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MicroRNAs as Mediators of Viral Immune Evasion

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Abstract

Cellular microRNAs play a key role in the post-transcriptional regulation of almost every cellular gene regulatory pathway and it therefore is not surprising that viruses have found ways to subvert this process. Several viruses encode microRNAs that directly downregulate the expression of innate immune factors, including proteins involved in promoting apoptosis and recruiting immune effector cells. Viruses have also evolved the ability to downregulate or upregulate specific cellular miRNAs in order to enhance their replication. This perspective provides an overview of our current knowledge of the complex interplay of viruses with the microRNA machinery of cells.

First identified as regulators of larval development in nematodes^{1,2}, it is now known that microRNAs (miRNAs) play key roles in the regulation of almost every important cellular process in all multicellular eukaryotes³. Human cells encode over 1000 miRNA species, and these have been implicated in cellular differentiation, innate immunity, apoptosis and oncogenic transformation, as well as many other cell fate decisions³. Almost all cellular miRNAs are first transcribed as capped, polyadenylated primary miRNA (pri-miRNA) transcripts that can encompass one or a cluster of ~22-nt miRNAs⁴. These miRNAs occupy the upper part of an ~33-bp imperfect stem that is crowned by a large (~10 nt) unstructured loop and flanked by single stranded RNA. This ~80-nt RNA structure is recognized by the nuclear microprocessor, consisting of the RNase III enzyme Drosha and the dsRNA binding protein DGCR8, which cleaves the stem ~22 bp from the stem:loop junction to generate the ~60-nt long pre-miRNA hairpin intermediate^{5,6}. Pre-miRNAs are bound by the nuclear export factor Exportin 5 (Exp5), which transports them into the cytoplasm⁷. Here, the pre-miRNA is bound by a second RNase III enzyme, called Dicer, which cleaves the pre-miRNA at the stem-loop junction⁸. The resultant miRNA duplex intermediate then interacts with one of the four mammalian Argonaute (Ago) proteins, which incorporates one RNA strand to form the RNA induced silencing complex (RISC), while the second RNA strand is degraded^{9–11}. Discrimination between the strands is thought to be regulated by the stability of the ends of the duplex, with the strand whose 5' end is less tightly base paired being favored for incorporation into RISC^{12,13}. However, this discrimination is rarely complete, so that both the major, miRNA strand and minor, star strand of the duplex intermediate are often detectable in RISC.

Once loaded into RISC, the miRNA serves as a guide RNA to target RISC to mRNAs bearing sequence complementarity¹⁴. If this complementarity is essentially complete, RISC binding can induce endonucleolytic cleavage and marked mRNA destabilization. However, if the target mRNA is only partially complementary, RISC binding induces translational inhibition, sometimes followed by deadenylation and mRNA destabilization^{15,16}. For mRNA targets that are only partially complementary, the key is the complementarity

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between the so-called miRNA seed region—positions 2 to 8 from the 5' end—and the mRNA target^{17,18}. As a result, miRNAs that share a common seed region have very similar mRNA targets, even if the rest of the miRNA is different in sequence.

Because seven nucleotides of sequence complementarity are sufficient, at least in principle, to allow inhibition of an mRNA by a given miRNA, it is easy to calculate that each miRNA has the potential to target large numbers of cellular mRNAs, even allowing for the fact that many complementary target sites on mRNAs may be occluded by RNA secondary structure or by bound proteins. Initial efforts to identify the “targetome” of a given miRNA by relying on bioinformatics gave long lists of potential mRNA targets and did not prove to be particularly reliable, though these methods continue to improve. A more sophisticated approach uses assays that measure global gene expression in the presence or absence of a given miRNA, for example using mRNA microarrays, to identify mRNAs whose expression is specifically inhibited. If these mRNAs also contain a computationally predicted target site for that miRNA, then this provides *a priori* evidence in favor of the hypothesis that this is indeed a target¹⁹. More recently, methods have been developed to directly recover and deep sequence RISC binding sites on mRNAs by using mRNA:protein crosslinking followed by immunoprecipitation with a RISC component, such as Ago2^{20–23}. These “CLIP” approaches have proven to be a very powerful tool for the global recovery of RISC target sites, with the major remaining difficulty being the accurate identification of which miRNA is actually responsible for mediating RISC recruitment.

