. The salivary gland replication defect could be partially restored through combined depletion of NK and CD4 + T cells; however, targets of miR-M23-2 and miR-m21-1 have not been identified. Given that the salivary gland is an immunologically unique site that is important for horizontal transmission, insights into the targets of these miRNAs would aid in understanding how CMV alters the cellular environment to establish a persistent infection while evading the host immune response.

To date, the only non-human primate model for which a viral miRNA mutant has been tested is RhCMV infection of Indian rhesus macaques. Similar to observations made in small animal models, deletion of a large region (RhCMV US2-US11) spanning the HCMV miR-US5-2 homolog, RhCMV miR-183-1, and other RhCMV miRNAs (Skalsky, Hancock et al., unpublished observations) showed no changes in viral replication or dissemination (Hansen et al. 2010a). For humans, epidemiological studies where viral genomic information is available can provide important links between viral miRNAs and disease phenotypes. As a notable example, natural KSHV strains contain SNPs in several of the pre-miRNA regions which affect miRNA processing (Han et al. 2013; Marshall et al. 2007); several pre-miRNA SNPs track with increased disease risk for AIDS-KS patients or are associated with Multicentric Castleman's disease (Marshall et al. 2010; Ray et al. 2012).

10.2 In Vivo Phenotypes of LncRNA Knockouts

For herpesvirus lncRNAs, in vivo phenotypes are more difficult to pinpoint as being a direct effect of the lncRNA since abrogation of the lncRNA requires larger deletion(s) from the virus that could disrupt protein and other non-coding regions of potentially unknown functions. Attempts have been made to characterize several herpesvirus lncRNAs in vivo. A homolog of HCMV RNA5.0, MCMV RNA7.2, is an extremely long-lived stable intron during MCMV infection (Kulesza and Shenk 2006; Schwarz et al. 2013). Although mutation of the RNA7.2 region had no effect on replication in vitro, RNA7.2 mutant viruses fail to persist in the mouse salivary gland at 14 days post-infection (Schwarz et al. 2013; Kulesza and Shenk 2006) suggesting that RNA7.2 is a virulence factor.

HSV LAT is the most abundantly transcribed RNA in latently infected cells in humans (Croen et al. 1987) and in small animal models (Stevens et al. 1987), and as such likely plays an important role during latency. Surprisingly then, it has been observed that LAT-negative viruses are still capable of establishing and reactivating from latency (Perng et al. 1994; Chen et al. 1997). However, a decrease in the number of latently infected trigeminal ganglia upon infection of mice with LAT-viruses (Thompson and Sawtell 1997; Devi-Rao et al. 1994) suggests at least a contributing role for the transcript in establishment or maintenance of latency. Many of the ascribed functions of LAT, including blocking apoptosis and heterochromatinization of the lytic promoters may contribute to this phenotype. Additionally, LAT has been shown to repress lytic gene expression (Chen et al. 1997), likely since the primary LAT transcript encodes for many of the HSV miRNAs that target ICP0 and ICP4 (Umbach et al. 2010; Tang et al. 2009; Umbach et al. 2008). Finally, LAT seems to be important for promoting spontaneous reactivation in rabbits (Perng et al. 1994; Hill et al. 1990), although this is not observed in mice (Margolis et al. 2007; Carr et al. 1998), indicating species-specific phenotypes (Margolis et al. 2007). The role of LAT in HSV latency in small animal models is complicated by the relatively short lifespan of these non-natural hosts and thus, the essential functions of LAT in humans may as yet be identified.

Phenotypes for EBV lncRNAs have also been difficult to elucidate in vivo. EBER1 and EBER2 knockout viruses were examined recently in a humanized mouse model. Compared to EBER-expressing viruses, there were no significant differences in viral loads in spleen and peripheral blood or reproducible phenotypes for CD8 + T cell responses (Gregorovic et al. 2015). Historical phenotypes associated with deletions or mutations in herpesvirus genomes may yet be linked to viral lncRNA functions as additional molecular and transcriptomic studies are performed. Furthermore, as more non-conventional molecular interactions are uncovered for viral lncRNAs, this information can guide the exploration for specific phenotypes in vivo.

11 Summary and Outlook

Much progress has been made in defining the ncRNAs that are produced by herpesviruses at various stages of their complex life cycles, and viral ncRNA functions are progressively being elucidated. The lncRNAs that are abundantly expressed during gamma-herpesvirus lytic replication play important roles at the epigenetic layer in modulating chromatin

structure; presumably, this allows access to the viral genome for lytic replication and/or lytic viral gene expression. For alpha and beta herpesviruses, lncRNAs have important roles in recruiting chromatin remodelers to silence viral gene expression; these chromatin marks can poise the lytic promoters for reactivation stimuli. Likely, this is further aided through the myriad of activities of the viral miRNAs (Fig. 3).

Many studies demonstrate that herpesviruses utilize their own miRNAs as well as co-opt host miRNAs to regulate the kinetics and level of viral transcripts; this contributes primarily towards the silencing of viral gene expression to promote an environment for latency. Viral and host miRNAs are further utilized to manipulate the host proteome which subsequently impacts signaling pathways, cell networks, and the micro-environment (Fig. 3). Important particularly for beta- and gamma-herpesviruses, and directly related to viral pathogenesis, many of these miRNA-regulated networks control cell lineage commitment and differentiation. The combinatorial targeting of signaling components as well as targeting of the transcription factors governing cell processes by viral miRNAs amplifies the subtle effects of individual miRNAs; furthermore, many viral miRNAs are expressed as clusters and thus can act simultaneously within a specific stage of the viral life cycle. Thus, these collective actions induce significant changes in the host transcriptome, amplified initially at the transcriptional level and further amplified at the post-translational level. In short, these tiny molecules have big impacts in providing a fit cellular environment to effectively support herpesvirus persistence.

New technologies to capture detailed molecular interactions between ncRNAs and binding partners should yield insight into novel functions during herpesvirus infection. Genome-wide meta-approaches to integrate the multiple layers of epigenetic, transcriptional, and post-transcriptional control facilitated by herpesvirus ncRNAs will help to further dissect the complexities of herpesvirus-host interactions and identify innovative opportunities for therapeutic intervention.

Questions Moving Forward

1. What viral ncRNAs are present and functional in vivo?
2. What is the viral ncRNA influence on the extracellular environment and how much does this contribute to pathogenesis?
3. How does the cell context contribute to viral ncRNA function(s)?
4. How can viral ncRNAs be used in novel ways to understand functions of host ncRNAs or probe unknown cellular functions?
5. How can viral ncRNAs be used as effective therapeutic targets?

Acknowledgments

We thank the past and present members of Jay Nelson's laboratory at VGTI for discussions, and apologize to the many scientists whose work we could not mention due to space limitations. This work was supported by a Pathway to Independence Award CA175181 from the National Cancer Institute to R.L.S. Support for M.H. comes from NIH grant AI21640.

Abbreviations

KSHV	Kaposi's sarcoma associated herpesvirus
HVS	Herpesvirus saimiri
HCMV	Human Cytomegalovirus
RhCMV	Rhesus Cytomegalovirus
MCMV	Mouse Cytomegalovirus
RCMV	Rat Cytomegalovirus
EBV	Epstein Barr virus
LCV	Lymphocryptovirus
HSV	Herpes simplex virus
miRNA	MicroRNA
snoRNA	Small nucleolar RNA
sisRNA	Stable intronic sequence RNA
ncRNA	Non-coding RNA
EBERs	EBV-encoded RNAs
BARTs	BamHI A rightward transcripts
BHRF1	BamHIright forward 1
PAN	Polyadenylated nuclear RNA
LAT	Latency-associated transcript
HSURs	HVSSm class U RNAs
UTR	Untranslated region
LMP	Latent membrane protein
RTA	Replication and transcription activator protein
EBNA	EBV nuclear antigen
RNA	Ribonucleic acid
RISC	RNA-induced silencing complex
PAR-CLIP	Photoactivatable ribonucleoside enhanced cross-linking and immunoprecipitation

HITS-CLIP	High throughput sequencing of RNA isolated by cross-linking and immunoprecipitation
ChIRP	Chromatin isolation by RNA purification
CHART	Capture hybridization analysis of RNA targets
TF	Transcription factor

