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Immunomodulatory roles of human herpesvirus-encoded microRNA in host-virus interaction

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Summary

Human herpesviruses (HHV) are large, double stranded, DNA viruses with high sero-prevalence across the globe. Clinical manifestation of primary HHV infection resolve shortly, however, this period is prolonged in immunocompromised patients or individuals with suppressed immunity. Examining molecular mechanisms of HHV-encoded virulence factors can provide finer details of HHV-host interaction. A unique genetic feature of most members of HHV is that they encode multiple microRNAs (miR). In this review, I will provide mechanistic insights into the immunomodulatory functions of herpesvirus-encoded viral miR (v-miR) that favor viral persistence and spread by ingenious immune evasion schemes. Similar to host miR, v-miR can simultaneously regulate expression of multiple transcripts including host- and virus-derived. V-miRs, by virtue of their direct interaction with various transcripts, can regulate expression of critical components of host innate and adaptive immune system. V-miRs are also exported through exosomal route and gain entry into various cells even at distant sites, thereby allowing HHV to manipulate cellular and tissue immunity. Targeting v-miR may serve as a novel and promising therapeutic candidate to mitigate HHV-mediated clinical manifestations.

Keywords

human herpesvirus; immune responses; viral microRNA

1 | INTRODUCTION

Human herpesviruses (HHV) are large, enveloped, linear double-stranded DNA (dsDNA) viruses that have evolved over millions of years to persist lifelong inside host. HHV establish lifelong infection, interchanging between latent (non-productive) and lytic (productive) infections.[1–3] The abilty of the virus to survive is dependent on its ability to evade host immunosurveillance.[2] The *Herpesviridae* family is subdivided into three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. Members of *Alphaherpesvirinae* family (HSV-1, HSV-2, and VZV) are cytolytic viruses that infect a variety of human cell-types and establish latency in neurons.[1,2] Frequent clinical

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CONFLICT OF INTEREST

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manifestations of HSV-1 infection are gingivostomatitis and pharyngitis, whereas herpes labialis is the most frequent sign of reactivation disease.[2] HSV-1 can also cause ocular infections (eg, herpetic keratitis) and serious systemic illnesses such as encephalitis and neonatal disease involving multiple organs. HCMV, HHV-6A, and HHV-6B are members of the *Betaherpesvirinae* family.[1] These viruses maintain latency in cells of myeloid and lymphoid compartments and exhibit clinical manifestation particularly in immunocompromised or immunosuppressed individuals. While latent infection may not have immediate ramifications to the host, latent infection of two gammaherpesviruses, Epstein-Barr Virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV), may lead to carcinogenesis. EBV and KSHV are two of seven oncoviruses, otherwise known as human cancer viruses.[4] Specifically, EBV is shown to cause Hodgkin lymphoma, Burkitt lymphoma, gastric cancer, and nasopharyngeal carcinoma.[5] KSHV is shown to cause Kaposi sarcoma, a cancer seen in Acquired Immunodeficiency Syndrome (AIDS) patients,[4] as well as primary effusion lymphoma (PEL)[6] and mulicentric Castleman disease.

With relatively large genomes (approximately 120–180 kb), these viruses encode a large repertoire of protein-coding genes to overcome numerous host defense and cellular functions. The discovery of herpesvirus encoded v-miRs by Pfeffer et al[7] showed that these viruses have evolved with yet another critical regulatory RNA called miR, previously thought to be restricted to metazoans and plants. Incorporation of tiny, multifunctional, non-protein coding RNAs that are tightly clustered within few viral transcripts, demonstrate a classical example of incessant host-pathogen co-evolution. Most of the HHV are reported to encode a large number of (22–44) v-miRs, indicating unique requirement of these noncoding RNAs in HHV-host interaction with numerous studies experimentally supporting the notion. Lack of v-miR sequence conservation across different HHV further supports these findings. Similar to host miR, v-miR can interact with the 3′ untranslated region (UTR) of the target mRNA to regulate gene expression.[8–10] This interaction results in translational inhibition and/or destabilization of cognate transcripts including host- and virus-encoded.[11,12]

Viruses develop ingenious mechanisms to overcome host defenses. In this review, critical functions of HHV-encoded v-miR in shaping host-pathogen interaction through modulation of host and viral functions are highlighted. Specifically, v-miR-mediated subversion of host immune responses (innate and adaptive) is discussed. HHV v-miRs are demonstrated to utilize host exosomal pathway to safely reach bystander cells and alter their transcriptome. Evidence demonstrating exosome-mediated delivery of functional v-miR and how this alters the function of recipient cells are provided. Figure 1 illustrates an overview of herpesvirus entry into target host cell, v-miR biogenesis and modulation of infected cell function and regulation of viral life cycle, secretion of v-miRs in exosomes and their entry and potential impact on bystander cells.

2 | VIRUS-ENCODED miRNAs

In 2004, the first v-miRs were discovered in human B cells that were latently infected with γ-herpesvirus EBV.[7] Subsequently, many other miRs were identified in human and animal herpesviruses.[13] To date, more than 250 different miR of viral origin have been identified and the list continues to expand (“miRBase: the microRNA database”). Both host and viral

miR are involved in viral tropism, pathogenesis, and latency.[14–17] Interestingly, while host miR can function as antiviral defenses, viruses encode suppressors (RNA/proteins) to counteract miR functions and have evolved mechanisms to utilize host miR for their benefit.[18–21] According to miRBase (version 22.1), more than 30 different viruses are known to encode miRs. These predominantly include members of herpesviruses representing both human- and animal-infecting viruses. The focus of this review primarily involves HHV derived v-miRs and their functions in host-pathogen interaction. Among human herpesviruses, miR are encoded by herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human cytomegalovirus (HCMV), human herpesvirus-6B (HHV-6B), Epstein-Barr virus (EBV), and Kaposi sarcoma-associated herpesvirus (KSHV).

Within the alpha subfamily of HHV, miR have been documented in HSV-1 and HSV-2. While the discovery of miR in HSV-1 was first reported in 2006,[22] 27 miR in HSV-1 have now been documented. Interestingly, over half of the miR are generated from latency-associated transcript (LAT) locus, while others are expressed during productive infection from other regions of the viral genome. It has been proposed that miR work in concert with other viral proteins during different life-cycle stages. More specifically, LAT-encoded miR may repress transcripts that are essential for lytic gene expression, thereby working to establish or maintain latency.[23] MiR have been isolated in betaherpesvirus family including HCMV and HHV-6B. While miR genes are seen throughout the viral genome, they are most commonly associated with the lytic stage of the viral lifecycle.[24,25] Finally, both gammaherpesviruses viz., KSHV and EBV encode large repertoire of miR. Interestingly, a cluster of 12 pre-miR are highly expressed in latently infected B cells.[24,26] These v-miRs may play a role in viral transformation of B cells, as multiple mRNA targets have been documented. In addition to the v-miRs associated with latency, others have been found to be associated with lytic reactivation and viral replication.[27] While some HHV express miR during all the phases of the viral life cycle, others may predominantly express them during latency. For instance, specific EBV miR are required in abundance during every stage of virus life cycle indicating their critical role in host-virus interaction.[28]

HHV miR biogenesis is completely dependent on host miR machinery and evidences clearly suggest that none of the viral protein is required to facilitate this process. Multiple EBV miR precursors were cloned in an expression vector to check if EBV miR are produced in the absence of viral protein. Interestingly, generation of mature EBV miR was observed using various quantitative expression and reporter assays indicating that host miR machinery is sufficient to process v-miR precursors.[12,27,29] However, viruses can obstruct miR biogenesis. Pan et al demonstrated impaired precursor-miR/mature miR ratios in HSV-1 infected cells or murine trigeminal ganglia during different life cycle stages.[30] Higher levels of precursor-miR were observed in nuclear fraction indicating that miR biogenesis is inhibited at the step of nuclear export. This inhibition was mediated by viral protein ICP27 with a known role in mRNA nuclear export and deletion of ICP27 enhances nuclear export of precursor-miR (both virus and host-derived) with almost no impact on mature miRs. This strategy allows HSV-1 to block nuclear export of host precursor-miR and eventually mature miRs which are antiviral in function. While generation of v-miR requires host machinery, virus-encoded proteins can impair miR biogenesis pathway to facilitate virus survival.