Discovery and expression pattern of viral microRNAs

Given the small size of miRNAs, their lack of antigenicity and their ability to post-transcriptionally inhibit the expression of specific mRNA species, they would seem to represent ideal tools for use by viruses to inhibit the expression of proteins that might act as inhibitors of viral replication, including especially mediators of antiviral innate immunity. The first viral miRNAs were discovered in 2003 in human B cells latently infected with the γ -herpesvirus Epstein-Barr virus (EBV)²⁴, and subsequent papers identifying miRNAs in several other human and animal herpesviruses rapidly followed²⁵ (Table 1). In addition, several human and animal polyomaviruses have been found to each encode a single pre-miRNA, while human adenoviruses have been proposed to encode two pre-miRNAs²⁵. On the other hand, no miRNAs have been identified in human papillomaviruses or poxviruses, which are also DNA viruses^{26,27}, and no human RNA virus, including human immunodeficiency virus (HIV-1), hepatitis C virus (HCV) and influenza B virus, has so far been shown to express any viral miRNAs^{28–30}. This may relate to the fact that excision of a pre-miRNA stem-loop from an RNA virus genome would result in the cleavage of that genome, which might inhibit virus replication. Indeed, insertion of pri-miRNA stem-loops into the genome of HIV-1-based vectors does reduce vector titre, especially if multiple stem-loops are inserted (B.R. Cullen, unpublished observations).

Because the large majority of known viral miRNAs are encoded by herpesviruses, I will focus this review on this viral genus and in particular on EBV, Kaposi's sarcoma-associated herpesvirus (KSHV) and human cytomegalovirus (HCMV), with only occasional reference to other human or animal virus species. In the case of EBV, we now know that this virus encodes 25 pre-miRNAs located in two distinct clusters, i.e., the 3 pre-miRNA BHRF1 cluster and the 22 pre-miRNA BART cluster^{24,31,32} (Fig. 1). Expression of these clusters is differential, depending on the latency stage of the virus. In primary B cells, EBV infection results in establishment of latency III, marked by high level expression of the three miR-BHRF1 miRNAs and moderate expression of the miR-BART miRNAs. In contrast, cells recovered from EBV-induced nasopharyngeal carcinomas (NPCs), as well as in B-cell primary effusion lymphomas (PELs), where EBV is in the latency II stage, are characterized

by high levels of EBV miR-BART miRNA expression and a total lack of miR-BHRF1 miRNA expression³¹. Mutational analysis of the EBV genome has demonstrated that the miR-BHRF1 miRNAs, which are expressed at high levels in the transformed lymphoblastoid cell lines (LCLs) that result from primary B-cell infection, are important but not essential for LCL outgrowth and that LCLs lacking these viral miRNAs grow more slowly^{33,34}. In contrast, the miR-BART miRNAs do not affect B-cell transformation by EBV in culture. As there is currently no *in vitro* model to study transformation of epithelial cells by EBV, it remains unclear whether the miR-BART miRNAs play a role in the initiation or maintenance of epithelial cell-derived tumors, such as NPCs.