References

- Abend JR, Ramalingam D, Kieffer-Kwon P, Uldrick TS, Yarchoan R, Ziegelbauer JM. 2012; KSHV microRNAs target two components of the TLR/IL-1R signaling cascade, IRAK1 and MYD88, to reduce inflammatory cytokine expression. *J Virol.* doi: 10.1128/JVI.01147-12
- Albanese M, Tagawa T, Bouvet M, Maliqi L, Lutter D, Hoser J, Hastreiter M, Hayes M, Sugden B, Martin L, Moosmann A, Hammerschmidt W. 2016; Epstein-Barr virus microRNAs reduce immune surveillance by virus-specific CD8 + T cells. *Proc Natl Acad Sci U S A.* 113(42):E6467–E6475. DOI: 10.1073/pnas.1605884113 [PubMed: 27698133]
- Ambros V. 2004; The functions of animal microRNAs. *Nature.* 431(7006):350–355. DOI: 10.1038/nature02871 [PubMed: 15372042]
- Amelio AL, Giordani NV, Kubat NJ, O'Neil JE, Bloom DC. 2006; Deacetylation of the herpes simplex virus type 1 latency-associated transcript (LAT) enhancer and a decrease in LAT abundance precede an increase in ICP0 transcriptional permissiveness at early times postexplant. *J Virol.* 80(4):2063–2068. DOI: 10.1128/JVI.01528-10 [PubMed: 16439563]
- Amoroso R, Fitzsimmons L, Thomas WA, Kelly GL, Rowe M, Bell AI. 2011; Quantitative studies of Epstein-Barr virus-encoded microRNAs provide novel insights into their regulation. *J Virol.* 85(2):996–1010. DOI: 10.1128/JVI.01528-10 [PubMed: 21068248]
- Arias C, Weisburd B, Stern-Ginossar N, Mercier A, Madrid AS, Bellare P, Holdorf M, Weissman JS, Ganem D. 2014; KSHV 2.0: a comprehensive annotation of the Kaposi's sarcoma-associated herpesvirus genome using next-generation sequencing reveals novel genomic and functional features. *PLoS Pathog.* 10(1):e1003847. doi: 10.1371/journal.ppat.1003847 [PubMed: 24453964]
- Arvey A, Tempera I, Tsai K, Chen HS, Tikhmyanova N, Klichinsky M, Leslie C, Lieberman PM. 2012; An atlas of the Epstein-Barr virus transcriptome and epigenome reveals host-virus regulatory interactions. *Cell Host Microbe.* 12(2):233–245. DOI: 10.1016/j.chom.2012.06.008 [PubMed: 22901543]
- Bartel DP. 2004; MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 116(2):281–297. [PubMed: 14744438]
- Bartel DP. 2009; MicroRNAs: target recognition and regulatory functions. *Cell.* 136(2):215–233. [PubMed: 19167326]
- Barth S, Pfuhl T, Mamiani A, Ehse C, Roemer K, Kremmer E, Jaker C, Hock J, Meister G, Grasser FA. 2008; Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. *Nucleic Acids Res.* 36(2):666–675. DOI: 10.1093/nar/gkm1080 [PubMed: 18073197]
- Bellare P, Ganem D. 2009; Regulation of KSHV lytic switch protein expression by a virus-encoded microRNA: an evolutionary adaptation that fine-tunes lytic reactivation. *Cell Host Microbe.* 6(6):570–575. DOI: 10.1016/j.chom.2009.11.008 [PubMed: 20006845]
- Bogerd HP, Karnowski HW, Cai X, Shin J, Pohlars M, Cullen BR. 2010; A Mammalian Herpesvirus Uses Noncanonical Expression and Processing Mechanisms to Generate Viral MicroRNAs. *Mol Cell.* 37:135–142. [PubMed: 20129062]
- Boss IW, Nadeau PE, Abbott JR, Yang Y, Mergia A, Renne R. 2011; A Kaposi's sarcoma-associated herpesvirus-encoded ortholog of microRNA miR-155 induces human splenic B-cell expansion in NOD/LtSz-scid IL2R γ manull mice. *J Virol.* 85(19):9877–9886. DOI: 10.1128/JVI.05558-11 [PubMed: 21813606]

- Britt W. 2008; Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. *Curr Top Microbiol Immunol.* 325:417–470. [PubMed: 18637519]
- Bruce AG, Ryan JT, Thomas MJ, Peng X, Grundhoff A, Tsai CC, Rose TM. 2013; Next-generation sequence analysis of the genome of RFHVMn, the macaque homolog of Kaposi's sarcoma (KS)-associated herpesvirus, from a KS-like tumor of a pig-tailed macaque. *J Virol.* 87(24):13676–13693. DOI: 10.1128/JVI.02331-13 [PubMed: 24109218]
- Buck AH, Perot J, Chisholm MA, Kumar DS, Tuddenham L, Cognat V, Marcinowski L, Dolken L, Pfeffer S. 2010; Post-transcriptional regulation of miR-27 in murine cytomegalovirus infection. *RNA.* 16(2):307–315. DOI: 10.1261/rna.1819210 [PubMed: 20047990]
- Buck AH, Santoyo-Lopez J, Robertson KA, Kumar DS, Reczko M, Ghazal P. 2007; Discrete clusters of virus-encoded micrornas are associated with complementary strands of the genome and the 7.2-kilobase stable intron in murine cytomegalovirus. *J Virol.* 81(24):13761–13770. DOI: 10.1128/JVI.01290-07 [PubMed: 17928340]
- Cai LM, Lyu XM, Luo WR, Cui XF, Ye YF, Yuan CC, Peng QX, Wu DH, Liu TF, Wang E, Marincola FM, Yao KT, Fang WY, Cai HB, Li X. 2015; EBV-miR-BART7-3p promotes the EMT and metastasis of nasopharyngeal carcinoma cells by suppressing the tumor suppressor PTEN. *Oncogene.* 34(17):2156–2166. DOI: 10.1038/onc.2014.341 [PubMed: 25347742]
- Cai X, Lu S, Zhang Z, Gonzalez CM, Damania B, Cullen BR. 2005; Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. *Proc Natl Acad Sci U S A.* 102(15):5570–5575. DOI: 10.1073/pnas.0408192102 [PubMed: 15800047]
- Cai X, Schafer A, Lu S, Bilello JP, Desrosiers RC, Edwards R, Raab-Traub N, Cullen BR. 2006; Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog.* 2(3):e23.doi: 10.1371/journal.ppat.0020023 [PubMed: 16557291]
- Campbell M, Kung HJ, Izumiya Y. 2014; Long non-coding RNA and epigenetic gene regulation of KSHV. *Viruses.* 6(11):4165–4177. DOI: 10.3390/v6114165 [PubMed: 25375882]
- Cao S, Strong MJ, Wang X, Moss WN, Concha M, Lin Z, O'Grady T, Baddoo M, Fewell C, Renne R, Flemington EK. 2015; High-throughput RNA sequencing-based virome analysis of 50 lymphoma cell lines from the Cancer Cell Line Encyclopedia project. *J Virol.* 89(1):713–729. DOI: 10.1128/JVI.02570-14 [PubMed: 25355872]
- Caposio P, Lukanini A, Hahn G, Landolfo S, Griboaldo G. 2007; Activation of the virus-induced IKK/NF-kappaB signalling axis is critical for the replication of human cytomegalovirus in quiescent cells. *Cell Microbiol.* 9(8):2040–2054. DOI: 10.1111/j.1462-5822.2007.00936.x [PubMed: 17419715]
- Carr DJ, Halford WP, Veress LA, Noisakran S, Perng GC, Wechsler SL. 1998; The persistent elevated cytokine mRNA levels in trigeminal ganglia of mice latently infected with HSV-1 are not due to the presence of latency associated transcript (LAT) RNAs. *Virus Res.* 54(1):1–8. [PubMed: 9660066]
- Cazalla D, Yario T, Steitz JA. 2010; Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. *Science.* 328(5985):1563–1566. DOI: 10.1126/science.1187197 [PubMed: 20558719]
- Cech TR, Steitz JA. 2014; The noncoding RNA revolution-trashing old rules to forge new ones. *Cell.* 157(1):77–94. DOI: 10.1016/j.cell.2014.03.008 [PubMed: 24679528]
- Chandriani S, Xu Y, Ganem D. 2010; The lytic transcriptome of Kaposi's sarcoma-associated herpesvirus reveals extensive transcription of noncoding regions, including regions antisense to important genes. *J Virol.* 84(16):7934–7942. DOI: 10.1128/JVI.00645-10 [PubMed: 20534856]
- Chen SH, Kramer MF, Schaffer PA, Coen DM. 1997; A viral function represses accumulation of transcripts from productive-cycle genes in mouse ganglia latently infected with herpes simplex virus. *J Virol.* 71(8):5878–5884. [PubMed: 9223477]
- Chen SJ, Chen GH, Chen YH, Liu CY, Chang KP, Chang YS, Chen HC. 2010; Characterization of Epstein-Barr virus miRNAome in nasopharyngeal carcinoma by deep sequencing. *PLoS ONE.* 5(9):0001.doi: 10.1371/journal.pone.0012745
- Chinen M, Tani T. 2012; Diverse functions of nuclear non-coding RNAs in eukaryotic gene expression. *Front Biosci (Landmark Ed).* 17:1402–1417. [PubMed: 22201811]