Comparative analysis of human and herpesviruses miR, genome size and gene/miR ratio highlights how efficiently these viruses have successfully integrated miR-mediated post-transcriptional regulation. The human genome contains approximately 3 billion bases and encodes approximately 73 000 transcripts and approximately 1900 miR precursors (approximately 2300 mature miR).[31,32] In humans, each miR precursor will have approximately 1.5 million bases of genomic landscape to encode for its gene and will regulate approximately 38 different transcripts among the whole transcriptome. However, it can be noted that several human miR have not been validated and are not considered as bona fide miR. This will likely further increase the gene/miR ratio.

Depending on the herpesvirus in context, genome size of human herpesvirus range from 140 to 235 kb and encode 74 to 213 genes and protein-coding genes and 8 to 44 mature miR.[33–35] For herpesvirus, the genomic space and gene/miR ratio vary remarkably indicating some herpesviruses have evolved more robust miR-mediated post-transcriptional regulation than others. Table 1 highlights the differences between the human and human herpesvirus with respect to integration of miR-encoding genes and their predictive post-transcriptional utility. For instance, the gene/miR ratio in HSV-1, HSV-2, EBV, and KSHV is close to approximately 3 to 6, indicating that viral transcripts are under a robust v-miR control. Other miR encoding HHV, HCMV, and HHV-6B (both beta family members) exhibit a gene/miR ratio of 14 and 28, respectively. Compared with humans, even HCMV and HHV-6B exhibit more efficient integration of miR genes and mature miR control of viral transcriptome. Indeed, *cis*- and *trans*-regulation of viral transcripts is a common feature of HHV-encoded v-miRs indicating their critical role in viral life cycle.[12–18] This in silico analysis shows human herpevirus have evolved this post-transcriptional mechanism to regulate large proportion of its transcriptome. However, further experimental testing is required to support the findings of bioinformatic analysis.

While research in the field has introduced the influence of herpesvirus-encoded miR in the regulation of virus lifecycle regulation, much is left to learn regarding their direct and indirect downstream functional impact on host defense responses. This is particularly significant as herpesvirus have mastered multiple ingenious mechanisms to escape host immune pathways. In the following sections, I discuss the role of HHV-derived miR in targeting various aspects of host innate and adaptive immunity pathways that play critical role in virus survival, spread, and evasion.

3 | ROLE OF VIRAL MIRS IN MODULATING HOST IMMUNITY

3.1 | Innate immune responses

All living organisms are subject to attack from disease-causing agents. Metazoans, over millions of years, have evolved to precisely recognize self from non-self. Acquisition of these mechanisms, in particular, have allowed them to fend off plethora of microbes that are ubiquitously present and pose danger to host. Orchestrated response by various cell types (both immune and non-immune cells) confer protection against incessant invasion of microbes (harmful or innocuous) by recognizing and eliciting efficient immune response to contain and clear microbes. Immune mechanisms that participate in the recognition and recruitment of leukocytes acts as first line of defense for host. Innate (non-specific) immune

system, which responds very quickly-within minutes to hours after infection, can halt spread of virus. For a successful viral pathogen, like HHV, it is required to subdue innate immune response in order to avoid robust adaptive immune responses. V-miRs play critical role in overcoming host innate immune response by suppressing various genes involved in the pathway.

3.1.1 | Pathogen recognition receptors—A key aspect of host defense is the ability to recognize and initiate responses to counter invading pathogens and destroy infected cells. Host and pathogen interactions has co-evolved over millennia and the host has evolved diverse mechanisms to elicit adept responses by distinguishing between self and non-self (pathogenic) antigens. This is achieved primarily by precise recognition of patterns displayed by pathogenic biomolecules. This includes proteins, carbohydrates moieties, DNA methylation patterns, and nucleic acid structure and sequence. Proteins present on host cell surface, endosomal membranes, and cytosol act as receptors for various pathogen associated molecular patterns (PAMPs). Upon recognition, these receptors can initiate a signaling cascade that consequently activates proinflammatory cytokine production.[36,37] On the basis of the motifs, these recognition molecules are categorized into three major categories viz, (a) toll-like receptors (TLR), (b) Nucleotide oligomerization domain (NOD)-like receptors (NLR), and (c) retinoid acid-inducible gene-I (RIG-I)-like receptors (RLR).[38–40] While TLR are localized on cellular and endosomal membranes, NLR and RLR are intracellular cytosolic sensors. Together, these molecules scan both extra and intracellular PAMPs and elicit adept immune response.

Toll-like receptors: TLRs are present on cell and endosomal surfaces and play a key role in innate immune activation. In general, extracellular pathogens are recognized by surface TLRs, while intracellular pathogens and their ligands are recognized by endosomal TLRs. Depending on the ligand binding and location of TLRs, a specific set of transcription factors are activated leading to expression of genes required to potentiate immune response against incumbent pathogens.[36,38,39,41]

Not surprisingly, HHV has devised mechanisms to thwart innate immune responses. HHV utilizes v-miRs as an important tool to suppress innate immunity. Figure 2 summarizes v-miR–mediated inhibition of selective host innate immune genes. Among the TLRs, TLR2 in conjunction with TLR1 or TLR6 is a key receptor in recognizing HCMV, EBV, HSV-1/2, and HSV-derived ligands.[42–44] HCMV encoded miR-UL-112–3p has been demonstrated to silence TLR2 which binds to HCMV glycoproteins B and H.[45] This is highly significant for viral persistence as it prevents NFκB-mediated proinflammatory cytokine production. The cytokines in turn will recruit other immune cells of both innate and adaptive arms of immunity that work in concert to clear virus-infected cells. Moreover, viral ligand-mediated internalization of TLR2 complex can also occur. This, unlike NFκB activation, triggers IRF2, IRF3, IRF7 activation by yet unknown signaling cascade, consequently upregulating production of antiviral genes IFNα (IRF2) and IFNβ (IRF3/7).[46–50]

Various components of TLR signaling proteins are also reported as direct targets of HHV miR. Myeloid differentiation primary-response gene (MyD88) and interleukin-1 receptor-associated kinase (IRAK1) are two key components of TLR signaling. Multiple KSHV-

encoded v-miRs are demonstrated to specifically interfere with TLR signaling, signifying the importance of innate responses in KSHV infection. KSHV miR-K12–5 and miR-K12–9, respectively, downregulate expression of MyD88 and IRAK1 and hence inhibit proinflammatory cytokine production.[51] Activation of NFκB is mediated by two IκB kinases (IKK), namely, IKKα and IKKβ that can phosphorylate IκBα and release the p50 and p65 (RelA) subunits. Two HCMV miR, viz, miR-US5–1 and miR-UL112–3p suppress IKKα and IKKβ levels thereby limiting NFκB activation allowing viral persistence by mitigating cellular inflammation.[52] Virus mutated for miR-US5–1 and miR-UL112–3p increased levels of IKKα and IKKβ proteins, impaired NF-κB signaling at late times of lytic infection, and increased proinflammatory cytokine production compared to wild-type virus in HCMV infection *in vivo*.

In addition, miR-K12–11 targets IKKε, a non-canonical NFκB activator and inducer of antiviral IRF3/7 signaling.[53] By silencing IKKε, which is involved in KSHV reactivation, viral miR suppresses antiviral pathway and also promotes latency maintenance. Similarly, KSHV miR-K12–3 and miR-K12–7 target an important transcriptional regulator C/EBPβ that suppresses IL-6 and IL-10 cytokines and promotes virus reactivation.[54] HHV miR not only target PRR but also downstream effector molecules consequently inhibiting inflammatory as well as interferon pathways. These findings clearly indicate that multiple KSHV miR have evolved to target different critical components of the innate signaling cascade. List of validated v-miR and their host targets are summarized in Table 2.