The oncogenic human γ -herpesvirus KSHV encodes a single cluster of 12 pre-miRNAs that are expressed at high levels in latently KSHV-infected B cells^{28,35} (Fig. 1). Ten of the 12 viral pre-miRNAs are located in an intron, while the other two, miR-K10 and miR-K12, are unusually located in the viral K12 open reading frame (ORF) and in the K12 mRNA 3' untranslated region (3'UTR), respectively. Processing of these two miRNAs appears to be inefficient, thus allowing expression of the viral K12 protein³⁶. Whether any of these miRNAs are important for B-cell transformation by KSHV remains unclear, as there is currently no *in vitro* model that accurately measures KSHV-mediated transformation. Nevertheless, several mRNA targets for the KSHV miRNAs have been identified and these data strongly suggest that this is indeed likely to be the case. Of interest, while all 12 KSHV pre-miRNAs are expressed during latency, this miRNA cluster also contains a lytic viral promoter, located immediately 5' to the viral K12 ORF, that strongly induces expression of not only the K12 protein but also the viral miR-K10 and miR-K12 miRNAs during lytic re-activation³⁶. However, it remains unclear what roles these two miRNAs play during productive KSHV replication.

Finally, HCMV is quite different from both EBV and KSHV in that the 12 known HCMV pre-miRNAs are scattered over the entire viral genome and, hence, transcribed by several different promoter elements^{28,37} (Fig. 1). Curiously, no report of HCMV miRNA expression during viral latency has so far appeared. However, all 12 HCMV pre-miRNAs are expressed at substantial levels during HCMV lytic replication. As there is no animal model available to analyze HCMV replication and pathogenicity *in vivo*, it is unclear whether these miRNAs indeed play a critical role in the viral life cycle. However, analysis of mutants of the related mouse CMV (MCMV) have shown that viral miRNAs promote MCMV replication and persistence in salivary glands, a key organ in terms of viral transmission³⁸.

Virally-encoded microRNAs as mediators of immune evasion

As noted above, obvious potential targets for virally encoded miRNAs include factors that form part of the innate antiviral immune response, including factors that promote apoptosis or cell cycle arrest, or factors that help to recruit immune effector cells to virus infected cells. A number of these have been proposed, but instead of providing a comprehensive list of these proposed targets, I will here focus on mRNA targets for miRNAs encoded by EBV, KSHV or HCMV that are supported by several different lines of evidence and/or by data from more than one laboratory and that also illustrate the kinds of viral miRNA targets that have the potential to enhance viral replication or pathogenicity *in vivo* (Table 2).

One of the first cellular mRNA targets reported for an EBV miRNA was the p53 up-regulated mediator of apoptosis (PUMA), which was proposed to be targeted by EBV miR-BART5³⁹. This in turn was proposed to protect EBV-infected NPC cells from apoptosis induced by DNA damaging reagents. EBV infection is, in fact, known to induce a cellular DNA damage response and attenuation of this response has been shown to substantially increase the efficiency of transformation of B cells by EBV⁴⁰. A second target for the EBV

miRNA miR-BART19 in the 3' UTR of PUMA mRNAs has recently been identified by CLIP²¹, further supporting the importance of this mRNA target.

Another pro-apoptotic cellular gene product that has been shown to be targeted by EBV miRNAs is BCL2L1/BIM, which appears to be targeted by both miR-BART4 and miR-BART15^{20,41}. This activity was proposed to correlate with the observed inhibition of apoptosis by miR-BART miRNAs in the human gastric carcinoma cell line AGS.

A third, well supported target for the EBV miR-BART miRNA is the major histocompatibility complex class I-related chain B (MICB), a stress-induced ligand of the natural killer (NK) cell activating receptor NKG2D that plays a key role in promoting NK-mediated killing of virus infected cells⁴². MICB was actually first identified as a target for the HCMV-encoded miRNA miR-UL112⁴³, but more recent data suggest the MICB is also targeted by KSHV miR-K7 and by EBV BART miRNAs⁴². As expected, miRNA-mediated downregulation of MICB has been shown to protect cells from NK cell killing *in vitro*⁴². CLIP analysis has more recently documented binding sites for miR-BART1, miR-BART3, miR-BART5 and miR-BART9 in the MICB 3'UTR^{20,21}. The fact that MICB is not only targeted by miRNAs encoded by several herpesvirus species but also by several distinct EBV miRNAs strongly suggests that attenuation of MICB function is highly advantageous to viruses *in vivo*. Indeed, in the case of HCMV, MICB expression is also inhibited by a virally encoded protein, UL16, which acts synergistically with miR-UL112⁴³.

