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Viral miRNAs and immune evasion

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Abstract

Viral miRNAs, ~22nt RNA molecules which post-transcriptionally regulate gene expression, are emerging as important tools in immune evasion. Viral infection is a complex process that requires immune evasion in order to establish persistent life-long infection of the host. During this process viruses express both protein-coding and non-coding genes, which help to modulate the cellular environment making it more favorable for infection. In the last decade, it was uncovered that DNA viruses express a diverse and abundant pool of small non-coding RNA molecules, called microRNAs (miRNAs). These virally encoded miRNAs are non-immunogenic and therefore are important tools used to evade both innate and adaptive immune responses. This review aims to summarize our current knowledge of herpesvirus- and polyomavirus-encoded miRNAs, and how they contribute to immune evasion by targeting viral and/or host cellular genes.

Introduction

All currently identified miRNAs are described as noncoding RNA molecules ~22 nucleotides in length that post-transcriptionally regulate gene expression by complementary binding to target mRNA transcripts. MiRNAs were first identified in 1993 by Ambros and colleagues in the nematode *Ceanorhabidtis elegans* and were originally thought to be a regulatory phenomenon specific for this organism [1]. Hallmark discoveries in 2000 by the Ruvkin laboratory showed that one C. elegans miRNA, let-7, was 100% conserved within the genomes of mice and humans [2]. This finding initiated a rapid discovery phase for novel miRNAs and now more than 15,000 different miRNAs have been identified in all metazoan and plant species investigated thus far. In 2004 this list was further expanded to include DNA viruses, when Tuschl and colleagues identified the first viral miRNAs encoded by Epstein-Barr virus (EBV), a human DNA tumor virus [3]. To date, over 200 viral miRNAs have now been identified, with most being encoded by herpesviruses.

Functionally, miRNAs regulate a vast set of targets that are involved in multiple cellular processes including development, immunity, and apoptosis. In mammalian species, it has been estimated that greater than half of all protein coding genes contain a miRNA target site, indicating their heavy impact on gene regulation [4]. Viral miRNAs have also been found to target a large number of host genes involved in regulating cell proliferation, apoptosis, and

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host immunity. In addition, viral miRNAs target their own genes, which also help the virus to remain hidden from the host immune response.

Viral miRNA biogenesis and functional targeting is 100% dependent on the host molecular miRNA maturation and silencing machinery. In brief, viral miRNA biogenesis initiates in the nucleus, where after transcription, the host RNase III endonuclease Drosha cleaves pri-miRNA hairpins into 60 to 80nts long pre-miRNAs. These pre-miRNAs are rapidly exported by the Exportin 5/Ran GTPase pathway into the cytoplasm where they are further processed by a second host RNase III endonuclease, Dicer, into a short dsRNA duplex. One strand of the duplex is incorporated into the host RNA-induced silencing complex (RISC), which guides the mature miRNA to 3′UTR's of mRNAs containing complementary sequences, leading to translational silencing and/or transcript degradation. An important parameter of targeting is complementary base pairing between the miRNA ‘seed’ sequence (5′ nucleotides 2-7) and the target transcript [5]. To date, no viral proteins have been described that directly contribute to either miRNA biogenesis or targeting mechanisms.

Based on the requirements of nuclear machinery and RNA cleavage for miRNA processing, it is no surprise that cytoplasmic replicating DNA viruses and RNA viruses have not been found to express miRNAs. In fact, out of the currently identified viral miRNAs (>200) the vast majority (>90%) are encoded by all three families of herpesviruses (α, β, and γ), which are DNA viruses that replicate in the nucleus of host cells. In addition to the herpesvirus miRNAs, a small number of miRNAs (7) have been found in other nuclear replicating DNA viruses, including adenovirus and several polyomaviruses, although for the former, miRNAs are processed in a Drosha-independent manner. Interestingly, all three of these DNA virus families establish persistent infections in a large percentage of the human population, indicating that immune evasion is essential for their lifecycles and that viral miRNAs may play an important role in promoting immune evasion. To date only herpesvirus and polyomavirus miRNA targets have been characterized (Table 1) and this review will focus on how miRNAs encoded by these viruses contribute to immune evasion.