- Cliffe AR, Coen DM, Knipe DM. 2013; Kinetics of facultative heterochromatin and polycomb group protein association with the herpes simplex viral genome during establishment of latent infection. *MBio*. 4(1):0001.doi: 10.1128/mBio.00590-12
- Cliffe AR, Garber DA, Knipe DM. 2009; Transcription of the herpes simplex virus latency-associated transcript promotes the formation of facultative heterochromatin on lytic promoters. *J Virol*. 83(16):8182–8190. DOI: 10.1128/JVI.00712-09 [PubMed: 19515781]
- Concha M, Wang X, Cao S, Baddoo M, Fewell C, Lin Z, Hulme W, Hedges D, McBride J, Flemington EK. 2012; Identification of new viral genes and transcript isoforms during Epstein-Barr virus reactivation using RNA-Seq. *J Virol*. 86(3):1458–1467. DOI: 10.1128/JVI.06537-11 [PubMed: 22090128]
- Conrad NK. 2016; New insights into the expression and functions of the Kaposi's sarcoma-associated herpesvirus long noncoding PAN RNA. *Virus Res*. 212:53–63. DOI: 10.1016/j.virusres.2015.06.012 [PubMed: 26103097]
- Conrad NK, Steitz JA. 2005; A Kaposi's sarcoma virus RNA element that increases the nuclear abundance of intronless transcripts. *EMBO J*. 24(10):1831–1841. DOI: 10.1038/sj.emboj.7600662 [PubMed: 15861127]
- Croen KD, Ostrove JM, Dragovic LJ, Smialek JE, Straus SE. 1987; Latent herpes simplex virus in human trigeminal ganglia. Detection of an immediate early gene “anti-sense” transcript by in situ hybridization. *N Engl J Med*. 317(23):1427–1432. DOI: 10.1056/NEJM198712033172302 [PubMed: 2825014]
- Dahlke C, Maul K, Christalla T, Walz N, Schult P, Stocking C, Grundhoff A. 2012; A microRNA encoded by Kaposi sarcoma-associated herpesvirus promotes B-cell expansion in vivo. *PLoS ONE*. 7(11):e49435.doi: 10.1371/journal.pone.0049435 [PubMed: 23185331]
- Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. 2012; The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res*. 22(9):1775–1789. DOI: 10.1101/gr.132159.111 [PubMed: 22955988]
- Devi-Rao GB, Bloom DC, Stevens JG, Wagner EK. 1994; Herpes simplex virus type 1 DNA replication and gene expression during explant-induced reactivation of latently infected murine sensory ganglia. *J Virol*. 68(3):1271–1282. [PubMed: 8107193]
- Diebel KW, Oko LM, Medina EM, Niemeyer BF, Warren CJ, Claypool DJ, Tibbetts SA, Cool CD, Clamby ET, van Dyk LF. 2015; Gammaherpesvirus small noncoding RNAs are bifunctional elements that regulate infection and contribute to virulence in vivo. *MBio*. 6(1):e01670–e01614. DOI: 10.1128/mBio.01670-14 [PubMed: 25691585]
- Diebel KW, Smith AL, van Dyk LF. 2010; Mature and functional viral miRNAs transcribed from novel RNA polymerase III promoters. *RNA*. 16(1):170–185. DOI: 10.1261/rna.1873910 [PubMed: 19948768]
- Dolken L, Krmpotic A, Kothe S, Tuddenham L, Tanguy M, Marcinowski L, Ruzsics Z, Elefant N, Altuvia Y, Margalit H, Koszinowski UH, Jonjic S, Pfeffer S. 2010a; Cytomegalovirus microRNAs facilitate persistent virus infection in salivary glands. *PLoS Pathog*. 6(10):e1001150.doi: 10.1371/journal.ppat.1001150 [PubMed: 20976200]
- Dolken L, Malterer G, Erhard F, Kothe S, Friedel CC, Suffert G, Marcinowski L, Motsch N, Barth S, Beitzinger M, Lieber D, Bailer SM, Hoffmann R, Ruzsics Z, Kremmer E, Pfeffer S, Zimmer R, Koszinowski UH, Grasser F, Meister G, Haas J. 2010b; Systematic analysis of viral and cellular microRNA targets in cells latently infected with human gamma-herpesviruses by RISC immunoprecipitation assay. *Cell Host Microbe*. 7(4):324–334. DOI: 10.1016/j.chom.2010.03.008 [PubMed: 20413099]
- Dolken L, Perot J, Cognat V, Alioua A, John M, Soutschek J, Ruzsics Z, Koszinowski U, Voinnet O, Pfeffer S. 2007; Mouse cytomegalovirus microRNAs dominate the cellular small RNA profile during lytic infection and show features of posttranscriptional regulation. *J Virol*. 81(24):13771–13782. DOI: 10.1128/JVI.01313-07 [PubMed: 17942535]

- Dunn W, Trang P, Zhong Q, Yang E, van Belle C, Liu F. 2005; Human cytomegalovirus expresses novel microRNAs during productive viral infection. *Cell Microbiol.* 7(11):1684–1695. DOI: 10.1111/j.1462-5822.2005.00598.x [PubMed: 16207254]
- Edwards RH, Marquitz AR, Raab-Traub N. 2008; Epstein-Barr virus BART microRNAs are produced from a large intron prior to splicing. *J Virol.* 82(18):9094–9106. DOI: 10.1128/JVI.00785-08 [PubMed: 18614630]
- Estep RD, Hansen SG, Rogers KS, Axthelm MK, Wong SW. 2013; Genomic characterization of Japanese macaque rhadinovirus, a novel herpesvirus isolated from a nonhuman primate with a spontaneous inflammatory demyelinating disease. *J Virol.* 87(1):512–523. DOI: 10.1128/JVI.02194-12 [PubMed: 23097433]
- Feederle R, Haar J, Bernhardt K, Linnstaedt SD, Bannert H, Lips H, Cullen BR, Delecluse HJ. 2011a; The members of a viral miRNA cluster co-operate to transform B lymphocytes. *J Virol.* doi: 10.1128/JVI.05100-11
- Feederle R, Linnstaedt SD, Bannert H, Lips H, Bencun M, Cullen BR, Delecluse HJ. 2011b; A viral microRNA cluster strongly potentiates the transforming properties of a human herpesvirus. *PLoS Pathog.* 7(2):e1001294.doi: 10.1371/journal.ppat.1001294 [PubMed: 21379335]
- Feldman ER, Kara M, Coleman CB, Grau KR, Oko LM, Krueger BJ, Renne R, van Dyk LF, Tibbetts SA. 2014; Virus-encoded microRNAs facilitate gammaherpesvirus latency and pathogenesis in vivo. *MBio.* 5(3):e00981–e00914. DOI: 10.1128/mBio.00981-14 [PubMed: 24865551]
- Feldman ER, Kara M, Oko LM, Grau KR, Krueger BJ, Zhang J, Feng P, van Dyk LF, Renne R, Tibbetts SA. 2016; A Gammaherpesvirus Noncoding RNA Is Essential for Hematogenous Dissemination and Establishment of Peripheral Latency. *mSphere.* 1(2)doi: 10.1128/mSphere.00105-15
- Flores O, Nakayama S, Whisnant AW, Javanbakht H, Cullen BR, Bloom DC. 2013; Mutational inactivation of herpes simplex virus 1 microRNAs identifies viral mRNA targets and reveals phenotypic effects in culture. *J Virol.* 87(12):6589–6603. DOI: 10.1128/JVI.00504-13 [PubMed: 23536669]
- Fok V, Mitton-Fry RM, Grech A, Steitz JA. 2006; Multiple domains of EBER 1, an Epstein-Barr virus noncoding RNA, recruit human ribosomal protein L22. *RNA.* 12(5):872–882. DOI: 10.1261/rna.2339606 [PubMed: 16556938]
- Forte E, Luftig MA. 2011; The role of microRNAs in Epstein-Barr virus latency and lytic reactivation. *Microbes and infection/Institut Pasteur.* 13(14–15):1156–1167. DOI: 10.1016/j.micinf.2011.07.007
- Fu M, Gao Y, Zhou Q, Zhang Q, Peng Y, Tian K, Wang J, Zheng X. 2014; Human cytomegalovirus latent infection alters the expression of cellular and viral microRNA. *Gene.* 536(2):272–278. DOI: 10.1016/j.gene.2013.12.012 [PubMed: 24361963]
- Gallagher AM, Das S, Xiao Z, Andresson T, Kieffer-Kwon P, Happel C, Ziegelbauer J. 2013; Proteomic screening of human targets of viral microRNAs reveals functions associated with immune evasion and angiogenesis. *PLoS Pathog.* 9(9):e1003584.doi: 10.1371/journal.ppat.1003584 [PubMed: 24039573]
- Gatherer D, Seirafian S, Cunningham C, Holton M, Dargan DJ, Baluchova K, Hector RD, Galbraith J, Herzyk P, Wilkinson GW, Davison AJ. 2011; High-resolution human cytomegalovirus transcriptome. *Proc Natl Acad Sci U S A.* 108(49):19755–19760. DOI: 10.1073/pnas.1115861108 [PubMed: 22109557]
- Gawn JM, Greaves RF. 2002; Absence of IE1 p72 protein function during low-multiplicity infection by human cytomegalovirus results in a broad block to viral delayed-early gene expression. *J Virol.* 76(9):4441–4455. [PubMed: 11932411]
- Gottwein E, Cai X, Cullen BR. 2006; A novel assay for viral microRNA function identifies a single nucleotide polymorphism that affects Drosha processing. *J Virol.* 80(11):5321–5326. DOI: 10.1128/JVI.02734-05 [PubMed: 16699012]
- Gottwein E, Corcoran DL, Mukherjee N, Skalsky RL, Hafner M, Nusbaum JD, Shamulailatpam P, Love CL, Dave SS, Tuschl T, Ohler U, Cullen BR. 2011; Viral microRNA targetome of KSHV-infected primary effusion lymphoma cell lines. *Cell Host Microbe.* 10(5):515–526. DOI: 10.1016/j.chom.2011.09.012 [PubMed: 22100165]