An interesting final point is that EBV miRNAs not only target cellular mRNAs but also viral mRNAs. For example, the EBV miR-BART2 miRNA lies directly antisense to the mRNA encoding the viral DNA polymerase BALF5²⁴. This antisense location has been observed previously for several viral miRNAs, including the single pre-miRNAs encoded by all members of the polyomavirus family, which are encoded antisense to the viral large T antigen (TAG)^{25,44}, and the miR-H2 miRNA encoded by Herpes Simplex Virus Type 1, which lies antisense to the ICP0 protein⁴⁵. In the case of the polyomavirus SV40, it was proposed that expression of this miRNA late in the viral replication cycle inhibited T antigen expression and thereby reduced killing of SV40 infected cells by cytotoxic T lymphocytes (CTLs) *in vivo*⁴⁴. In contrast, inhibition of ICP0 expression by miR-H2 and inhibition of BALF5 expression by miR-BART2 has been proposed to stabilize viral latency^{45,46}, though neither of these proposals has so far been experimentally validated.

The miRNAs encoded by KSHV have been perhaps the most intensely studied viral miRNAs, and a number of mRNA targets for KSHV miRNAs have been defined (Table 2). In addition to MICB, as noted above, these include:

1. The Tumor Necrosis Factor-Like Weak Inducer of Apoptosis receptor protein TWEAKR, which is targeted by miR-K10⁴⁷. This targeting, which reduces the sensitivity of expressing cells to pro-apoptotic stimuli, has been further validated by CLIP²³.
2. IL-1 receptor associated kinase 1 (IRAK1) and MYD88, which are components of the human Toll-like receptor (TLR)/IL-1 receptor signaling cascade, are downregulated by KSHV miR-K9 and miR-K5, respectively⁴⁸. The expression of these two miRNAs in endothelial cells was shown to inhibit the production of the pro-inflammatory cytokines IL-6 and IL-8 after IL-1 α treatment, and this activity may inhibit the immune clearance of KSHV-infected cells *in vivo*⁴⁸.
3. The KSHV miRNA miR-K10 has also been proposed to target the transforming growth factor- β type II receptor (TGFR β), which was shown to inhibit TGF- β induced apoptosis in culture⁴⁹. This may again promote the survival of KSHV-infected endothelial cells in culture.

4. Another protein involved in apoptosis that is targeted by KSHV miRNAs is the key effector protein Caspase 3, whose expression is attenuated by miR-K1, miR-K3 and miR-K4⁵⁰. When the activity of these viral miRNAs was inhibited, both Caspase 3 and apoptosis levels increased significantly.
5. A final cellular mRNA target for KSHV miRNAs that merits discussion is the cyclin-dependent protein kinase inhibitor p21⁵¹. As noted above, infection of cells by herpesviruses induces a DNA damage response that activates not only pro-apoptotic proteins but can also induce cell-cycle arrest via the p53-mediated induction of p21 expression⁴⁰. The KSHV miRNA miR-K1 binds two targets in the 3' UTR of the p21 mRNA and this can prevent cell-cycle arrest by factors that activate p53⁵¹. Therefore, miR-K1 may play an important role in KSHV-mediated oncogenesis *in vivo*.

As noted above, HCMV strongly inhibits the expression of MICB using both protein and miRNA-mediated mechanisms⁴³. Other cellular factors targeted by HCMV miRNAs include ERAP1, an aminopeptidase that is important for appropriate presentation of antigenic viral peptides to CTLs⁵². As a result, the relevant HCMV miRNA, termed miR-US4-1, was able to reduce the susceptibility of HCMV-infected cells to CTL-mediated killing. Finally, the HCMV miRNA miR-UL148D has been shown to target mRNAs encoding the important chemokine RANTES, which attracts immune cells to sites of inflammation and tissue damage⁵³. Blocking RANTES expression obviously could prevent the recruitment of immune effector cells to infected cells *in vivo*.