Anti-viral host defenses

The host defense against viral infection is mediated by two main components: innate and adaptive immunity. Considered the first line of defense, innate immunity primarily involves early viral detection which elicits an interferon response and activation of natural killer (NK) cells. At later time points adaptive immunity is induced which elicits antigen-specific responses, including antibody production by B cells and the activation of cytotoxic T lymphocytes (CTL). To counteract these hosts defense mechanisms both herpesvirus and polyomavirus have coevolved miRNAs to regulate a variety of host-immune modulatory pathways (Fig. 1).

Viral miRNA inhibition of cell mediated immunity

Cell mediated immunity is essential for viral clearance of infected cells. The effectors of this mechanism involve both antigen specific CTLs and non-specific NK cells, both are cytotoxic mediators which eliminate infected cells through processes that induce apoptosis and ultimately result in cell lysis. Several viral miRNAs have been found that work to inhibit these cell mediated responses by targeting viral antigens or proteins involved in antigen presentation and activation pathways.

The clearance of virally infected cells by CTLs requires viral antigen presentation by infected target cells which are recognized by antigen specific CTL receptors (Fig. 1a). In murine models it has been shown that expression of the polyomavirus viral protein, T-antigen, can elicit such a response [6]. Studies done with the primate polyomavirus, simian

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virus 40 (SV40), identified miRNAs encoded antisense to the T-antigen that mediate its transcripts cleavage during infection [7]. Subsequent studies found that the human polyomaviruses JCV and BKV, as well as a murine Polyomavirus muPyV also encode miRNAs that cleave T-antigens [8]. Using mutant SV40 viruses, which do not express the miRNAs and as a result express increased levels of large T-antigens, it was shown in cell culture that these viruses had increased susceptibility to CTL lysis compared to wild type virus [7]. This study provided the first evidence that viral miRNAs can inhibit CTL recognition by directly targeting viral antigens. However, when a miRNA mutant of muPyV virus was used to infect mice there was no detectable change in the CTL response or viral clearance, indicating that more studies are needed to fully understand the function of polyomavirus miRNA mediated T-antigen regulation during natural infection [9].

MiRNAs expressed by the gammaherpesvirus EBV have also been shown to suppress the expression of viral antigens [10,11]. During latency EBV expresses the latent membrane proteins 1 and 2a (LMP1 and LMP2A); both proteins are crucial for EBV transformation of B cells and have been shown to elicit a CTL response [12,13]. LMP1 expression was found to be targeted by three EBV miRNAs (miR-BART16, miR-BART17-5p, and miR-BART1-5p) in nasopharyngeal carcinoma (NPC), an epithelial cancer associated with EBV infection [10]. However, in this study a link between miRNA targeting of LMP1 and the CTL response was not made, rather, it was shown that LMP1 downregulation was important in attenuating a pro-apoptotic affect caused by abundant LMP1 expression. Members of this same group later found that EBV miR-BART22, is responsible for targeting LMP2a [11]. Bioinformatic analysis of possible miR-BART22 targets identified high sequence complementarity (16 nucleotides) between mir-BART-22 and LMP-2a. Like LMP1 expression in NPC, low protein levels of LMP2A correlated well with high levels of miR-BART22 in patient samples, indicative of miRNA targeting within these tumors. Based on the immunogenicity of these viral proteins the authors believe that EBV miRNAs regulate their expression to inhibit immune surveillance; this hypothesis will need to be further validated by demonstrating miRNA targeting in appropriate *in vitro* and *in vivo* models.