NOD-like receptors and RIG-1–like receptors: As mentionend earlier, NLR and RLR are a family of multiple cytosolic helicases that detect invading pathogens or host-derived damage signals in the cytosol and trigger innate immune response primarily by NFκB and MAPK singaling cascade.[37] A key feature of NLR proteins is they oligomerize to form large complexes called inflammasomes. EBV miR have been shown to influence inflammation and chemotaxis and thereby contribute to host immune system evasion. EBV-miR-BART15 binds and controls levels of NLR family, pyrin domain containing 3 (NLRP3). NLRP3 inflammasomes are among the well-characterized NLR-containing inflammasomes. NLRP3 is involved in the production of numerous proinflammatory cytokines such as IL-1β and IL-18, suggesting that downregulation of this protein by EBV miR can suppress innate immune activation.[56]

RLR are important in detecting viral infection (RNA or replication transcripts) and initiate the production of IFN and induction of an antiviral response.[65,66] Thus, silencing the components of this pathway is crucial for viral persistence. Not surprisingly, EBV miR-BART6–3p directly bind to and post-transcriptionally suppresses RIG-1 mRNA. Overexpression of miR-BART6–3p was able to suppress EBV-triggered IFN-β response production.[55] Another EBV miR, miR-BART16, also inhibits type I IFN signaling by targeting CREB-binding protein (CREBBP), a transcriptional coactivator required for IFN pathway.[57] Overall, these studies clearly support the role of HHV miR in the early phase of virus infection by simultaneously interfering with multiple host defense systems involved in virus sensing and immune activation.

Cytokine receptors: Cytokine-mediated autocrine and paracrine immune signaling is critical in containing virus infection. IL-1 and IL-6 are two major proinflammatory cytokines that are induced during initial herpesvirus infection both in vitro and in vivo implying their role in paracrine antiviral immunity.[67,68] Cytokine signaling cascade is triggered upon binding of cytokine to its cognate receptors which in turn activate other cytokine/chemokine production. In order to block the spread of antiviral cytokines, it is critical for HHV to interfere with cytokine signaling. Indeed, different HHV are reported to block the receptors for IL-1 and IL-6 cytokines, namely, IL-1R and IL-6R.

Using a combinatorial approach of miR targetome analysis, v-miR pathway analysis and reporter-based assays, Skinner et al. showed that EBV encoded miR-BHRF-1-2-5p is shown to target interleukin 1 receptor (IL-1R1).[58] Ectopic expression of miR-BHRF-1-2-5p downregulated IL-1R1 and hence IL-1 signaling, while inhibition of miRBHRF-1-2-5p augments IL-1 activation in latently infected B cells.

Zhang et al showed that IL-6R transcript was downregulated in EBV-positive BL.[59] IL6/IL-6R signaling is critical in acute phase response, immune activation, T cell growth, and NK cell activity. They predicted EBV-BART-6–3p binding site on IL-6R and validated that v-miR directly regulate IL-6R levels by reporter gene assays.[59] Inhibtion of IL-6 pathway is also important for latent HCMV infection in myeloid cells, which are important latent HCMV reservoirs. Lau et al showed that miR-UL148D targets cellular receptor ACVR1B, an important component of the activin signaling axis and promotes monocyte-to-dendritic cell differentiation.[60] Monocytes challenged with activin A secrete proinflammatory cytokines including IL-1 and IL-6.[69] Comparative analysis of activin A-treated monocytes challenged with wild type HCMV and ΔmiR-UL148D HCMV showed higher expression of ACVR1B and concomitantly induced IL-6 production in cell infected with miR-UL148D deleted HCMV. Yet another HCMV miR-UL112–1 also impairs activation of inflammatory signaling by binding to and inhibiting IL-32, a key cytokine that participates in the induction of other proinflammatory cytokines including IL-1β, IL-6, IL-8, and TNF-α through the p38MAPK, NF-kappa B, and AP-1 signaling pathways.[63] On the other hand, HSV-2 encoded miR-H9–5p targets suppressors of cytokine signaling 2 (SOCS2), a multifaceted immunoregulatory protein involved in immune cell differentiation, NK cell function, and resolution of inflammatory processes.[61]

KSHV-encoded miR target genes involved in the secretion of cytokines. More specifically, KSHV miR-K10 was found to target TNF-like weak inducer of apoptosis receptor (TWEAKR), which is the receptor for the proinflammatory cytokine TNF-like weak inducer of apoptosis (TWEAK).[62] This leads to a decrease in proinflammatory cytokine expression.[70] Another example of v-miR involvement in inflammation and chemotaxis is found in KSHV miR-K6 and miR-K9, which were found to target transcripts involved in the NFκB signaling pathway, and thereby decrease the expression of cytokines involved in inflammation.[71] V-miR-mediated suppression of these critical, early proinflammatory and antiviral cytokines are not only helpful in blocking cytokine signaling but also enhances survival of HHV infected cell by dampening inflammatory microenvironment.

3.1.2 | Phagocytosis and immune activation—

Studies from our lab have identifed v-miR in diseased oral tissues including gingiva and dental pulp, which are also sites of herpesvirus infection and reactivation.[72–75] Global mRNA and miR profiling of miR-H1 and miR-K12–3 expressing human oral keratinocytes and primary macrophages together reveal that these v-miRs specifically target pathways related to endosomal trafficking, suggesting their role in deregulation of endocytic pathways which are required for phagocytosis and immune activation.[64,74,76] Genes downregulated by miR-H1 and miR-K12–3 harbors one or more potential binding sites for genes involved in secretory pathway and some of these sites were validated using dual luciferase assays.[64,74] miR-K12–3 regulate RAB3B and RAB3D, while miR-H1 target sortilin 1 (SORT1) transcripts by directly binding to the 3′UTR. Targeting of endosomal/secretory pathway-related genes is also common in other HHV members with various HCMV and KSHV miR known to specifically target cellular genes associated with endosomal pathways.[71,77]

Macrophages expressing miR-H1 and miR-K12–3 exhibit reduced uptake of florescently labeled *Escherichia coli*, strongly suggesting impaired phagocytosis in presence of v-miRs. Furthermore, this translates to dysregulated immune response against incumbent pathogen as observed by cytokine profile analysis. Levels of multiple cytokines/ chemokines (including TNF-α, IL-1β, GM-CSF, IL12-p70, and IL-10) were significantly altered in miR-H1 and miR-K12–3 expressing macrophages.[64]

Overexpression of miR-H1 in HOK showed inhibitory role of v-miR in entry and infection. Given that miR-H1 is expressed in copious amounts during productive infection, our results suggest that this v-miR likely facilitate optimization of virion generation during the productive phase. Two other HSV-1 miRs, viz, miR-H28 and miR-H29, are also reported to regulate HSV-1 infection and spread.[78] Similar to miR-H1, both v-miRs are produced during productive infection, are exported into exosomes and prevent spread and infection of HSV-1. Thus, v-miRs act as multifunctional viral factors that regulate viral transcripts, participate in viral life cycle switching, suppress host immune response and facilitate viral spread in a contained manner.

3.2 | Adaptive immune responses

Adaptive (specific) immune responses are initiated by activation of naïve lymphocytes by cells of the innate immune pathway, in particular dendritic cells and macrophages. Proper induction of this pathway takes a long time—days to weeks—and is critical in acquiring immunity. However, the adaptive immune system specializes in retaining memory of foreign antigens. Adaptive immunity effectively contains herpesviruses in immunocompetent individuals, while high prevalence of herpesvirus and clinical manifestations are observed in immunocompromised individuals. Blocking activation of adaptive immune response is therefore critical to herpesvirus persistence and spread. The contribution of v-miR in evading adaptive immunity is discussed in this section.