Viral subversion of cellular microRNA function

Cellular miRNAs are highly conserved during evolution—for example, the let-7 miRNA family is conserved from nematodes to humans⁵⁴—and many mRNA targets for cellular miRNAs are also conserved³. In this way, cells are able to use miRNAs to regulate logically coherent sets of mRNA species despite the fact that miRNA seed targets are only ~7 nt in length and hence predicted to occur by chance every 4⁷=16,384 nt. In contrast, viral miRNAs presumably promote viral replication at the expense of the host and viral miRNA targets are therefore, if anything, subject to negative selection. As a result, one might have predicted that viral miRNA would have evolved to be perfectly complementary to perhaps only a single deleterious cellular mRNA that they would then strongly inhibit by endonucleolytic cleavage. In fact, however, viral miRNAs do not generally show perfect complementarity to any mRNAs, the main exception being the antisense viral mRNAs mentioned previously. One can therefore hypothesize that viral miRNAs have likely evolved seed sequences that target non-conserved 3'UTR sequences present on a small number of functionally relevant cellular mRNAs, as well as a larger number of other cellular mRNAs whose downregulation at least does not significantly inhibit viral replication.

While *de novo* evolution of a useful viral miRNA, especially in the face of possible host cell adversarial evolution, is therefore challenging, viruses do have the option of simply expressing a miRNA that mimics a cellular miRNA, if that cellular miRNA has an activity that is potentially helpful to the virus. Analysis of the seed sequences of known viral and cellular miRNAs has in fact revealed that the former have a tendency to share the seed sequences of cellular miRNAs that is substantially higher than predicted by chance alone²⁵. While shared seed sequences obviously predict a shared miRNA targetome, this has only been demonstrated for a small number of viral miRNAs⁵⁵. The most intensively investigated example of viral mimics of a cellular miRNA arises in the case of cellular miR-155, a miRNA that is transiently induced upon activation of both lymphoid and myeloid cells and that has been shown to be important during antigenic stimulation of T- and B-lymphocytes^{56,57}. Moreover, there is extensive evidence linking the constitutive expression

of miR-155 to oncogenic transformation of, especially, lymphoid cells^{58–60}. The first viral miR-155 mimic to be described was KSHV miR-K11, which was shown by two groups to both share the full seed sequence of miR-155 (Fig. 2) and to also target a very similar set of cellular mRNAs^{23,61,62}. Indeed, miR-K11 can even substitute for miR-155 in promoting hematopoiesis *in vivo*⁶³! More recently, a second miR-155 analog, miR-M4, was identified in the avian α -herpesvirus Marek's Disease Virus Type 1 (MDV1)⁶⁴. MDV1 causes T-cell lymphomas in chickens and miR-M4 is highly expressed in these tumors. Strikingly, an MDV1 mutant lacking miR-M4 was found to replicate normally in culture but failed to induce any tumors *in vivo*, while a "revertant" MDV1 mutant in which cellular miR-155 was substituted in place of miR-M4 regained oncogenic potential. This striking result therefore suggests that the miR-155 analog encoded by KSHV is also likely to play a critical role in the transformation of infected human B cells *in vivo*.

While a virus can certainly evolve a viral miRNA that mimics the function of a cellular miRNA, it is also possible for a virus to simply induce the expression of a cellular miRNA in infected cells. While viral infection has been shown to affect cellular mRNA expression in a number of experimental systems, and this has been proposed to potentially facilitate viral replication⁶⁵, this is perhaps most clearly demonstrated in the case of EBV. EBV strongly induces several cellular miRNAs after infection of primary B cells in culture, and this infection, as noted above, leads to the outgrowth of transformed LCLs^{66,67}. One cellular miRNA that is particularly strongly induced is miR-155, which increases by ~200-fold within days of EBV infection^{66–68}. This is probably due to activation of cellular NF- κ B by the EBV LMP1 oncoprotein, which in turn results in increased pri-miR-155 transcription⁶⁹. EBV does not encode a miR-155 analog, so this finding suggested the possibility that EBV had evolved an alternative mechanism to inhibit the cellular mRNAs targeted by miR-155 and promote B-cell growth and transformation. Indeed, inhibition of miR-155 function in both EBV-infected LCLs as well as EBV-positive diffuse large B-cell lymphoma cells was found to induce cell cycle arrest and strongly promote apoptosis⁷⁰. These data therefore reveal that miR-155 plays a critical role in the transformation of lymphoid cells by several human and non-human oncogenic herpesviruses and suggest that manipulation of the pattern of miRNA expression by viruses may play a critical role in viral replication and pathogenesis in several different disease contexts.