Elimination of virally infected cells by NK cells involves NK cell receptor recognition of ligands expressed by target cells (Fig. 1b). Elegant genetic studies on the betaherpesvirus human cytomegalovirus (HCMV) demonstrated that HCMV miRNA miR-UL112-1 targets the major histocompatibility complex class l-related chain B (MICB), a stress induced ligand recognized by the NKG2D receptor expressed by NK cells and CD8+ T-cells [14]. Using a HCMV mutant virus containing a miR-UL112-1 deletion, the authors showed that mutant virus infected cells were more efficiently recognized and killed by NK cells *in vitro*, providing direct evidence that MICB repression mediated by this miRNA is important for NK cell evasion. Later studies discovered that gammaherpesvirus miRNAs, EBV miR-BART2-5p and Kaposi's sarcoma-associated herpesvirus (KSHV) miR-K12-7, also regulate MICB expression, and inhibition of MICB miRNA targeting with miRNA sponges in virally infected cells increased NK cell killing [15]. Interestingly, it was recently shown that miR-UL112-1 acts in combination with a host miRNA, miR-376a, to more efficiently regulate MICB expression, and that this synergistic action is important for inhibiting NK cell killing *in vitro*. These data strongly suggest that miRNA-dependent regulation of MICB is important for herpesviral persistence, which is further underscored by the fact that both HCMV and KSHV encode proteins that inhibit MICB surface expression [16, 17]. In addition, the observation that HCMV-, EBV-, and KSHV-encoded miRNAs target the MICB gene by completely different sequences raises a very interesting question about the co-evolution of viral miRNAs and their corresponding cellular targets.

A separate stress induced ligand (ULBP3), also recognized by the NKG2D receptor, is targeted by miR-J1-3p, a miRNA that is conserved between two different polyomaviruses,

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JCV and BKV [18]. Suppressing miR-J1-3p using a miRNA sponge leads to increased ULBP3 expression in JCV infected cells and subsequently enhanced NK cell killing. The identification of these multiple targets confirms that miRNAs expressed by both herpesvirus and polyomavirus represent important tools for suppressing immune surveillance by dampening NK cell target cell recognition.

In addition to inhibiting recognition by cell mediated effectors, experimental evidence suggests that herpesvirus miRNAs can inhibit cell mediated immunity by directly modulating cytokine expression (Fig. 1C). KSHV miRNAs miR-K12-3 and miR-K12-7, when ectopically expressed in human myelomonocytic and murine macrophage cell lines can increase secretion of host cytokines IL-6 and IL-10, which are highly expressed in KS lesions [19]. Bioinformatic analysis in combination with antagomir-based derepression assays demonstrated that miR-K12-3 and miR-K12-7 downregulates LIP, an isoform of C/EBPβ that functions as a negative transcriptional regulator of IL-6. Although these cytokines have broad functions in suppressing the activity of multiple immune cell types including T-cells, NK cells, and dendritic cells their impact during natural KSHV infection needs to be further tested [20-22].

Recently, it was shown that KSHV miR-K12-11 targets l-kappa-B kinase epsilon (IKKε), an important signaling molecule in the antiviral interferon response pathway [23]. To test the impact of IKKε targeting by miR-K12-11 without any confounding effects of other KSHV immune regulatory proteins, miR-K12-11 transduced lung cancer cells were infected with two RNA viruses, Sendai virus (SeV) and vesicular stomatitis virus (VSV), which strongly induce the interferon response. Results showed that upon infection, miR-K12-11 expressing cells had markedly attenuated interferon signaling and enhanced VSV titers.

An additional study demonstrated that ectopic expression of KSHV miR-K10a, in primary endothelial cells, markedly reduced production of pro-inflammatory cytokines, IL-8 and monocyte chemoattractant protein 1 (MCP-1), by targeting tumor necrosis factor (TNF)-like weak inducer of apoptosis receptor (TWEAKR), a target identified by a elegant tandem-array screen using ectopic expression in combination with miRNA inhibition in KSHV-infected lymphoma cells [24]. Curiously, these pro-inflammatory cytokines are induced by KSHV proteins (vFLIP and vGPCR) and may promote tumorigenesis [25, 26]. To integrate these paradoxical observations, the authors hypothesize that miR-K10a dependent regulation of IL-8 and MCP-1 may provide a mechanism that fine tunes cytokine expression to levels beneficial for the virus without eliciting a strong immune response. Hence, this regulatory loop is analogous to the fine tuning of LMP-1 expression by EBV miRNAs to balance proliferation and apoptotic activities.