- Gottwein E, Mukherjee N, Sachse C, Frenzel C, Majoros WH, Chi JT, Braich R, Manoharan M, Soutschek J, Ohler U, Cullen BR. 2007; A viral microRNA functions as an orthologue of cellular miR-155. *Nature*. 450(7172):1096–1099. DOI: 10.1038/nature05992 [PubMed: 18075594]
- Gregorovic G, Boulden EA, Bosshard R, Elgueta Karstegl C, Skalsky R, Cullen BR, Gujer C, Ramer P, Munz C, Farrell PJ. 2015; Epstein-Barr Viruses (EBVs) Deficient in EBV-Encoded RNAs Have Higher Levels of Latent Membrane Protein 2 RNA Expression in Lymphoblastoid Cell Lines and Efficiently Establish Persistent Infections in Humanized Mice. *J Virol*. 89(22):11711–11714. DOI: 10.1128/JVI.01873-15 [PubMed: 26339045]
- Grey F. 2015; Role of microRNAs in herpesvirus latency and persistence. *J Gen Virol*. 96(Pt 4):739–751. DOI: 10.1099/vir.0.070862-0 [PubMed: 25406174]
- Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC, Nelson J. 2005; Identification and characterization of human cytomegalovirus-encoded microRNAs. *J Virol*. 79(18):12095–12099. DOI: 10.1128/JVI.79.18.12095-12099.2005 [PubMed: 16140786]
- Grey F, Meyers H, White EA, Spector DH, Nelson J. 2007; A human cytomegalovirus-encoded microRNA regulates expression of multiple viral genes involved in replication. *PLoS Pathog*. 3(11):e163.doi: 10.1371/journal.ppat.0030163 [PubMed: 17983268]
- Grey F, Tirabassi R, Meyers H, Wu G, McWeeney S, Hook L, Nelson JA. 2010; A viral microRNA down-regulates multiple cell cycle genes through mRNA 5' UTRs. *PLoS Pathog*. 6(6):e1000967.doi: 10.1371/journal.ppat.1000967 [PubMed: 20585629]
- Grundhoff A, Sullivan CS. 2011; Virus-encoded microRNAs. *Virology*. 411(2):325–343. DOI: 10.1016/j.virol.2011.01.002 [PubMed: 21277611]
- Grundhoff A, Sullivan CS, Ganem D. 2006; A combined computational and microarray-based approach identifies novel microRNAs encoded by human gamma-herpesviruses. *RNA*. 12(5):733–750. DOI: 10.1261/rna.2326106 [PubMed: 16540699]
- Guo YE, Riley KJ, Iwasaki A, Steitz JA. 2014; Alternative capture of noncoding RNAs or protein-coding genes by herpesviruses to alter host T cell function. *Mol Cell*. 54(1):67–79. DOI: 10.1016/j.molcel.2014.03.025 [PubMed: 24725595]
- Gutilla IK, White BA. 2009; Coordinate regulation of FOXO1 by miR-27a, miR-96, and miR-182 in breast cancer cells. *J Biol Chem*. 284(35):23204–23216. DOI: 10.1074/jbc.M109.031427 [PubMed: 19574223]
- Ha M, Kim VN. 2014; Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 15(8):509–524. DOI: 10.1038/nrm3838 [PubMed: 25027649]
- Haecker I, Gay LA, Yang Y, Hu J, Morse AM, McIntyre LM, Renne R. 2012; Ago HITS-CLIP Expands Understanding of Kaposi's Sarcoma-associated Herpesvirus miRNA Function in Primary Effusion Lymphomas. *PLoS Pathog*. 8(8):e1002884.doi: 10.1371/journal.ppat.1002884 [PubMed: 22927820]
- Han SJ, Marshall V, Barsov E, Quinones O, Ray A, Labo N, Trivett M, Ott D, Renne R, Whitby D. 2013; Kaposi's sarcoma-associated herpesvirus microRNA single-nucleotide polymorphisms identified in clinical samples can affect microRNA processing, level of expression, and silencing activity. *J Virol*. 87(22):12237–12248. DOI: 10.1128/JVI.01202-13 [PubMed: 24006441]
- Hancock MH, Tirabassi RS, Nelson JA. 2012; Rhesus cytomegalovirus encodes seventeen microRNAs that are differentially expressed in vitro and in vivo. *Virology*. 425(2):133–142. DOI: 10.1016/j.virol.2012.01.009 [PubMed: 22305624]
- Hansen A, Henderson S, Lagos D, Nikitenko L, Coulter E, Roberts S, Gratrix F, Plaisance K, Renne R, Bower M, Kellam P, Boshoff C. 2010a; KSHV-encoded miRNAs target MAF to induce endothelial cell reprogramming. *Genes Dev*. 24:195–205. [PubMed: 20080955]
- Hansen SG, Powers CJ, Richards R, Ventura AB, Ford JC, Siess D, Axthelm MK, Nelson JA, Jarvis MA, Picker LJ, Fruh K. 2010b; Evasion of CD8 + T cells is critical for superinfection by cytomegalovirus. *Science*. 328(5974):102–106. DOI: 10.1126/science.1185350 [PubMed: 20360110]
- Hill JM, Sedarati F, Javier RT, Wagner EK, Stevens JG. 1990; Herpes simplex virus latent phase transcription facilitates in vivo reactivation. *Virology*. 174(1):117–125. [PubMed: 2152989]
- Hook LM, Grey F, Grabski R, Tirabassi R, Doyle T, Hancock M, Landais I, Jeng S, McWeeney S, Britt W, Nelson JA. 2014; Cytomegalovirus miRNAs target secretory pathway genes to facilitate

formation of the virion assembly compartment and reduce cytokine secretion. *Cell Host Microbe*. 15(3):363–373. DOI: 10.1016/j.chom.2014.02.004 [PubMed: 24629342]

Hutchinson NI, Tocci MJ. 1986; Characterization of a major early gene from the human cytomegalovirus long inverted repeat; predicted amino acid sequence of a 30-kDa protein encoded by the 1.2-kb mRNA. *Virology*. 155(1):172–182. [PubMed: 3022468]

Hutzinger R, Feederle R, Mrazek J, Schiefermeier N, Balwierz PJ, Zavolan M, Polacek N, Delecluse HJ, Huttenhofer A. 2009; Expression and processing of a small nucleolar RNA from the Epstein-Barr virus genome. *PLoS Pathog*. 5(8):e1000547.doi: 10.1371/journal.ppat.1000547 [PubMed: 19680535]

Iizasa H, Wulff BE, Alla NR, Maragkakis M, Megraw M, Hatzigeorgiou A, Iwakiri D, Takada K, Wiedmer A, Showe L, Lieberman P, Nishikura K. 2010; Editing of EBV-encoded BART6 microRNAs controls their dicer targeting and consequently affects viral latency. *J Biol Chem*. doi: 10.1074/jbc.M110.138362

Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans JR, Zhao S, Poliakov A, Cao X, Dhanasekaran SM, Wu YM, Robinson DR, Beer DG, Feng FY, Iyer HK, Chinnaiyan AM. 2015; The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet*. 47(3):199–208. DOI: 10.1038/ng.3192 [PubMed: 25599403]

Javier RT, Stevens JG, Disette VB, Wagner EK. 1988; A herpes simplex virus transcript abundant in latently infected neurons is dispensable for establishment of the latent state. *Virology*. 166(1):254–257. [PubMed: 2842950]

Jiang X, Chentoufi AA, Hsiang C, Carpenter D, Osorio N, BenMohamed L, Fraser NW, Jones C, Wechsler SL. 2011; The herpes simplex virus type 1 latency-associated transcript can protect neuron-derived C1300 and Neuro2A cells from granzyme B-induced apoptosis and CD8 T-cell killing. *J Virol*. 85(5):2325–2332. DOI: 10.1128/JVI.01791-10 [PubMed: 21177822]

Jung YJ, Choi H, Kim H, Lee SK. 2014; MicroRNA miR-BART20-5p Stabilizes Epstein-Barr Virus Latency by Directly Targeting BZLF1 and BRLF1. *J Virol*. 88(16):9027–9037. DOI: 10.1128/JVI.00721-14 [PubMed: 24899173]

Jurak I, Hackenberg M, Kim JY, Pesola JM, Everett RD, Preston CM, Wilson AC, Coen DM. 2014; Expression of herpes simplex virus 1 microRNAs in cell culture models of quiescent and latent infection. *J Virol*. 88(4):2337–2339. DOI: 10.1128/JVI.03486-13 [PubMed: 24307587]