3.2.1 | Antigen processing and presentation—

To persist inside the host, it is imperative for HHV to interfere with antigenic presentation of viral proteins. This is achieved by encoding proteins that have specifically evolved to block the antigen processing

pathway. There are two distinct antigen processing and presentation pathways. In general, major histocompatibility complex (MHC) I and MHC II process intracellular and extracellular antigens, respectively.[79–81] MHC-II presentation to CD4+ T cells results in polarized cells with specific antiviral immunity.[82–85]

Several HHV-encoded proteins inhibit both pathways, however, the propensity to interfere with cytotoxic T lymphocyte (CTL) activation is remarkably common. Indeed, EBV-specific CD8+ T cells are frequently detected in asymptomatic individuals and decrease in the EBV-specific T cells is associated with the emergence of EBV-related clinical manifestations in immunocompromised subjects.[86] Multiple HHV-encoded proteins target the generation of peptides (including viral antigens) by blocking the activity of host proteins required in the process.[87,88] HHV proteins have also been shown to interfere with proteasome activity, transport of viral peptide to MHC I, degradation of MHC I, secretion of mature MHC I-peptide complex into the golgi, directing of MHC I to cytosolic degradation, and redirecting surface-bound MHC I-peptide complex to lysosome for degradation.[89,90] For instance, HSV-1 ICP47, HCMV US3 and US6, and EBV BNLF2a all target Tapasin, a protein that monitors the integrity of peptide loading to MHC I.[91–93] Tagawa et al showed that two different EBV miRs, viz, BHRF1–3 and BART17, directly target TAP2 mRNA and downregulate its protein levels. Given that these v-miR are expressed in early infection (BHRF1–3) as well as latency (BART17) cleary suggest that v-miR assure suppression of antigenic peptide transport.[94]

The role of v-miRs in interfering antigen processing/presentation pathway is reported. MHC class I and II pathways and selected genes of the pathways targeted by v-miRs are highlighted in Figure 3. An increasing number of HHV-encoded v-miRs are demonstrated to specifically target genes that participate in antigen presentation and activation of T cells as well as NK cells. Different HHV encode miR that target the same gene, while multiple v-miRs from the same HHV exhibit sequence complementarity for a single gene. MHC class I polypeptide-related sequence A/B (MICA/MICB), a stress-induced or virus infection-induced glycosylated protein is targeted by v-miRs encoded by HCMV (miR-UL112), EBV (BART-2–5p), and KSHV (miR-K12–1).[95,96] MICB is a ligand for C-type lectin receptor NKG2D expressed on NK cells and γδ T cells. Signals from NKG2D induces cytolytic activity of NK or T cells. HCMV utilizes another ingenious strategy to downmodulate MICA by miR-US25–2–3p targeting of TIMP3 transcript, leading to enhanced shedding of MICA.[97] Silencing MICA/ MICB by v-miR prevents clearance of virus-infected cells.

HCMV miR-US4–1 binds to and suppresses endoplasmic reticulum aminopeptidase 1 (ERAP1) expression.[98] This gene is responsible for trimming peptides to be properly loaded onto MHC I. In this regard, ERAP1 is important in activating cytolysis of virus-infected cells through activation of CD8+ T cells. Tagawa et al showed multiple genes involved in antigen processing directly regulated by EBV miR.[94] Three critical endosomal genes involved in antigen processing, namely, interferon gamma-inducible protein 30 (IFI30), cathepsin B (CTSB), and asparagine endopeptidase (LGMN), were targeted by multiple EBV miR to restrict viral antigen processing to MHC pathway.[94] Expression of CTSB, a lysosomal cysteine protease, is directly targeted by two different miRs encoded by EBV, viz, BART-2 and BHRF-1–2. CTSB is an important protein involved in antigen processing of

intracellular antigens. Downregulation of IFI30 and CTSB by miR-BART1 and miR-BART2 assures reduced antigen presentation of exogenously loaded protein in presence of v-miR as observed by impaired recognition of virus-infected B cells by EBV-specific CD4+ T cells.[94] V-miR are therefore capable of restricting the antigen porcessing pathway at multiple levels including lysosomal protein degradation, HLA class II, and co-stimulatory molecule expression. By targeting multiple proteins of the same pathway, v-miR facilitate successful immune evasion by virus. These examples and others of v-miR targeting of antigen processing/presentation pathways reveal that they exhibit a propensity toward the same targets as shown for viral proteins. For example, MICB is targeted by KSHV kK3 and kK5, VZV ORF66, HCMV Il18, and also by KSHV BART2–5p and miR-K12–7. These observations indicate that viruses have evolved multi-prong approaches to thwart antigen processing and presentation to T/NK cells, suggesting the importance of this pathway in HHV persistence.

LY75, a C-type lectin receptor is a key molecule involved in antigen presentation on MHC. PAR-CLIP–based EBV miR target identification yielded two potential EBV miR with putative binding sites on LY75 3′ UTR. Using mutation analysis, miR-BART1–5p was confirmed as a bonafide regulator of LY75 transcript.[12] Overexpression of miR-BART1–5p can evade recognition of EBV-infected cells by $CD4^+$ or $CD8^+$ T cells. Interestingly, bioinformatic analysis also predicted binding sites of miR from other HHV including KSHV, and HCMV indicating LY75 (like many other host targets) are selectively targeted by HHV encoded miR to evade immune responses. Studies from our lab have reported increased accumulation and prevalence of various HHV-encoded miR in healthy and diseased gingiva, suggesting their role in the pathogenesis of oral inflammatory diseases. miR-K12–3 identified in our analysis was assessed for its potential role in antigen processing and uptake. We observed that ectopic expression of miRK12–3 in macrophages and DC interferes with the processing of antigen as observed by reduced processing of ovalbumin, conjugated with a quencher dye (Naqvi et al, Unpublished data).

Interestingly, viral miR not only suppress host genes involved in antigen processing/ presentation, but also actively degrade viral transcripts that generate highly immunogenic proteins. For instance, EBV-encoded latency-associated membrane proteins LMP1 and LMP2A are post-transcriptionally silenced by v-miR. LMP1 is regulated by miR-BART16, miR-BART17–5p, and miR-BART1–5p, while LMP2A expression levels are controlled by miR-BART22.[99,100] Down-regulation of LMP protein levels by EBV miRs allow evasion of host antigen responses against latently infected cells. List of validated v-miR and their host targets involved in shaping adaptive immunity are summarized in Table 3.

3.2.2 | T cell activation and polarization—Antigen-presenting cells (APCs) display viral epitopes that are recognized by T cells leading to their activation. This is mediated by a set of stimulatory and costimulatory molecules present on APC and T cells. MHCI/II (APCs) and TCR (T cells) are primary stimulatory molecules, while multiple factors present on APCs (CD86/CD80/ICAM1/CD40/PD-L1/2) and T cells (CD28/CD40L/CTLA/PD-1) act as costimulatory molecules.[103,104]

In order to survive inside the host, it is imperative for HHV to interfere with cytokine-mediated feed-forward activity. Recently, Tagawa et al compared the impact of EBV miR on B cell function, an important APC.[94] Using an EBV wild-type strain that expresses 13 v-miRs and a deletion strain lacking any miRs, they demonstrated that v-miRs can modulate various pathways including apoptosis, cell cycle, cytokine signaling, and antigen processing relevant to viral persistence. Several proinflammatory cytokines (TNF-α, IL-6, IL-8, IL-12, IL-23) but not the anti-inflammatory cytokine IL-10, were also observed to be downregulated in wild-type EBV strain compared with the v-miR deleted strain. Five different EBV encoded miRs, viz, BART-1, −2, −10, −22, and BHRF1 were shown to directly target IL12B by binding to the 3′ UTR. Reduced IL-12 production by B cells interferes with polarization of Th cells to the Th1 subtype. Antibody against IL-12 relieved Th1 suppression caused by v-miRs indicating a specific role of this cytokine in T cell polarization. These findings indicate that targeting of IL-12–mediated activation of Th1 cells is critical for EBV survival. IL-12 also activates expression of IFN responsive genes required for antiviral immunity. Furthermore, IL-12 not only derives Th1 differentiation but also suppresses Th2 polarization by opposing IL-4 activity.[94]

Further examples of the role of v-miR in immune evasion via altered chemotaxis can be found in HCMV. It has been demonstrated that HCMV-miR-UL148D may contribute to evasion of the immune response via targeting of the gene encoding the chemokine (C-C Motif) ligand 5 (CCL5) transcript.[101] This protein acts as an inducer of cell activation as well as proliferation in NK cells.[3,105] EBV miR-BHRF1–3 is yet another example of v-miR regulating chemotaxis. This v-miR targets CXCL11, a T cell attracting chemokine.[102] By interfering with chemotaxis, HHV can simultaneously minimize the intensity of the innate response and block efficient activation of adaptive responses.