Cellular microRNAs as regulators of viral replication

As noted above in the case of EBV, viruses can certainly use cellular miRNAs to their advantage and indeed, in the case of Hepatitis C virus (HCV), it is known that the liver-specific cellular miRNA miR-122 is essential for productive virus replication⁷¹. Interestingly, the ability of miR-122 to enhance HCV replication results from a non-conventional activity of miR-122, which binds two sites near the HCV genomic RNA 5' end and appears to prevent recognition of this 5' end, which bears a terminal triphosphate, by cellular innate immune factors⁷². One can also envision that cellular miRNAs could act to significantly inhibit viral replication. Cellular miRNAs are expressed in a highly tissue specific manner and the pattern of miRNA expression can therefore vary dramatically across not only different tissue types but also, for example, depending on whether a cell is growth arrested or actively dividing³. Each cell expresses dozens of miRNAs, each of which has the potential to bind to and inhibit viral mRNA species. The question of how viruses are able to replicate in the face of this potential innate cellular restriction mechanism is therefore of considerable interest.

One possible explanation is that viruses could simply block cellular miRNA function, either selectively or globally. Two examples are now known of viruses that use RNA "decoys" to bind and destabilize a specific cellular miRNA^{73,74}. Interestingly the same miRNA, miR-27,

is inhibited by both the simian Herpesvirus saimiri and MCMV. In the latter case, restoration of miR-27 expression was found to inhibit MCMV replication⁷⁴, although this seemed to be due to targeting of cellular, not viral, mRNA species.

Two virus families have also been found to globally block miRNA expression or function. The best documented case is the global degradation of miRNAs induced by members of the poxvirus family, which encode a protein that induces 3' polyadenylation of miRNAs, which then induces degradation by the host cell²⁶. In the case of adenovirus, it has been demonstrated that the viral non-coding VA1 RNA competitively inhibits both Exp5-mediated pre-miRNA export and Dicer-mediated pre-miRNA processing, thus strongly inhibiting miRNA biogenesis^{75,76}.

Despite these interesting examples of virus-mediated inhibition of cellular miRNA function, it is clear that the majority of virus-infected cells retain the ability to use miRNAs to regulate mRNA function. Indeed, several groups have used this fact to control virus tropism by engineering perfect target sites for particular tissue-specific cellular miRNAs into the viral genome, thus preventing the virus from being able to replicate effectively in that tissue^{77–79}. This approach shows considerable promise for the development of attenuated virus vaccines and in the design of oncolytic viruses that can grow in, and kill, cancer cells but spare adjoining normal cells. We are then left with the question of how viruses normally evade inhibition by cellular miRNAs, given that a cursory bioinformatic analysis reveals that viruses often contain large numbers of potential seed-target sites for cellular miRNAs, including miRNAs expressed in their normal target tissues⁸⁰. One hypothesis that can explain why this is not, apparently, a general problem is that viral RNA transcripts are highly structured and that potential miRNA target sites are therefore occluded. Indeed, several RNA viruses, including HIV-1, appear to be highly structured, while others, such as poliovirus, appear to be fairly unstructured^{81,82}. As poliovirus RNAs are uncapped, and the lack of a cap structure has been reported to prevent translational inhibition by miRNAs^{83,84}, it is interesting to speculate that there might be a correlation between a high level of viral RNA secondary structure and predicted susceptibility to inhibition by RISC. Regardless, it will certainly be interesting to determine whether cellular miRNAs are indeed able to bind viral mRNAs effectively and to what degree this binding affects viral replication either positively, as seen for HCV⁷¹, or negatively, as one would predict from the repressive activity that is normally characteristic of miRNAs^{80,85}. Clearly, our understanding of how viruses interact with the cellular miRNA machinery remains at an early stage, and research in this area may still present us with a number of surprises.