Juranic Lisnic V, Babic Cac M, Lisnic B, Trsan T, Mefferd A, Das Mukhopadhyay C, Cook CH, Jonjic S, Trgovcich J. 2013; Dual analysis of the murine cytomegalovirus and host cell transcriptomes reveal new aspects of the virus-host cell interface. *PLoS Pathog*. 9(9):e1003611.doi: 10.1371/journal.ppat.1003611 [PubMed: 24086132]

Kang D, Skalsky RL, Cullen BR. 2015; EBV BART MicroRNAs Target Multiple Pro-apoptotic Cellular Genes to Promote Epithelial Cell Survival. *PLoS Pathog*. 11(6):e1004979.doi: 10.1371/journal.ppat.1004979 [PubMed: 26070070]

Kapranov P, Cheng J, Dike S, Nix DA, Duttgupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammanna H, Gingeras TR. 2007; RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science*. 316(5830):1484–1488. DOI: 10.1126/science.1138341 [PubMed: 17510325]

Kenney SC, Mertz JE. 2014; Regulation of the latent-lytic switch in Epstein-Barr virus. *Semin Cancer Biol*. 26:60–68. DOI: 10.1016/j.semcancer.2014.01.002 [PubMed: 24457012]

Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL. 2009; Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A*. 106(28):11667–11672. DOI: 10.1073/pnas.0904715106 [PubMed: 19571010]

Kim S, Seo D, Kim D, Hong Y, Chang H, Baek D, Kim VN, Lee S, Ahn K. 2015; Temporal Landscape of MicroRNA-Mediated Host-Virus Crosstalk during Productive Human Cytomegalovirus Infection. *Cell Host Microbe*. 17(6):838–851. [PubMed: 26067606]

Kincaid RP, Sullivan CS. 2012; Virus-encoded microRNAs: an overview and a look to the future. *PLoS Pathog*. 8(12):e1003018.doi: 10.1371/journal.ppat.1003018 [PubMed: 23308061]

- Krug LT. 2013; Complexities of gammaherpesvirus transcription revealed by microarrays and RNAseq. *Curr Opin Virol.* 3(3):276–284. DOI: 10.1016/j.coviro.2013.04.006 [PubMed: 23684513]
- Kulesza CA, Shenk T. 2004; Human cytomegalovirus 5-kilobase immediate-early RNA is a stable intron. *J Virol.* 78(23):13182–13189. DOI: 10.1128/JVI.78.23.13182-13189.2004 [PubMed: 15542670]
- Kulesza CA, Shenk T. 2006; Murine cytomegalovirus encodes a stable intron that facilitates persistent replication in the mouse. *Proc Natl Acad Sci U S A.* 103(48):18302–18307. DOI: 10.1073/pnas.0608718103 [PubMed: 17105807]
- Kurkewich JL, Bikorimana E, Nguyen T, Klopfenstein N, Zhang H, Hallas WM, Stayback G, McDowell MA, Dahl R. 2016; The miR-23a microRNA cluster antagonizes B cell development. *J Leukoc Biol.* 100(4):665–677. DOI: 10.1189/jlb.1HI0915-398RR [PubMed: 27084569]
- Kwiatkowski DL, Thompson HW, Bloom DC. 2009; The polycomb group protein Bmi1 binds to the herpes simplex virus 1 latent genome and maintains repressive histone marks during latency. *J Virol.* 83(16):8173–8181. DOI: 10.1128/JVI.00686-09 [PubMed: 19515780]
- Lan K, Kuppers DA, Verma SC, Robertson ES. 2004; Kaposi's sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen inhibits lytic replication by targeting Rta: a potential mechanism for virus-mediated control of latency. *J Virol.* 78(12):6585–6594. DOI: 10.1128/JVI.78.12.6585-6594.2004 [PubMed: 15163750]
- Lau B, Poole E, Krishna B, Sellart I, Wills MR, Murphy E, Sinclair J. 2016; The Expression of Human Cytomegalovirus MicroRNA MiR-UL148D during Latent Infection in Primary Myeloid Cells Inhibits Activin A-triggered Secretion of IL-6. *Sci Rep.* 6:31205.doi: 10.1038/srep31205 [PubMed: 27491954]
- Lee N, Moss WN, Yario TA, Steitz JA. 2015; EBV noncoding RNA binds nascent RNA to drive host PAX5 to viral DNA. *Cell.* 160(4):607–618. DOI: 10.1016/j.cell.2015.01.015 [PubMed: 25662012]
- Lee N, Pimienta G, Steitz JA. 2012; AUFI/hnRNP D is a novel protein partner of the EBER1 noncoding RNA of Epstein-Barr virus. *RNA.* 18(11):2073–2082. DOI: 10.1261/rna.034900.112 [PubMed: 23012480]
- Lee N, Yario TA, Gao JS, Steitz JA. 2016; EBV noncoding RNA EBER2 interacts with host RNA-binding proteins to regulate viral gene expression. *Proc Natl Acad Sci U S A.* 113(12):3221–3226. DOI: 10.1073/pnas.1601773113 [PubMed: 26951683]
- Lee S, Song J, Kim S, Kim J, Hong Y, Kim Y, Kim D, Baek D, Ahn K. 2013; Selective degradation of host MicroRNAs by an intergenic HCMV noncoding RNA accelerates virus production. *Cell Host Microbe.* 13(6):678–690. DOI: 10.1016/j.chom.2013.05.007 [PubMed: 23768492]
- Lei X, Bai Z, Ye F, Huang Y, Gao SJ. 2010; Regulation of herpesvirus lifecycle by viral microRNAs. *Virulence.* 1(5):433–435. DOI: 10.4161/viru.1.5.12966 [PubMed: 21170300]
- Lei X, Zhu Y, Jones T, Bai Z, Huang Y, Gao SJ. 2012; A Kaposi's sarcoma-associated herpesvirus microRNA and its variants target the transforming growth factor beta pathway to promote cell survival. *J Virol.* 86(21):11698–11711. DOI: 10.1128/JVI.06855-11 [PubMed: 22915806]
- Lerner MR, Andrews NC, Miller G, Steitz JA. 1981; Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc Natl Acad Sci U S A.* 78(2):805–809. [PubMed: 6262773]
- Li CX, Shan GZ, Fan B, Tao PZ, Zhao LX, Jiang JD, Li YH, Li ZR. 2011; Synthesis and antiviral activities of geldanamycin analog TC-GM in vitro. *Yao Xue Xue Bao.* 46(6):683–687. [PubMed: 21882529]
- Li J, Zhao Y, Lu Y, Ritchie W, Grau G, Vadas MA, Gamble JR. 2016; The Poly-cistronic miR-23-27-24 Complexes Target Endothelial Cell Junctions: Differential Functional and Molecular Effects of miR-23a and miR-23b. *Molecular therapy Nucleic acids.* 5(8):e354.doi: 10.1038/mtna.2016.62 [PubMed: 27741223]
- Lin Z, Wang X, Strong MJ, Concha M, Baddoo M, Xu G, Baribault C, Fewell C, Hulme W, Hedges D, Taylor CM, Flemington EK. 2013; Whole-genome sequencing of the Akata and Mutu Epstein-Barr virus strains. *J Virol.* 87(2):1172–1182. DOI: 10.1128/JVI.02517-12 [PubMed: 23152513]