4 | IMPACT OF VIRAL miRNA MUTANTS ON HERPESVIRUS PATHOGENESIS

Deletion or mutation of HHV miRs has shown contrasting results in vitro and in vivo. Phenotypic effects of v-miR knockout differed on the basis of the infection model used, viral strain used, number of v-miRs targeted, and experimental conditions. In HSV-1, impact of mutation or deletion of three highly expressed and RISC-associated v-miRs, viz, miR-H2, miR-H3, and miR-H4 on viral replication was examined in different cell types.[106] Mutation/deletion of these miR-H2 and miR-H4 also impacted expression of other v-miRs. Surprisingly, it was observed that disruption of miR-H3 and miR-H4 significantly enhanced viral replication in Neuro2A cells but not in NIH 3 T3 fibroblasts. On the other hand, mutational inactivation of miR-H2 reduced viral replication only in Neuro2A. These findings indicate that v-miR have a critical role to play in viral replication, but the effects may be specific to cell type or lifecycle associated. The impact of mutated miR-H2 HSV-1 was evaluated for viral replication and reactivation in vivo by the same research group. Contrary to the previous in vitro findings, no significant changes in viral replication, virulence, or reactivation was observed in the mice trigeminal ganglia acutely or latently infected with wild type or miR-H2 mutant virus.[107] Furthermore, expression of ICP0, a well-characterized miR-H2 target in vitro, were not significantly changed in TG infected

with mutant virus, suggesting it is a weak regulator of ICP0 in vivo. On the contrary, Jiang et al showed miR-H2 mutant virus exhibits increased ICP0 during productive infection and higher virulence and rate of reactivation, implying that targeting of v-miR could be a promising antiviral strategy.[108,109] The differences observed could likely be attributed to the redundant functions of v-miRs. Simultaneous deletion of multiple miR might be required to observe more consistent phenotypes. Different viral strains, mutation impact on viral miRs or transcripts, and HHV-specific differences in humans and mice could contribute to disparities observed in the v-miR mutation analysis.

Mutational analysis of EBV miRs strongly support their oncogenic role. The BHRF region encodes three v-miR, viz, BHRF1–1, BHRF1–2, and BHRF1–3. To evaluate thier role in EBV infection and oncogenic potential, Feederle et al deleted the BHRF1 miR cluster from the B95.8 genome. B cells infected with mutant showed reduced transformation compared with wild type or revertant virus, suggesting that BHRF1 miR cluster markedly increases the efficiency of transformation.[110] Cell cycle analysis revealed that in the absence of these genetic elements, infected B cells grow markedly more slowly. Using a humanized mouse model, Wahl et al demonstrated a role of v-miR in EBV infection.[111] Compared with the wild type virus, systemic EBV infection is delayed in mice infected with mutant virus lacking BHRF miR cluster. However, v-miR mutant virus had no effect on the generation of tumors.

Employing CRISPR/Cas9 to directly mutate v-miR has shown promising results indicating their role as critical viral genetic elements required for both productive and latent herpesvirus infection.[112–115] Guide RNAs (gRNA) targeting v-miRs BART5, BART6, and BART15 in latently infected gastric carcinoma cell line NSU-719-generated viruses with mutant v-miR genes. Using a florescent reporter-based assay, direct editing of v-miR on the EBV genome impaired their function by preventing v-miR binding to their target transcripts.[112] Comparing different mutation techniques for assessing v-miR function both in vivo and in vitro will provide better and novel insights of their biological roles.

KSHV miRs are highly expressed in latency and KS tumors implicating their role in oncogenesis. The oncogenic role of KSHV miR cluster was assessed by deleting 10 pre-miRs. Cells infected with mutated virus showed similar anchorage-dependence and contact-inhibition, and thus were not transformed.[116] Mutant virus infected cells fail to develop tumors in nude mice. Genetic complementation by stably expressing the miR cluster rescues KSHV-induced cellular transformation. Mechanistically, miRs in this cluster promote cell cycle progression and inhibit apoptosis.

Mutating the viral gene targets of v-miRs further highlight their role in viral persistence. In HCMV, immediate early 72 (IE72) is a target of miR-UL112–1. Mutating miR-binding site on IE72 remarkably enhanced expression of IE72 and allowed productive virus infection.[117] Monocytes latently infected with mutant virus are recognized specifically by IE-specific CD8+ T cells suggesting an immunosuppressive role of v-miR by inhibiting recognition of latently infected cells by the host's CTL response. Overall, v-miR mutation/deletion studies have emphasized the role of these regulatory RNAs in pathogenesis, viral spread and persistence but the observations are not consistent in different models. Development of

tractable and consistent disease models to measure in vivo impact of v-miR in disease pathogenesis, oncogenesis, and herpesvirus reactivation will provide conclusive information on their biological functions and mechanisms through which they contribute to these pathways.

5 | VIRAL miRNA AND EXOSOME PATHWAY

Similar to cellular miR, v-miRs can be packaged into exosomes and delivered to various cell types. Exosomes are membrane bound nanovesicles (20–100 nm in diameter) secreted ubiquitously by various cell types. These vesicles contain RNA, miR, proteins, and DNA and can reach other cell types through membrane fusion.[118] To exert systemic modulation of cellular functions, viruses have hijacked the host exosomal pathway to disseminate their modulatory biomolecules, including proteins and v-miR.[119–121] This allows viruses to shut down key host cell functions. In terms of immune responses, this may allow viruses to persist. Furthermore, unlike proteins, these v-miRs are nonimmunogenic thus providing viruses an advantage. Studies from our lab and others have shown the presence of v-miRs in host cell– derived exosomes in vivo and in vitro, further signifying the functional relevance of v-miRs in viral immunoevasion.[64,122–124] EBV miR-BART-15 is secreted in exosomes of EBV-infected B cells as well as macrophages ectopically expressing v-miR. This v-miR share the same seed region as that of cellular miR-223 for its target NLRP3.[56] Such interactions provide an evolutionary benefit as these sequences are less likely to undergo mutation to avoid v-miR regulation. B cell–derived exosome mediated delivery of BART-15 into recipient macrophages suppresses inflammasome formation by silencing NLRP3. Considering that exosomal v-miRs can theoretically be delivered to any cell type, these can be considered as broad spectrum virulence factors. While copious amounts of v-miRs are produced in both the latency as well as lytic phase of viral infection, the functional relevance of v-miRs as virulence factors is plausible in latency where they have ample time to downmodulate target genes in a wide range of host cells, particularly immune cells. Overall, non-immunogenic v-miRs are employed as tools by HHV to modulate functions of a wide range of non-permissive cells.