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REFERENCES

1. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell*. 1993; 75:855–862. [PubMed: 8252622]
2. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*. 1993; 75:843–854. [PubMed: 8252621]
3. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136:215–233. [PubMed: 19167326]
4. Cullen BR. Transcription and processing of human microRNA precursors. *Mol Cell*. 2004; 16:861–865. [PubMed: 15610730]

5. Zeng Y, Yi R, Cullen BR. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. *EMBO J.* 2005; 24:138–148. [PubMed: 15565168]
6. Han J, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell.* 2006; 125:887–901. [PubMed: 16751099]
7. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* 2003; 17:3011–3016. [PubMed: 14681208]
8. Hutvagner G, et al. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science.* 2001; 293:834–838. [PubMed: 11452083]
9. Su H, Trombly MI, Chen J, Wang X. Essential and overlapping functions for mammalian Argonautes in microRNA silencing. *Genes Dev.* 2009; 23:304–317. [PubMed: 19174539]
10. Peters L, Meister G. Argonaute proteins: mediators of RNA silencing. *Mol Cell.* 2007; 26:611–623. [PubMed: 17560368]
11. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature.* 2000; 404:293–296. [PubMed: 10749213]
12. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell.* 2003; 115:209–216. [PubMed: 14567918]
13. Schwarz DS, et al. Asymmetry in the assembly of the RNAi enzyme complex. *Cell.* 2003; 115:199–208. [PubMed: 14567917]
14. Martinez J, Patkaniowska A, Urlaub H, Luhrmann R, Tuschl T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell.* 2002; 110:563–574. [PubMed: 12230974]
15. Wu L, Fan J, Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. *Proc Natl Acad Sci U S A.* 2006; 103:4034–4039. [PubMed: 16495412]
16. Behm-Ansmant I, et al. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev.* 2006; 20:1885–1898. [PubMed: 16815998]
17. Doench JG, Sharp PA. Specificity of microRNA target selection in translational repression. *Genes Dev.* 2004; 18:504–511. [PubMed: 15014042]
18. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell.* 2005; 120:15–20. [PubMed: 15652477]
19. Lim LP, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature.* 2005; 433:769–773. [PubMed: 15685193]
20. Skalsky RL, et al. The viral and cellular microRNA targetome in lymphoblastoid cell lines. *PLoS Pathog.* 2012; 8:e1002484. [PubMed: 22291592]
21. Riley KJ, et al. EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. *EMBO J.* 2012; 31:2207–2221. [PubMed: 22473208]
22. Haecker I, et al. Ago HITS-CLIP expands understanding of Kaposi's sarcoma-associated herpesvirus miRNA function in primary effusion lymphomas. *PLoS Pathog.* 2012; 8:e1002884. [PubMed: 22927820]
23. Gottwein E, et al. Viral microRNA targetome of KSHV-infected primary effusion lymphoma cell lines. *Cell Host Microbe.* 2011; 10:515–526. [PubMed: 22100165]
24. Pfeffer S, et al. Identification of virus-encoded microRNAs. *Science.* 2004; 304:734–736. [PubMed: 15118162] This is the first report of viral miRNAs
25. Grundhoff A, Sullivan CS. Virus-encoded microRNAs. *Virology.* 2011; 411:325–343. [PubMed: 21277611]
26. Backes S, et al. Degradation of host microRNAs by poxvirus poly(A) polymerase reveals terminal RNA methylation as a protective antiviral mechanism. *Cell Host Microbe.* 2012; 12:200–210. [PubMed: 22901540] This paper describes a unique viral mechanism that globally degrades cellular miRNAs.
27. Cai X, Li G, Laimins LA, Cullen BR. Human papillomavirus genotype 31 does not express detectable microRNA levels during latent or productive virus replication. *J Virol.* 2006; 80:10890–10893. [PubMed: 17041229]