The first *in vivo* phenotype of a viral miRNA knock-out was recently reported using a murine cytomegalovirus (MCMV) mutant [27]. In this study, mice infected with mutant viruses, lacking miR-M23-1 and miR-M23-2, showed a significant reduction in viral load in salivary glands. Furthermore, when both NK cells and CD4+ T-cells were depleted the ability of the mutant virus to replicate in salivary glands was partly restored, indicating that these viral miRNAs are important immune modulators. A number of potential immune targets were predicted by bioinformatics, and subsequent *in vitro* analysis confirmed that miR-M23-2 targets CXCL16, a host chemokine involved in T-cell activation and NK cell migration. Hence, the loss of miR-M23-2-dependent down-regulation of CXCL16 is believed to lead to rapid viral immune clearance.

Currently, the only other viral miRNA reported to modulate cytokine expression is EBV miR-BHRF1-3, which was found to target the T-cell attractant chemokine CXCL11 [28].

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However, the functional relevance of this regulation during EBV infection *in vivo* has yet to be reported.

Herpesvirus latent infection

To avoid recognition from host immune surveillance herpesviruses have co-evolved a mode of infection known as latency, during which the virus remains hidden by expressing only a small number of genes including viral miRNAs. One mechanism for controlling latent gene expression is to utilize viral miRNAs to suppress both viral and/or cellular genes that can trigger reactivation. Unlike viral proteins, which can elicit strong immune responses, viral miRNAs are indistinguishable from their cellular counterparts, nonimmunogenic, and therefore ideal tools for latent gene regulation. Mounting evidence, summarized in this section, shows that herpesvirus miRNAs play an important role in maintaining latent infection, thereby contributing to immune evasion resulting in life-long persistent infections, a hallmark of all herpesviruses.

The first direct evidence that herpesvirus miRNAs play a role in the maintenance of latency was provided by two independent studies in HCMV, which demonstrated that HCMV miR-UL112-1 can target IE72, a major immediate early viral transactivator that promotes lytic replication [29, 30]. Furthermore, Grey et al. showed that introduction of a miR-UL112-1 mimic into cells prior to HCMV infection significantly attenuates viral replication. Additionally, miR-UL112-1 was found to target UL114, a DNA glycosylase involved in viral replication and encoded antisense to miR-UL112-1 [31]. While Grey et al. showed that miR-UL112-1 did not mediate UL114 transcript cleavage; a separate study reported that ectopic expression of miR-UL112-1 can indeed target UL114 and reduce its expression in various cell model systems [32]. However, although mutant viruses lacking UL114 lead to a moderate decrease in viral replication, probably due to decreased DNA replication, the authors suggest that miR-UL112-1 targeting of IE72 is the main mechanism for reducing viral replication.

Unlike HCMV, where miRNA expression is mainly characterized during productive infection, miRNA expression for EBV and KSHV during latent and lytic replication has been well characterized using a combination of approaches (Northern blot, tiled arrays, and sequence analysis) [3, 33-41]. While subsets of EBV miRNAs have been found to be differentially expressed during latent and lytic lifecycles, KSHV miRNAs are predominantly expressed during latency [42, 43]. Early studies of EBV miRNAs found that latently expressed miR-BART2 is encoded antisense to the viral DNA polymerase BALF5, which is only expressed during lytic replication [3]. Later studies by a separate group provided evidence that miR-BART2 mediates regulation of BALF5 by antisense directed cleavage of the BALF5 transcript [44]. While ectopic miR-BART2 expression in EBV infected 293 cells modestly reduced virus production, inhibition of miR-BART2 with antagomirs did not induce lytic reactivation. Based on these observations the authors propose that instead of playing a central role in maintaining latency the targeting of BALF5 by miR-BART2 may help to safeguard against inadvertent lytic reactivation.

Most recently, it was shown that latently expressed EBV miR-BART6-5p can target the cellular miRNA processing enzyme Dicer, leading to increased expression of the two major IE transactivators RTA and Zta [45]. However, the direct functional consequence of Dicer targeting needs to be further validated by measuring viral replication after miR-BART6-5p inhibition.