- Liu Q, Fu H, Sun F, Zhang H, Tie Y, Zhu J, Xing R, Sun Z, Zheng X. 2008; miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. *Nucleic Acids Res.* 36(16):5391–5404. DOI: 10.1093/nar/gkn522 [PubMed: 18701644]
- Lo AK, To KF, Lo KW, Lung RW, Hui JW, Liao G, Hayward SD. 2007; Modulation of LMP1 protein expression by EBV-encoded microRNAs. *Proc Natl Acad Sci U S A.* 104(41):16164–16169. DOI: 10.1073/pnas.0702896104 [PubMed: 17911266]
- Lu CC, Li Z, Chu CY, Feng J, Sun R, Rana TM. 2010; MicroRNAs encoded by Kaposi's sarcoma-associated herpesvirus regulate viral life cycle. *EMBO Rep.* 11(10):784–790. DOI: 10.1038/embor.2010.132 [PubMed: 20847741]
- Lu X, Li X, He Q, Gao J, Gao Y, Liu B, Liu F. 2013; miR-142-3p regulates the formation and differentiation of hematopoietic stem cells in vertebrates. *Cell Res.* 23(12):1356–1368. DOI: 10.1038/cr.2013.145 [PubMed: 24165894]
- Lung RW, Tong JH, Sung YM, Leung PS, Ng DC, Chau SL, Chan AW, Ng EK, Lo KW, To KF. 2009; Modulation of LMP2A expression by a newly identified Epstein-Barr virus-encoded microRNA miR-BART22. *Neoplasia.* 11(11):1174–1184. [PubMed: 19881953]
- Lung RW, Tong JH, To KF. 2013; Emerging roles of small Epstein-Barr virus derived non-coding RNAs in epithelial malignancy. *Int J Mol Sci.* 14(9):17378–17409. DOI: 10.3390/ijms140917378 [PubMed: 23979421]
- Ma J, Nie K, Redmond D, Liu Y, Elemento O, Knowles DM, Tam W. 2016; EBV-miR-BHRF1-2 targets PRDM1/Blimp1: potential role in EBV lymphomagenesis. *Leukemia.* 30(3):594–604. DOI: 10.1038/leu.2015.285 [PubMed: 26530011]
- Mahjoub N, Dhorne-Pollet S, Fuchs W, Endale Ahanda ML, Lange E, Klupp B, Arya A, Loveland JE, Lefevre F, Mettenleiter TC, Giuffra E. 2015; 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.* 89(1):428–442. DOI: 10.1128/JVI.02181-14 [PubMed: 25320324]
- Manzano M, Forte E, Raja AN, Schipma MJ, Gottwein E. 2015; Divergent target recognition by coexpressed 5'-isomiRs of miR-142-3p and selective viral mimicry. *RNA.* 21(9):1606–1620. DOI: 10.1261/rna.048876.114 [PubMed: 26137849]
- Manzano M, Shamulailatpam P, Raja AN, Gottwein E. 2013; Kaposi's sarcoma-associated herpesvirus encodes a mimic of cellular miR-23. *J Virol.* 87(21):11821–11830. DOI: 10.1128/JVI.01692-13 [PubMed: 23986579]
- Marcinowski L, Tanguy M, Krmpotic A, Radle B, Lisnic VJ, Tuddenham L, Chane-Woon-Ming B, Ruzsics Z, Erhard F, Benkartek C, Babic M, Zimmer R, Trgovcich J, Koszinowski UH, Jonjic S, Pfeffer S, Dolken L. 2012; Degradation of cellular mir-27 by a novel, highly abundant viral transcript is important for efficient virus replication in vivo. *PLoS Pathog.* 8(2):e1002510.doi: 10.1371/journal.ppat.1002510 [PubMed: 22346748]
- Margolis TP, Elfman FL, Leib D, Pakpour N, Apakupakul K, Imai Y, Voytek C. 2007; Spontaneous reactivation of herpes simplex virus type 1 in latently infected murine sensory ganglia. *J Virol.* 81(20):11069–11074. DOI: 10.1128/JVI.00243-07 [PubMed: 17686862]
- Marquitz AR, Mathur A, Chugh PE, Dittmer DP, Raab-Traub N. 2014; Expression profile of microRNAs in Epstein-Barr virus-infected AGS gastric carcinoma cells. *J Virol.* 88(2):1389–1393. DOI: 10.1128/JVI.02662-13 [PubMed: 24227849]
- Marshall V, Martro E, Labo N, Ray A, Wang D, Mbisa G, Bagni RK, Volfovsky N, Casabona J, Whitby D. 2010; Kaposi sarcoma (KS)-associated herpesvirus microRNA sequence analysis and KS risk in a European AIDS-KS case control study. *J Infect Dis.* 202(7):1126–1135. DOI: 10.1086/656045 [PubMed: 20715927]
- Marshall V, Parks T, Bagni R, Wang CD, Samols MA, Hu J, Wyvil KM, Aleman K, Little RF, Yarchoan R, Renne R, Whitby D. 2007; Conservation of virally encoded microRNAs in Kaposi sarcoma-associated herpesvirus in primary effusion lymphoma cell lines and in patients with Kaposi sarcoma or multicentric Castlemans disease. *J Infect Dis.* 195(5):645–659. DOI: 10.1086/511434 [PubMed: 17262705]
- Mayr C, Bartel DP. 2009; Widespread shortening of 3' UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell.* 138(4):673–684. DOI: 10.1016/j.cell.2009.06.016 [PubMed: 19703394]

- McCaskill J, Prahirunkit P, Sharp PM, Buck AH. 2015; RNA-mediated degradation of microRNAs: A widespread viral strategy? *RNA Biol.* 12(6):579–585. DOI: 10.1080/15476286.2015.1034912 [PubMed: 25849078]
- McDonough SH, Spector DH. 1983; Transcription in human fibroblasts permissively infected by human cytomegalovirus strain AD169. *Virology.* 125(1):31–46. [PubMed: 6187127]
- McDonough SH, Staprans SI, Spector DH. 1985; Analysis of the major transcripts encoded by the long repeat of human cytomegalovirus strain AD169. *J Virol.* 53(3):711–718. [PubMed: 2983083]
- McSharry BP, Tomasec P, Neale ML, Wilkinson GW. 2003; The most abundantly transcribed human cytomegalovirus gene ($\beta 2$. 7) is non-essential for growth in vitro. *J Gen Virol.* 84(9):2511–2516. DOI: 10.1099/vir.0.19298-0 [PubMed: 12917473]
- Meshesha MK, Bentwich Z, Solomon SA, Avni YS. 2016; In vivo expression of human cytomegalovirus (HCMV) microRNAs during latency. *Gene.* 575(1):101–107. DOI: 10.1016/j.gene.2015.08.040 [PubMed: 26302752]
- Meyer C, Grey F, Kreklywich CN, Andoh TF, Tirabassi RS, Orloff SL, Streblow DN. 2011; Cytomegalovirus microRNA expression is tissue specific and is associated with persistence. *J Virol.* 85(1):378–389. DOI: 10.1128/JVI.01900-10 [PubMed: 20980502]
- Mogilyansky E, Rigoutsos I. 2013; The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ.* 20(12):1603–1614. DOI: 10.1038/cdd.2013.125 [PubMed: 24212931]
- Moss WN, Steitz JA. 2013; Genome-wide analyses of Epstein-Barr virus reveal conserved RNA structures and a novel stable intronic sequence RNA. *BMC Genom.* 14:543.doi: 10.1186/1471-2164-14-543
- Murphy E, Vanicek J, Robins H, Shenk T, Levine AJ. 2008; Suppression of immediate-early viral gene expression by herpesvirus-coded microRNAs: implications for latency. *Proc Natl Acad Sci U S A.* 105(14):5453–5458. DOI: 10.1073/pnas.0711910105 [PubMed: 18378902]
- Nachmani D, Lankry D, Wolf DG, Mandelboim O. 2010; The human cytomegalovirus microRNA miR-UL112 acts synergistically with a cellular microRNA to escape immune elimination. *Nat Immunol.* 11(9):806–813. DOI: 10.1038/ni.1916 [PubMed: 20694010]
- O'Connor CM, Vanicek J, Murphy EA. 2014; Host microRNA regulation of human cytomegalovirus immediate early protein translation promotes viral latency. *J Virol.* 88(10):5524–5532. DOI: 10.1128/JVI.00481-14 [PubMed: 24599990]
- O'Grady T, Cao S, Strong MJ, Concha M, Wang X, Splinter Bondurant S, Adams M, Baddoo M, Srivastav SK, Lin Z, Fewell C, Yin Q, Flemington EK. 2014; Global bidirectional transcription of the Epstein-Barr virus genome during reactivation. *J Virol.* 88(3):1604–1616. DOI: 10.1128/JVI.02989-13 [PubMed: 24257595]
- Pan D, Flores O, Umbach JL, Pesola JM, Bentley P, Rosato PC, Leib DA, Cullen BR, Coen DM. 2014; A neuron-specific host microRNA targets herpes simplex virus-1 ICP0 expression and promotes latency. *Cell Host Microbe.* 15(4):446–456. DOI: 10.1016/j.chom.2014.03.004 [PubMed: 24721573]
- Parnas O, Corcoran DL, Cullen BR. 2014; Analysis of the mRNA targetome of microRNAs expressed by Marek's disease virus. *MBio.* 5(1):e01060–e01013. DOI: 10.1128/mBio.01060-13 [PubMed: 24449754]
- Perng GC, Ghiasi H, Kaiwar R, Nesburn AB, Wechsler SL. 1994; An improved method for cloning portions of the repeat regions of herpes simplex virus type 1. *J Virol Methods.* 46(2):111–116. [PubMed: 8188808]
- Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, van Dyk LF, Ho CK, Shuman S, Chien M, Russo JJ, Ju J, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zavolan M, Tuschl T. 2005; Identification of microRNAs of the herpesvirus family. *Nat Methods.* 2(4):269–276. DOI: 10.1038/nmeth746 [PubMed: 15782219]
- Pfeffer S, Zavolan M, Grasser FA, Chien M, Russo JJ, Ju J, John B, Enright AJ, Marks D, Sander C, Tuschl T. 2004; Identification of virus-encoded microRNAs. *Science.* 304(5671):734–736. DOI: 10.1126/science.1096781304/5671/734 [PubMed: 15118162]
- Plaisance-Bonstaff K, Choi HS, Beals T, Krueger BJ, Boss IW, Gay LA, Haecker I, Hu J, Renne R. 2014; KSHV miRNAs decrease expression of lytic genes in latently infected PEL and endothelial

cells by targeting host transcription factors. *Viruses*. 6(10):4005–4023. DOI: 10.3390/v6104005 [PubMed: 25341664]