In a recent study, HCMV virions are shown to carry different RNA molecules, both of viral and cellular origin.[125] Purified virions and dense bodies (DB) isolated from HCMV infected lung fibroblast cells MRC-5 were profiled for RNA contents. Interestingly, various small RNAs including cellular and viral miR, protein coding, and long non-coding RNA were identified in virions and DB. Among the miRs, 14 HCMV-encoded miR (more than half of the HCMV repertoire) and two cellular miR, viz, miR-218–5p and miR-21–5p, were detected. This indicates that v-miRs are preferentially sorted into the virions; however, the underlying mechanisms remain obscure. Using dual luciferase reporter assays, it was shown that virion contained miR were functionally delivered to the recipient cells. Virion mediated delivery of miR-US25-1-5p into recipient cells expressing luciferase-tagged CCNE2 5′UTR, a known target of candidate v-miR,[126] was functionally assessed by silencing of reporter gene expression. Overall, both exosomes and virions are key sources for virus to modulate host cell functions, primarily by sorting large repertoire of v-miRs.

6 | CONCLUSIONS AND FUTURE PERSPECTIVE

Numerous mechanisms of HHV protein mediated immune evasion and modulation of host cell function are well-known. However, these viruses maintain latent life cycle during which there is little or no viral protein production except LAT transcripts that also encode for miR. Incessant production of v-miRs over a long period might even alter cellular functions. Ability of v-miRs to reach distant sites through exosome pathway can further cause a ripple effect in impairing functions of various cells at tissue or even systemic level. Altered genetic and immune function of host can further facilitate this long-term take-over. Dissecting molecular mechanisms underlying each v-miR will be critical in our understanding of their contribution in viral tropism, persistence and disease manifestation. While the discovery of v-miRs in most of the human viruses have become stagnant, a more challenging path forward is to annotate biological functions to these enigmatic molecules deployed by virus to evade host defenses.

Association of HHV in diverse inflammation-related diseases and/or disorders is increasingly being recognized. For instance, increased prevalence of various members of HHV have been demonstated in Alzheimer's disease, cancers, multiple sclerosis, periodontitis, peri-apical periodontitis, etc, indicating their contribution in manifesting chronic inflammation, a common feature of most of these diseases.[127–131] This is not surprising given that most of these viruses are acquired during childhood, possess multiple immune subversion strategies and persist lifelong. Therefore, v-miRs may serve as a reliable diagnostic marker for HHV infection. A thorough examination of HHV genome detection and its correlation with v-miR may further shed light on how expression levels of these promising viral biomolecules correlate in health and disease.[72–74,132–134] This will facilitate future research avenues on employing v-miRs as therapeutic targets, not only for HHV-mediated infections but likely for other diseases as well. How v-miRs can alter the life cycle of HHV in host is another key question that remains unanswered. If exogenous supply of disease-associated v-miRs can support HHV infection and/or persistence, how this changes the microenvironment for other diseases (eg, oral cancers) and pathogens (bacteria and fungi) will be critical questions to address. Studies focusing on chronic inflammatory diseases with HHV association will unravel new etiological role of these elusive viruses. Future studies examining HHV association with other known etiological factors in chronic diseases like periodontitis, Alzheimer's, multiple sclerosis, and cancers could provide a novel perspective of dynamic players in the etiopathogenesis of multifactorial diseases.

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Abbreviations used

AIDS	Acquired Immunodeficiency Syndrome
APC	antigen presenting cells
BART	BamHI fragment A rightward transcript
BHRF1	BamHI fragment H rightward reading frame 1
C/EBP	CCAAT-enhancer-binding proteins
CCL5	chemokine (C–C motif) ligand 5
CREBBP	CREB-binding protein
CTL	cytotoxic T lymphocyte
EBV	Epstein-Barr virus
ERAP1	endoplasmic reticulum aminopeptidase 1
HCMV	human cytomegalovirus
HHV 6B	human herpesvirus 6B
HHV	human herpesviruses
HSV-1	herpes simplex virus 1
HSV-2	herpes simplex virus 2
IFI30	interferon gamma-inducible protein 30
IFN	interferon
IKK	inhibitor of kappa B kinase
IL	interleukin
IRAK1	interleukin-1 receptor–associated Kinase
IRF	interferon regulatory factor
KSHV	Kaposi sarcoma-associated herpesvirus
LAT	latency-associated transcript
LMP	latency-associated membrane proteins
MHC	major histocompatibility complex
MICA/MICB	MHC class I polypeptide-related sequence A/B
MyD88	myeloid differentiation primary-response gene
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells

NLR	nucleotide oligomerization domain (NOD)-like receptors
NLRP3	NLR family, pyrin domain containing 3
PAMP	pathogen associated molecular patterns
PEL	primary effusion lymphoma
RLR	retinoid acid-inducible gene-I (RIG-I)-like receptors
SOCS2	suppressors of cytokine signaling 2
Th	T helper
TIMP	tissue inhibitor of metalloproteinases
TLR	toll-like receptors
TNF	tumor necrosis factor
TWEAKR	TNF-like weak inducer of apoptosis receptor
UTR	untranslated region

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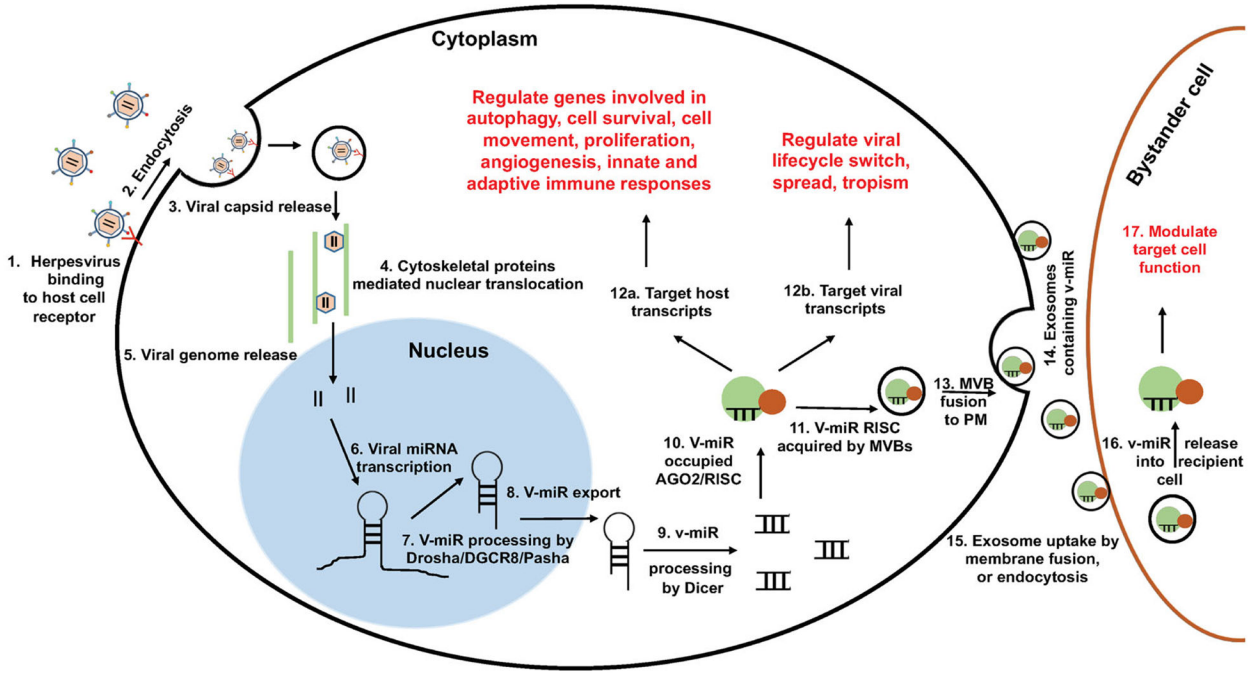

FIGURE 1.