In KSHV, the viral replication and transcription activator (RTA), a master regulator of lytic reactivation, has been shown to be regulated either directly or indirectly by multiple viral

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miRNAs. Two independent studies, using similar KSHV bacmid 36 derived recombinant viruses that lack 10 of 12 miRNA genes, reported elevated expression of lytic genes, including RTA, during *de novo* infection in separate cell lines [46, 47]. To determine the mechanism leading to increased lytic gene expression Lu et al. screened the individual KSHV miRNAs, using miRNA expression plasmids, for their ability to target a RTA luciferase construct and found that miR-K5 can repress RTA expression, albeit the 3′UTR of RTA lacks a canonical miR-K5 seed sequence. Additionally, Lu et al. carried out genome wide epigenetic analysis of the miRNA knock-out virus and found drastically reduced repressive marks on histones along with a global reduction of DNA methylation, suggesting that epigenetic modifications induced by viral miRNAs may contribute to the maintenance of latency. Searching for a mechanism to explain these modifications Lu et al. found that miR-K12-5p targets retinoblastoma (Rb)-like protein 2 (Rbl2), a negative regulator of DNA methyltransferases, thereby leading to an increase in DNA methylation. This is the first reported evidence that viral miRNAs can directly impact the epigenetic status of herpesvirus genomes during latency.

In the second study, Lei et al. also found an increase in RTA mRNA expression in cells infected with a very similar KSHV miRNA knock-out virus, but they did not identify direct targeting of the RTA 3′UTR by miR-K12-5p or any other KSHV miRNA [46]. Instead, Lei et al showed that miR-K1 targets the host gene lkBα, an inhibitor of NFκB, leading to activation of NFκB, which is known to inhibit lytic reactivation and, in the case of PELs, contributes to cell survival [48].

In addition to targeting lkBα, two independent studies reported that lytic reactivation can be regulated by KSHV miR-K12-11 targeting of IKKε and nuclear factor I/B (NFIB) [23, 49]. As mentioned before miR-K12-11 targeting of IKKε leads to attenuation of the interferon response [23]. This same study found that inhibiting miR-K12-11, with an anti-miR-K12-11 sponge, leads to an increase in lytic gene expression (RTA and ORF65) in bacmid infected A549 cells. The authors also showed that IKKε overexpression enhanced lytic replication when TPA, a chemical agent that triggers lytic reactivation, was used.

Using lentiviruses to express individual KSHV miRNAs in BC3 cells, Lu et al found that miR-K1, K3, K7, and K11 were all capable of moderately decreasing RTA mRNA levels [49]. MiR-K3 showed the greatest effect on RTA, and further investigation found that it directly targets NFIB, a cellular transcription factor that had previously been shown to reactivate KSHV when overexpressed [50]. Further analysis identified that the promoter of RTA contains a putative NFIB binding site and that ectopic NFIB expression could activate an RTA promoter construct. Additionally, shRNA knockdown of NFIB resulted in decreased RTA expression. This study provides indirect evidence that miR-K3 maintains latency by targeting NFIB, but further experiments using anti-miR-K3 antagomirs or a miR-K3 knockout virus are needed to prove this mechanism.

In addition to indirectly regulating RTA expression two separate studies have demonstrated that miR-K12-9* and miR-K12-7-5p can directly target and regulate RTA expression through seed match binding [51, 52]. Using luciferase constructs, containing the 3′UTR of RTA, and KSHV miRNA mimics, Bellare et al. identified that miR-K9* directly targets RTA through a canonical 6mer seed match site. Furthermore, when miR-K9* function in latently infected cells was inhibited with specific antagomirs a moderate increase in lytic reactivation, was observed. In a separate study by Lin et al., which used KSHV miRNA expression plasmids instead of miRNA mimics, miR-K9* and miR-K12-7-5p were also found to target RTA [52]. Lin et al. further show that miR-K12-7-5p targeting of RTA is mediated through a 7mer seed match site and that ectopic expression of miR-K12-7-5p in latently infected cell lines reduces the amount of progeny virus produced. In summary, these

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studies lend further credence that KSHV miRNAs directly regulate RTA expression during latency. However, while some studies hypothesize that KSHV miRNAs function as major regulators of latency, Bellare et al. suggests that these miRNAs may provide a mechanism for fine tuning and/or sensitizing latently infected cells to stimuli that trigger lytic replication.