- Puig-Barbera J, Burtseva E, Yu H, Cowling BJ, Badur S, Kyncl J, Sominina A. 2016; Influenza epidemiology and influenza vaccine effectiveness during the 2014–2015 season: annual report from the Global Influenza Hospital Surveillance Network. *BMC Public Health*. 16(Suppl 1): 757.doi: 10.1186/s12889-016-3378-1 [PubMed: 27556802]
- Qiu J, Smith P, Leahy L, Thorley-Lawson DA. 2015; The Epstein-Barr virus encoded BART miRNAs potentiate tumor growth in vivo. *PLoS Pathog*. 11(1):e1004561.doi: 10.1371/journal.ppat.1004561 [PubMed: 25590614]
- Ray A, Marshall V, Uldrick T, Leighty R, Labo N, Wyvill K, Aleman K, Polizzotto MN, Little RF, Yarchoan R, Whitby D. 2012; Sequence analysis of Kaposi sarcoma-associated herpesvirus (KSHV) microRNAs in patients with multicentric Castleman disease and KSHV-associated inflammatory cytokine syndrome. *J Infect Dis*. 205(11):1665–1676. DOI: 10.1093/infdis/jis249 [PubMed: 22448005]
- Reeves MB, Davies AA, McSharry BP, Wilkinson GW, Sinclair JH. 2007; Complex I binding by a virally encoded RNA regulates mitochondria-induced cell death. *Science*. 316(5829):1345–1348. DOI: 10.1126/science.1142984 [PubMed: 17540903]
- Rennekamp AJ, Lieberman PM. 2011; Initiation of Epstein-Barr virus lytic replication requires transcription and the formation of a stable RNA-DNA hybrid molecule at OriLyt. *J Virol*. 85(6): 2837–2850. DOI: 10.1128/JVI.02175-10 [PubMed: 21191028]
- Reusch JA, Nawandar DM, Wright KL, Kenney SC, Mertz JE. 2015; Cellular differentiation regulator BLIMP1 induces Epstein-Barr virus lytic reactivation in epithelial and B cells by activating transcription from both the R and Z promoters. *J Virol*. 89(3):1731–1743. DOI: 10.1128/JVI.02781-14 [PubMed: 25410866]
- Riley KJ, Rabinowitz GS, Steitz JA. 2010; Comprehensive analysis of Rhesus lymphocryptovirus microRNA expression. *J Virol*. 84(10):5148–5157. DOI: 10.1128/JVI.00110-10 [PubMed: 20219930]
- Riley KJ, Rabinowitz GS, Yario TA, Luna JM, Darnell RB, Steitz JA. 2012; EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. *EMBO J*. 31(9):2207–2221. DOI: 10.1038/emboj.2012.63 [PubMed: 22473208]
- Rossetto CC, Pari G. 2012; KSHV PAN RNA associates with demethylases UTX and JMJD3 to activate lytic replication through a physical interaction with the virus genome. *PLoS Pathog*. 8(5):e1002680.doi: 10.1371/journal.ppat.1002680 [PubMed: 22589717]
- Rossetto CC, Tarrant-Elorza M, Pari GS. 2013; Cis and trans acting factors involved in human cytomegalovirus experimental and natural latent infection of CD14 (+) monocytes and CD34 (+) cells. *PLoS Pathog*. 9(5):e1003366.doi: 10.1371/journal.ppat.1003366 [PubMed: 23717203]
- Samols MA, Hu J, Skalsky RL, Renne R. 2005; Cloning and identification of a microRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus. *J Virol*. 79(14): 9301–9305. DOI: 10.1128/JVI.79.14.9301-9305.2005 [PubMed: 15994824]
- Samols MA, Skalsky RL, Maldonado AM, Riva A, Lopez MC, Baker HV, Renne R. 2007; Identification of cellular genes targeted by KSHV-encoded microRNAs. *PLoS Pathog*. 3(5):e65.doi: 10.1371/journal.ppat.0030065 [PubMed: 17500590]
- Sandberg R, Neilson JR, Sarma A, Sharp PA, Burge CB. 2008; Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. *Science*. 320(5883):1643–1647. DOI: 10.1126/science.1155390 [PubMed: 18566288]
- Santhakumar D, Forster T, Laqtom NN, Fragkoudis R, Dickinson P, Abreu-Goodger C, Manakov SA, Choudhury NR, Griffiths SJ, Vermeulen A, Enright AJ, Dutia B, Kohl A, Ghazal P, Buck AH. 2010; Combined agonist-antagonist genome-wide functional screening identifies broadly active antiviral microRNAs. *Proc Natl Acad Sci U S A*. 107(31):13830–13835. DOI: 10.1073/pnas.1008861107 [PubMed: 20643939]
- Saxena A, Carninci P. 2011; Long non-coding RNA modifies chromatin: epigenetic silencing by long non-coding RNAs. *BioEssays*. 33(11):830–839. DOI: 10.1002/bies.201100084 [PubMed: 21915889]

- Scarpini CG, May J, Lachmann RH, Preston CM, Dunnett SB, Torres EM, Efstathiou S. 2001; Latency associated promoter transgene expression in the central nervous system after stereotaxic delivery of replication-defective HSV-1-based vectors. *Gene Ther.* 8(14):1057–1071. DOI: 10.1038/sj.gt.3301497 [PubMed: 11526453]
- Schafer A, Cai X, Bilello JP, Desrosiers RC, Cullen BR. 2007; Cloning and analysis of microRNAs encoded by the primate gamma-herpesvirus rhesus monkey rhadinovirus. *Virology.* 364(1):21–27. DOI: 10.1016/j.virol.2007.03.029 [PubMed: 17451774]
- Schwarz TM, Volpe LA, Abraham CG, Kulesza CA. 2013; Molecular investigation of the 7.2 kb RNA of murine cytomegalovirus. *Virol J.* 10:348.doi: 10.1186/1743-422X-10-348 [PubMed: 24295514]
- Seo GJ, Kincaid RP, Phanaksri T, Burke JM, Pare JM, Cox JE, Hsiang TY, Krug RM, Sullivan CS. 2013; Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. *Cell Host Microbe.* 14(4):435–445. DOI: 10.1016/j.chom.2013.09.002 [PubMed: 24075860]
- Shen ZZ, Pan X, Miao LF, Ye HQ, Chavanas S, Davrinche C, McVoy M, Luo MH. 2014; Comprehensive analysis of human cytomegalovirus microRNA expression during lytic and quiescent infection. *PLoS ONE.* 9(2):e88531.doi: 10.1371/journal.pone.0088531 [PubMed: 24533100]
- Skalsky RL, Barr SA, Jeffery AJ, Blair T, Estep R, Wong SW. 2016; Japanese Macaque Rhadinovirus Encodes a Viral MicroRNA Mimic of the miR-17 Family. *J Virol.* 90(20):9350–9363. DOI: 10.1128/JVI.01123-16 [PubMed: 27512057]
- Skalsky RL, Corcoran DL, Gottwein E, Frank CL, Kang D, Hafner M, Nusbaum JD, Feederle R, Delecluse HJ, Luftig MA, Tuschl T, Ohler U, Cullen BR. 2012; The viral and cellular microRNA targetome in lymphoblastoid cell lines. *PLoS Pathog.* 8(1):e1002484.doi: 10.1371/journal.ppat.1002484 [PubMed: 22291592]
- Skalsky RL, Cullen BR. 2015; EBV Noncoding RNAs. *Curr Top Microbiol Immunol.* 391:181–217. DOI: 10.1007/978-3-319-22834-1_6 [PubMed: 26428375]
- Skalsky RL, Kang D, Linnstaedt SD, Cullen BR. 2014; Evolutionary conservation of primate lymphocryptovirus microRNA targets. *J Virol.* 88(3):1617–1635. DOI: 10.1128/JVI.02071-13 [PubMed: 24257599]
- Skalsky RL, Samols MA, Plaisance KB, Boss IW, Riva A, Lopez MC, Baker HV, Renne R. 2007; Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. *J Virol.* 81(23):12836–12845. DOI: 10.1128/JVI.01804-07 [PubMed: 17881434]
- Stark TJ, Arnold JD, Spector DH, Yeo GW. 2012; High-resolution profiling and analysis of viral and host small RNAs during human cytomegalovirus infection. *J Virol.* 86(1):226–235. DOI: 10.1128/JVI.05903-11 [PubMed: 22013051]
- Stern-Ginossar N, Saleh N, Goldberg MD, Prichard M, Wolf DG, Mandelboim O. 2009; Analysis of human cytomegalovirus-encoded microRNA activity during infection. *J Virol.* 83(20):10684–10693. DOI: 10.1128/JVI.01292-09 [PubMed: 19656885]
- Stern-Ginossar N, Weisburd B, Michalski A, Le VT, Hein MY, Huang SX, Ma M, Shen B, Qian SB, Hengel H, Mann M, Ingolia NT, Weissman JS. 2012; Decoding human cytomegalovirus. *Science.* 338(6110):1088–1093. DOI: 10.1126/science.1227919 [PubMed: 23180859]
- Stevens JG, Wagner EK, Devi-Rao GB, Cook ML, Feldman LT. 1987; RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. *Science.* 235(4792):1056–1059. [PubMed: 2434993]
- Sun Z, Wang L, Eckloff BW, Deng B, Wang Y, Wampfler JA, Jang J, Wieben ED, Jen J, You M, Yang P. 2014; Conserved recurrent gene mutations correlate with pathway deregulation and clinical outcomes of lung adenocarcinoma in never-smokers. *BMC Med Genomics.* 7:32.doi: 10.1186/1755-8794-7-32 [PubMed: 24894543]
- Tagawa T, Albanese M, Bouvet M, Moosmann A, Mautner J, Heissmeyer V, Zielinski C, Lutter D, Hoser J, Hastreiter M, Hayes M, Sugden B, Hammerschmidt W. 2016; Epstein-Barr viral miRNAs inhibit antiviral CD4 + T cell responses targeting IL-12 and peptide processing. *J Exp Med.* 213(10):2065–2080. DOI: 10.1084/jem.20160248 [PubMed: 27621419]