Schematic depiction of viral miR biogenesis and their impact on infected and bystander host cells. A series of events leading to the production of viral miR and modulation of host functions is summarized. Virus bind to cell surface receptors via glycoproteins leading to fusion at the plasma membrane or fusion with an endocytic membrane after endocytosis. Internalized virion sheds envelop and is directed towards nucleus, facilitated by host cytoskeleton proteins. In nucleus, viral transcripts (including v-miR precursors), are generated. Host miR biogenesis machinery (eg, DGCR8 and Dicer) recognize viral miR stem-loop structure and generate mature v-miRs. These molecules acts as autocrine and paracrine modulators. In the infected cells, v-miRs can regulate both host and viral transcripts thereby shifting host transcriptome more favorable towards virus. Additionally, v-miRs can be packaged into exosomes and gain entry into bystander cells. Steady influx of v-miRs in non-infected bystander cells can also modulate their cellular functions, including immune responses and thus contribute to viral persistence and immune evasion

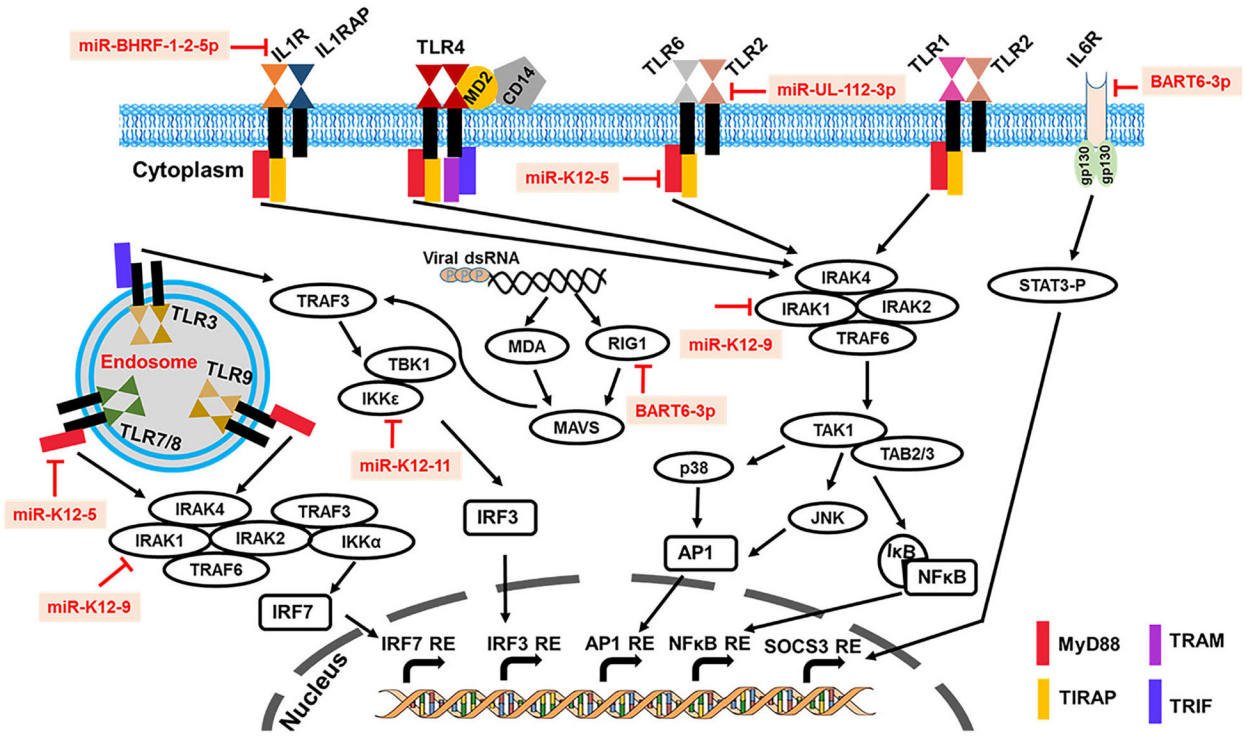

FIGURE 2.

Overview of viral miR-mediated suppression of host innate immune pathways. The illustration summarizes known mechanisms by which individual viral miR interact with host genes involved in virus recognition and activation of innate immune responses. Viral miR promote immune evasion by directly targeting host genes involved in the recognition of virion, virus-derived nucleic acids (DNA/RNA) or proteins to suppress antiviral pathways. Viral miR have evolved to target, essentially, all of the known viral defense mechanisms by targeting one or more critical components of the innate immune pathway. Functions of selected validated herpesvirus-encoded miR and host innate immunity genes are highlighted in the schematic. Viral miR inhibit production of proinflammatory cytokines and interferons that are required for recruitment and activation of adaptive arm of immunity, which can elicit robust antiviral response to contain herpesvirus infection by clearing infected cells

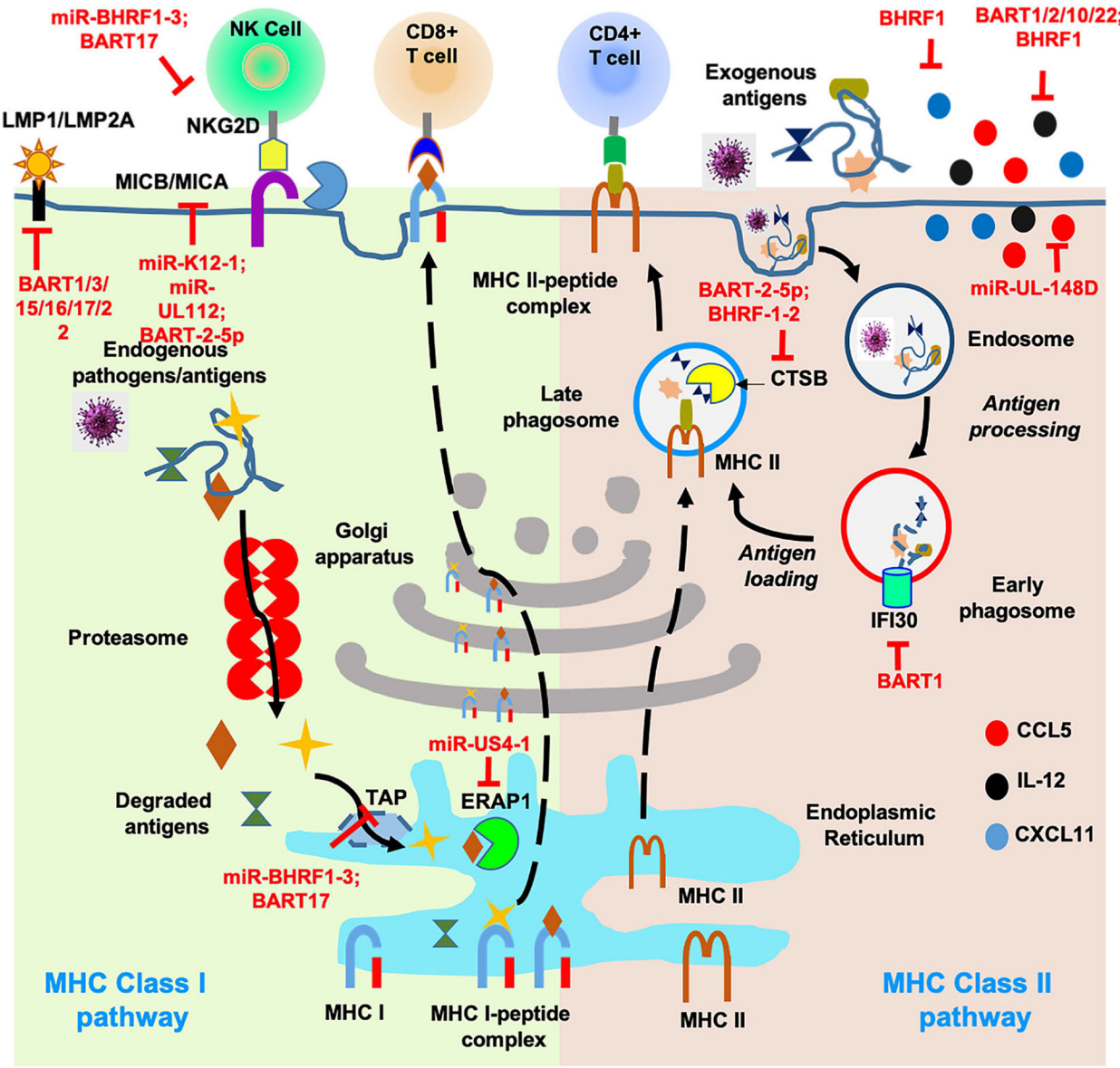

FIGURE 3.