We note that most of the KSHV miRNAs found to target RTA appear to differ between studies, with the exception of miR-K9* and miR-K7 [51, 52]. One possible reason for these differing results is that the miRNA targeting screens were not done using the same parameters, miRNA expression systems, and/or identical cellular backgrounds making it difficult to directly compare these studies. Nonetheless, in summary the evidence indicates that KSHV miRNA regulation of lytic reactivation is a complex process and that KSHV miRNAs do indeed play a synergistic role in the maintenance of latency.

The human alphaherpesvirus HSV-1 and HSV-2, are closely related neurotropic viruses which establish latency in sensory neurons and have recently been found to express miRNAs that contribute to the regulation of latency. Interestingly, the majority of genes in both viruses share similar genomic positions, including the viral miRNAs, many of which are encoded antisense to protein coding genes. Early studies of HSV-1 and HSV-2 found that only one viral product, the non-coding latency-associated transcript (LAT), is abundantly expressed during latency and that its expression is important for establishing and maintaining latency in HSV-1, as well as affecting lytic reactivation in HSV-1 and HSV-2 [53-55]. However, the mechanisms governing LAT function in these processes were not fully understood. Insight into LATs function was recently uncovered by the discovery that LAT encodes a number of viral miRNAs, some of which are located antisense to genes important for lytic reactivation [56-58]. First, Umbach et al. reported that HSV-1 ICP0, an important lytic transcriptional regulator, is targeted by the antisense encoded miR-H2, but curiously it does not mediate antisense cleavage of the ICP0 transcript [58]. In addition to LAT encoded miRNAs, Umbach et al. identified a separately expressed latent miRNA, miR-H6, which targets ICP4, a major viral transactivator. Although HSV-1 and HSV-2 miRNAs are not homologous in sequence, their relative genomic positions are very similar. The HSV-2 miR-lll was found to be positionally conserved to HSV-1 miR-H2, and like miR-H2 it was also found to regulate ICP0 expression [57]. Interestingly, although HSV-2 miR-H6 is positionally conserved to the HSV-1 miR-H6, it does not target or regulate the expression of ICP4 [59, 60]. Lastly, the HSV-2 LAT miRNAs, miR-l and miR-ll, were found in similar locations to the HSV-1 miRNAs, miR-H3 and miR-H4, all being encoded antisense to ICP34.5, a lytic neurovirulence factor [56, 57]. Published reports have shown that HSV-2 miR-1 and miR-ll can target and regulate ICP34.5 while unpublished reports by Tang et al have confirmed targeting by HSV-1 miR-H3 and miR-H4 [56, 57, 60]. The antisense locations and positional conservation of HSV miRNAs indicate that these viruses have co-evolved miRNAs to maintain latency in neuronal cells; however, more studies using miRNA knockout viruses and *in vivo* models will be required to further delineate the role of specific alphaherpesvirus miRNAs in latency control, reactivation and viral persistence of the infected host. With respect to immune evasion alphaherpesviruses have evolved the perfect “stealth mechanism” in that they do not express any latency-associated proteins that could trigger an immune response but instead seem to establish and maintain latency exclusively by expressing non-immunogenic miRNAs.

The future of viral miRNA research and new emerging questions

Since the discovery of EBV miRNAs in 2004, our advancement in understanding viral miRNA function has been considerably rapid. With hundreds of targets now reported, and many more unreported, the impact that these small RNAs have on virus/host interactions is

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unquestionable. Recently developed ribonomics-based assays, that combine in vivo UV crosslinking with RISC-specific immunoprecipitation, provide powerful experimental tools to probe for miRNA/mRNA interactions under physiological conditions. Analyzing RISC complexes from virus infected cells will allow to catalogue miRNA/target gene interaction within specific cell types. HITS-CLIP (High throughput sequencing UV cross linking Immunoprecipitation) uses 254 nm UV to directly cross-link RNA protein complexes prior to immunoprecipitation [61]. In a second method, PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation), cells are first labeled with photoreactive nucleoside analogs that are incorporated into nascent mRNAs in living cells [62]. An advantage of PAR-CLIP over conventional HITS-CLIP is that upon cDNA cloning of the recovered RNA, the cross-linking induces base transition, which creates a RISC footprint within the recovered mRNA tag [62].