- Tang S, Bertke AS, Patel A, Margolis TP, Krause PR. 2011; Herpes simplex virus 2 microRNA miR-H6 is a novel latency-associated transcript-associated microRNA, but reduction of its expression does not influence the establishment of viral latency or the recurrence phenotype. *J Virol.* 85(9): 4501–4509. DOI: 10.1128/JVI.01997-10 [PubMed: 21325410]
- Tang S, Bertke AS, Patel A, Wang K, Cohen JI, Krause PR. 2008; An acutely and latently expressed herpes simplex virus 2 viral microRNA inhibits expression of ICP34.5, a viral neurovirulence factor. *Proc Natl Acad Sci U S A.* 105(31):10931–10936. DOI: 10.1073/pnas.0801845105 [PubMed: 18678906]
- Tang S, Patel A, Krause PR. 2009; 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.* 83(3):1433–1442. DOI: 10.1128/JVI.01723-08 [PubMed: 19019961]
- Thompson RL, Sawtell NM. 1997; The herpes simplex virus type 1 latency-associated transcript gene regulates the establishment of latency. *J Virol.* 71(7):5432–5440. [PubMed: 9188615]
- Tirabassi R, Hook L, Landais I, Grey F, Meyers H, Hewitt H, Nelson J. 2011; Human cytomegalovirus US7 is regulated synergistically by two virally encoded microRNAs and by two distinct mechanisms. *J Virol.* 85(22):11938–11944. DOI: 10.1128/JVI.05443-11 [PubMed: 21900172]
- Toth Z, Maglinte DT, Lee SH, Lee HR, Wong LY, Brulois KF, Lee S, Buckley JD, Laird PW, Marquez VE, Jung JU. 2010; Epigenetic analysis of KSHV latent and lytic genomes. *PLoS Pathog.* 6(7):e1001013.doi: 10.1371/journal.ppat.1001013 [PubMed: 20661424]
- Tuddenham L, Jung JS, Chane-Woon-Ming B, Dolken L, Pfeffer S. 2012; Small RNA deep sequencing identifies microRNAs and other small noncoding RNAs from human herpesvirus 6B. *J Virol.* 86(3):1638–1649. DOI: 10.1128/JVI.05911-11 [PubMed: 22114334]
- Tycowski KT, Guo YE, Lee N, Moss WN, Vallery TK, Xie M, Steitz JA. 2015; Viral noncoding RNAs: more surprises. *Genes Dev.* 29(6):567–584. DOI: 10.1101/gad.259077.115 [PubMed: 25792595]
- Tycowski KT, Shu MD, Borah S, Shi M, Steitz JA. 2012; Conservation of a triple-helix-forming RNA stability element in noncoding and genomic RNAs of diverse viruses. *Cell Rep.* 2(1):26–32. DOI: 10.1016/j.celrep.2012.05.020 [PubMed: 22840393]
- Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. 2008; MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature.* 454(7205):780–783. DOI: 10.1038/nature07103 [PubMed: 18596690]
- Umbach JL, Nagel MA, Cohrs RJ, Gilden DH, Cullen BR. 2009; Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. *J Virol.* 83(20):10677–10683. DOI: 10.1128/JVI.01185-09 [PubMed: 19656888]
- Umbach JL, Wang K, Tang S, Krause PR, Mont EK, Cohen JI, Cullen BR. 2010; Identification of viral microRNAs expressed in human sacral ganglia latently infected with herpes simplex virus 2. *J Virol.* 84(2):1189–1192. DOI: 10.1128/JVI.01712-09 [PubMed: 19889786]
- Wahl A, Linnstaedt SD, Esoda C, Krisko JF, Martinez-Torres F, Delecluse HJ, Cullen BR, Garcia JV. 2013; 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.* 87(10): 5437–5446. [PubMed: 23468485]
- Walz N, Christalla T, Tessmer U, Grundhoff A. 2010; A global analysis of evolutionary conservation among known and predicted gammaherpesvirus microRNAs. *J Virol.* 84(2):716–728. DOI: 10.1128/JVI.01302-09 [PubMed: 19889779]
- Wang QY, Zhou C, Johnson KE, Colgrove RC, Coen DM, Knipe DM. 2005; Herpesviral latency-associated transcript gene promotes assembly of heterochromatin on viral lytic-gene promoters in latent infection. *Proc Natl Acad Sci U S A.* 102(44):16055–16059. DOI: 10.1073/pnas.0505850102 [PubMed: 16247011]
- Wang Y, Li H, Tang Q, Maul GG, Yuan Y. 2008; Kaposi's sarcoma-associated herpesvirus ori-Lyt-dependent DNA replication: involvement of host cellular factors. *J Virol.* 82(6):2867–2882. DOI: 10.1128/JVI.01319-07 [PubMed: 18199640]
- Xie M, Zhang W, Shu MD, Xu A, Lenis DA, DiMaio D, Steitz JA. 2015; The host Integrator complex acts in transcription-independent maturation of herpesvirus microRNA 3' ends. *Genes Dev.* 29(14):1552–1564. DOI: 10.1101/gad.266973.115 [PubMed: 26220997]

- Xing L, Kieff E. 2011; cis-Acting effects on RNA processing and Drosha cleavage prevent Epstein-Barr virus latency III BHRF1 expression. *J Virol.* 85(17):8929–8939. DOI: 10.1128/JVI.00336-11 [PubMed: 21697496]
- Zhao Y, Xu H, Yao Y, Smith LP, Kgosana L, Green J, Petherbridge L, Baigent SJ, Nair V. 2011; Critical role of the virus-encoded microRNA-155 ortholog in the induction of Marek's disease lymphomas. *PLoS Pathog.* 7(2):e1001305.doi: 10.1371/journal.ppat.100130 [PubMed: 21383974]
- Zhou C, Xie Z, Gao L, Liu C, Ai J, Zhang L, Shen K. 2015; Profiling of EBV-Encoded microRNAs in EBV-Associated Hemophagocytic Lymphohistiocytosis. *Tohoku J Exp Med.* 237(2):117–126. DOI: 10.1620/tjem.237.117 [PubMed: 26423217]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Non-coding RNAs expressed by herpesviruses. **a** Genomic locations (not to scale) of large and small ncRNAs (highlighted) encoded by the alpha, beta, and gamma-herpesviruses. Shown are representative human herpesviruses from each family and approximate locations of viral ncRNAs. miRNA precursors are represented by arrows that indicate the direction in which they are transcribed. For HSV-1, only miRNAs reported to be RISC-associated are shown (Flores 2013). **b** Drosha dependent (canonical) and Drosha independent miRNA biogenesis. The majority of herpesvirus miRNAs are generated via the canonical pathway. MHV68 and HVS bypass Drosha/DGCR8 cleavage and utilize alternative methods to generate pre-miRNAs that are co-transcribed with other viral ncRNAs

Fig. 2.

Viral long ncRNA regulation of gene expression. **a** KSHV PAN RNA can serve as a decoy by sequestering LANA from binding to and repressing KSHV lytic promoters **b** KSHV PAN can also guide various cellular factors (i.e. demethylases such as JMJD3 and UTX or methylases such as Ezh2) to sites on the KSHV genome which consequently alter histone modifications that mediate transcriptional activation or repression. **c** EBV EBER2 forms a complex with cellular PAX5 and an unknown bridging factor to regulate transcription from the terminal repeat (TR) regions of the EBV genome. Nascent TR transcripts pair with EBER2 to stabilize the interaction. **d** HCMV RNA4.9 interacts with the PRC2 complex and may bind regions of the viral genome to regulate the major immediate early promoter

Fig. 3.

Intrinsic and extrinsic modulation of host signaling pathways by viral ncRNAs. Pleiotropic herpesvirus post-transcriptional silencers (miRNAs) and nuclear modifiers (lncRNAs) of the internal host cell transcriptional environment can subsequently alter the external micro-environment through the manipulation of cell surface receptors, signaling pathways that activate or repress transcription, and subsequent trafficking and display or secretion of effector factors (such as cytokines) that are capable of influencing neighboring uninfected cells. The combined activities of viral ncRNAs (and viral proteins) provide a fit environment to support prolonged virus persistence