Viral miR-mediated evasion of adaptive immune responses. Antigens are presented by two major pathways, MHC class I and MHC class II. In MHC class I pathway, endogenous viral antigens are degraded via proteasome machinery, translocated to endoplasmic reticulum through TAP proteins and are processed by proteases like ERAP. Processed peptides are assembled with MHC-I complex to be presented predominantly to CD8+ T cells. MHC class II pathway involves exogenous antigen internalization by several pathways, including phagocytosis, macropinocytosis, and endocytosis. Antigens are trafficked to a mature or late endosomal compartment where they are processed and loaded onto MHC-II molecules that present antigens to CD4+ T cells. Numerous viral miR are known to impair both MHC class I and II pathways by targeting one or more components of the same pathways. In addition, some critical genes (eg, MICB) are targeted by multiple herpesvirus miR indicating selective evolution of miR sequences that can interfere with antigen processing and presentation

pathway. Inhibition by viral miR is shown as blocked line highlighted in red. By virtue of their direct interaction with host and viral transcripts, v-miRs can impair components of MHC class I (antigenic peptide transporter TAP, and protease ERAP1) and class II (cathepsin B); inhibit release of proinflammatory and antiviral cytokines; protect infected cell from CD4+/CD8+ T cell response and NK cell-mediated cytolysis; reduce highly antigenic viral antigens (LMP1/ LMP2A)

TABLE 1

Comparative analysis of human and human herpesviruses highlights integration of miR-mediated post-transcriptional regulation by most herpesviruses. Computed genomic landscape occupancy (per kb per miR) and protein coding gene to miR ratio of human and miR-encoding HHV

		Genome Size (Transcripts Encoded)	Precursor miR Expressed (Mature miR)	Ratio of Genome size/ miR	Ratio of Gene/miR
Human		3 billion base (~73 000)	1917 (2300)	1.5 Mb/miR	~38
Human Herpesvirus	Common Clinical Manifestations				
HSV-1 (α)	gingivostomatitis, herpetic keratitis, dermal whitlows; encephalitis in neonates	152 kb (90)	18 (27)	8.4 kb/miR	5
HSV-2 (α)	Genital herpes	155 kb (74)	18 (24)	8.6 kb/miR	4
HCMV (β)	mononucleosis syndrome, systemic diseases observed in congenital infection or immunocompromised individuals	235 kb (213)	15 (26)	15 kb/miR	14
HHV-6B (β)	exanthem subitum	161 kb (115)	4 (8)	40 kb/miR	28
EBV(γ)	Infectious mononucleosis, oral hairy leukoplakia, lymphoproliferative syndrome in immunocompromised hosts	172 kb (85)	25 (44)	6.8 kb/miR	3.4
KSHV (γ)	Kaposi's sarcoma, Primary effusion lymphomas and Castleman's disease	140 kb (87)	13 (25)	10.7 kb/miR	6.6

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TABLE 2

List of validated viral miR targets involved in the innate immune subversion

Viral miR Modulation of Innate Immune Responses

Viral miR	Target	Function	References
HCMV miR-UL112–3p	TLR2	Inhibition of proinflammatory responses by HCMV ligand	Landais et al[45]
KSHV miR-K12–5	MYD88	Suppression of TLR-mediated activation of cytokine production	Abend et al[51]
KSHV miR-K12–9	IRAK1	Suppression of TLR-mediated activation of cytokine production	Abend et al[51]
HCMV miR-US5–1 and miR-US112–3p	IKKA and IKKB	Blocks NFκB activation	Hancock et al[52]
KSHV miR-K12–11	IKKε	Inhibition of NFκB and antiviral cytokine activating IRF3	Liang et al[53]
EBV BART6–3p	RIG-1	Impairs RLR pathway-mediated activation of antiviral cytokines	Lu et al[55]
KSHV miR-K12–3, −7	C/EBPβ	Modulate cytokine secretion by immune cells	Qin et al[54]
EBV miR-BART-15	NLRP3	Suppress inflammasome formation and hence IL-1p/IL-18/ROS production	Haneklaus et al[56]
EBV miR-BART16	CREBBP	Inhibits type I interferon signaling	Hooykas et al[57]
EBV miR-BHRF-1-2-5p	IL1R1	Dampens proinflammatory IL-1 signaling	Skinner et al[58]
EBV BART-6–3p	IL6R	Impairs IL-6 signaling	Zhang et al[59]
HCMV miR-UL148D	ACVR1B	Suppress cytokine production by blocking activing A signaling	Lau et al[60]
HSV2 miR-H9–5p	SOCS2	Impaired immune cell activation and differentiation	Wang et al[61]
KSHV miR-K12–10	TWEAKR	Reduced induction of IL-6 and IL-10	Abend et al[62]
HCMV miR-UL112–1	IL-32	Reduced NK cell activity & Mϕ proinflammatory function	Huang et al[63]
HSV-1 miR-H1	SORT1/Integrin	Attenuates bacterial phagocytosis and cytokine production	Naqvi et al[64]
KSHV miR-K12–3	RAB3B/RAB3D	Attenuates bacterial phagocytosis and altered cytokine production	Naqvi et al[64]

TABLE 3

List of validated v-miR targets involved in the regualtion of antigen processing and presentation and T cell activation and polarization

Viral miR Modulation of Adaptive Immune Responses			
Antigen processing and presentation			
Viral miR	Target	Functional impact	References
HCMV miR-UL112; EBV miR-BART-2–5p; KSHV miR-K12–1	MICB	NK cell/CD8+ T cell/γδ T cell cytolytic evasion by reducing stimulatory signals through NKG2D receptor	Nachmani et al[95]; Stern-Ginossar et al[96]
HCMV miR-US25-2-3p	TIMP3	Degradation of MICA and hence reduced NKG2D activation	Esteso et al[97]
BHRF1–3; BART17	TAP2	Blocks peptide transport	Tagawa et al[94]
EBV miR-BART2 BHRF1–2	CTSB	Interferes with MHC class I antigen processing	Tagawa et al[94]
HCMV miR-US4–1	ERAP1	Reduced antigen degradation and presentation to CD8 + T cells	Kim et al[98]
EBV miR-BART1–5p; BART3; BART15; BART16; BART17–5p	LMP1 (viral)	Downregulation of highly immunogenic viral latency membrane protein 1	Lo et al[99]
EBV miR-BART22	LMP2A	Downregulation of highly immunogenic viral latency membrane protein 2A	Lung et al[100]
EBV miR-BART2	LGMN	Reduced processing of MHC II bound pathogenic antigens	Tagawa et al[94]
EBV miR-BART1	IFI30	Inhibition of MHC class II-restricted antigen processing	Tagawa et al[94]
EBV miR-BART1–5p	LY75	Impaired antigen presentation on MHC	Skalsky et al[12]
T cell activation and polarization			
EBV BART1; BART2; BART10; BART22; BHRF1	IL12B	Prevents polarization of Th cells to antiviral Th1 subtype	Tagawa et al[94]
HCMV-UL-148D	CCL5	Reduced NK cell activation and T cell recruitment	Kimet al[101]
EBV BHRF1–3	CXCL11	Inhibition of chemotaxis of activated T cells	Xia et al[102]