While these new techniques will allow to determine detailed miRNA targetomes in virally infected cells, determining the importance of each target on for virus biology remains a large task [63, 64]. Furthermore, while the identification of viral miRNA targets is an important step, the use of appropriate model systems (i.e recombinant viruses, *in vivo* infection models, NK killing assays) are still needed to decipher their functional relevance, especially with respect to immune evasion and other aspects of host/viral interactions. In turn, many new questions (Listed in Table 2) have been raised from published viral miRNA studies. So as new discoveries in viral miRNA function are made new questions emerge, making the complexities governing viral-host interactions, at the least, a little more transparent.

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Highlights

>We review currently known viral miRNA targets. >We examine viral miRNA function in regulating immune evasion. >The future of viral miRNA research is described.

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Emerging Questions

- Is polyoma virus miRNA regulation of T-antigen important for immune evasion *in vivo*?
- Does regulation of viral antigens and cytokines by EBV and KSHV miRNAs inhibit immune evasion *in vivo*?
- Are HCMV miRNAs essential for maintaining latent infection?
- Most studies show modest inhibition of RTA by KSHV miRNAs, so are miRNAs essential for regulation of latency or do the act more as a secondary mechanism?
- Because multiple viral miRNAs can regulate the immune response how do they work synergistically?
- How do viral miRNAs work synergistically with viral proteins to regulate the host immune response?

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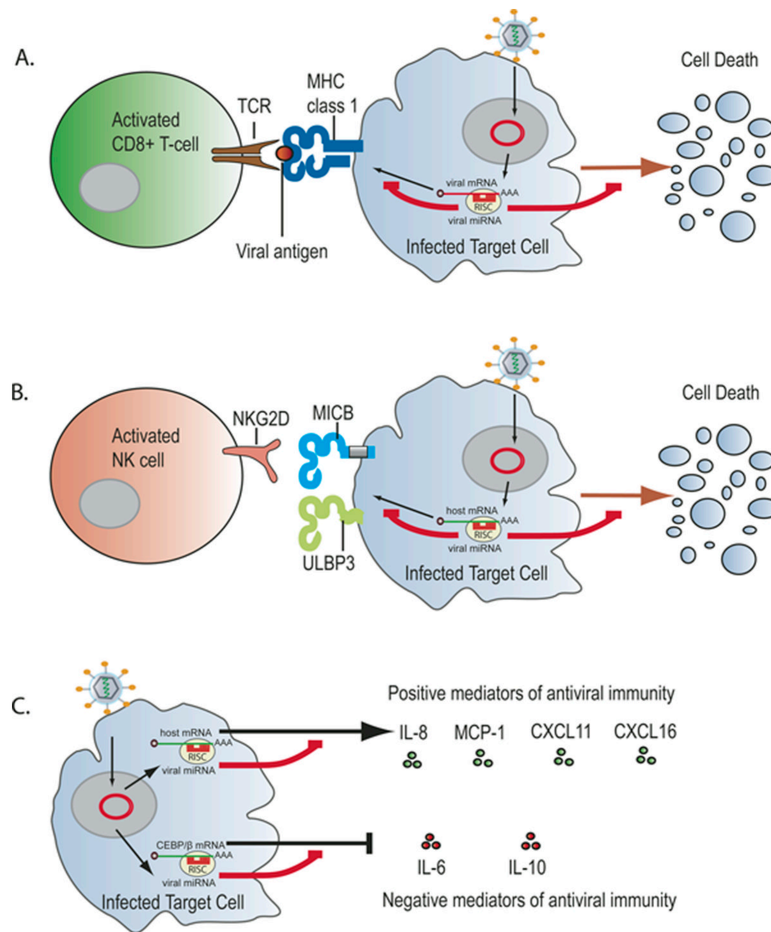

Fig. 1.

Viral miRNAs can inhibit cell mediated immunity by modulating the recognition and activity of effector cells. (a) A CTL response to virally infected cells is initiated when the T-cell receptor (TCR) of CD8+T cells recognizes viral antigenic peptides presented by class I MHC molecules on the surface of infected cells. Viral miRNAs can block this initiating step by inhibiting the expression of viral peptides before they are presented. (b) NK cell killing of virally infected cells is initiated when an activation receptor (i.e. NKG2D) engages ligands (i.e. MICB or ULBP3) expressed by virally infected cells. Viral miRNAs can block this step by inhibiting the expression of these ligands. (c) Viral miRNAs can regulate the activities of leukocytes involved in the immune response by inhibiting the production of cytokines/chemokines which promote anti-viral immunity or by inducing production of cytokines that may inhibit anti-viral immunity.

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Table 1

Immunomodulatory viral miRNAs

DNA Virus Family	Virus	Target	miRNA	Function	References
				Latency	
Alphaherpesvirus	HSV-1	Viral ICP0	miR-H2-3p	Immediate-early transactivator	[58]
		Viral ICP4	miR-H6	Immediate-early transactivator	[58]
		ICP34.5	miR-H3 and -H4	Lytic neurovirulence factor	[60]**
	HSV-2	Viral ICP0	miR-III	Immediate-early transactivator	[57]
		ICP34.5	miR-I and -II	Lytic neurovirulence factor	[56,57]
				Cell-mediated Immunity	
Betaherpesvirus	HCMV	Host MICB	miR-UL112-1	NK cell ligand	[14]
				Latency	
		Viral IE72	miR-UL112-1	Immediate-early transactivator	[29,30]
				Cell-mediated Immunity	
	MCMV	Host CXCL16	miR-M23-2	Chemokine	[27]
				Cell-mediated Immunity	
Gammaherpesvirus	EBV	Host MICB	miR-BART2-5p	NKG2D ligand	[15]
		Host CXCL11	miR-BHRF1-3	Chemokine, T-cell attractant	[28]
		Viral LMP1	miR-BART1-5p	Transforming factor	[10]
			miR-BART16		
			miR-BART17-5p		
		Viral LMP2a	miR-BART22	Transforming factor	[11]
				Latency	
		Viral BALF5	miR-BART2	DNA polymerase	[44]
		Host Dicer	miR-BART6-5p	miRNA processing enzyme	[45]
				Cell-mediated Immunity	
	KSHV	Host MICB	miR-K12-7	NKG2D ligand	[15]
		Host C/EBPβ (LIP)	miR-K12-3	Inhibits IL6 and IL10 expression	[19]
			miR-K12-7		
		Host TWEAKR	miR-K10a	Tumor necrosis factor receptor	[24]

DNA Virus Family	Virus	Target	miRNA	Function	References
				Cell-mediated Immunity/Latency	
		Host IKKε	miR-K12-11	Interferon signaling molecule	[23]
				Latency	
		Viral RTA	miR-K12-5	Master lytic switch	[47]
			miR-K9*		[51]
			miR-K12-7-5p		[52]
		Host Rbl2	miR-K12-4-5p	Transcriptional repressor	[47]
		Host IκBα	miR-K1	Inhibits NF-κβ	[48]
		Host NFIB	miR-K3	Transcriptional activator	[49]
				Cell-mediated Immunity	
Polyomavirus	SV40	Viral Large TAg	miR-S1	Transforming factor	[7]
	JCV	Viral Large Tag	miR-J1	Transforming factor	[8]
		Host ULBP3	miR-J1-3p	NKG2D ligand	[18]
	BKV	Viral Large Tag	miR-B1	Transforming factor	[8]
		Host ULBP3	miR-B1-3p*	NKG2D ligand	[18]

* miR-Bl-3p and miR-Jl-3p are identical and designated miR-Jl-3p in [18]

** miR-H3 and miR-H4 targeting of ICP34.5 is reported as an unpublished experiment in [60]

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