28. Pfeffer S, et al. Identification of microRNAs of the herpesvirus family. *Nat Methods*. 2005; 2:269–276. [PubMed: 15782219]
29. Lin J, Cullen BR. Analysis of the interaction of primate retroviruses with the human RNA interference machinery. *J Virol*. 2007; 81:12218–12226. [PubMed: 17855543]
30. Umbach JL, Yen HL, Poon LL, Cullen BR. Influenza A virus expresses high levels of an unusual class of small viral leader RNAs in infected cells. *mBio*. 2010; 1:e00204–e00210. [PubMed: 20842206]
31. Cai X, et al. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog*. 2006; 2:e23. [PubMed: 16557291]
32. Zhu JY, et al. Identification of novel Epstein-Barr virus microRNA genes from nasopharyngeal carcinomas. *J Virol*. 2009; 83:3333–3341. [PubMed: 19144710]
33. Feederle R, et al. A viral microRNA cluster strongly potentiates the transforming properties of a human herpesvirus. *PLoS Pathog*. 2011; 7:e1001294. [PubMed: 21379335]
34. Seto E, et al. Micro RNAs of Epstein-Barr virus promote cell cycle progression and prevent apoptosis of primary human B cells. *PLoS Pathog*. 2010; 6:e1001063. [PubMed: 20808852] These two papers^{33,34} provide the first demonstration that viral miRNAs play an important role in virus-mediated transformation of human cells.
35. Cai X, et al. Kaposi's sarcoma-associated herpesvirus expresses an array of viral microRNAs in latently infected cells. *Proc Natl Acad Sci U S A*. 2005; 102:5570–5575. [PubMed: 15800047]
36. Lin YT, Sullivan CS. Expanding the role of Drosha to the regulation of viral gene expression. *Proc Natl Acad Sci U S A*. 2011; 108:11229–11234. [PubMed: 21690333]
37. Stark TJ, Arnold JD, Spector DH, Yeo GW. High-resolution profiling and analysis of viral and host small RNAs during human cytomegalovirus infection. *J Virol*. 2012; 86:226–235. [PubMed: 22013051]
38. Dölken L, et al. Cytomegalovirus microRNAs facilitate persistent virus infection in salivary glands. *PLoS Pathog*. 2010; 6:e1001150. [PubMed: 20976200]
39. Choy EY, et al. An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. *J Exp Med*. 2008; 205:2551–2560. [PubMed: 18838543]
40. Nikitin PA, Luftig MA. At a crossroads: human DNA tumor viruses and the host DNA damage response. *Future virology*. 2011; 6:813–830. [PubMed: 21927617]
41. Marquitz AR, Mathur A, Nam CS, Raab-Traub N. The Epstein-Barr Virus BART microRNAs target the pro-apoptotic protein Bim. *Virology*. 2011; 412:392–400. [PubMed: 21333317]
42. Nachmani D, Stern-Ginossar N, Sarid R, Mandelboim O. Diverse herpesvirus microRNAs target the stress-induced immune ligand MICB to escape recognition by natural killer cells. *Cell Host Microbe*. 2009; 5:376–385. [PubMed: 19380116] This paper reveals that three different herpesviruses, KSHV, EBV and HCMV, all use distinct miRNAs to target the same immune factor.
43. Stern-Ginossar N, et al. Host immune system gene targeting by a viral miRNA. *Science*. 2007; 317:376–381. [PubMed: 17641203]
44. Sullivan CS, Grundhoff AT, Tevethia S, Pipas JM, Ganem D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. *Nature*. 2005; 435:682–686. [PubMed: 15931223]
45. Umbach JL, et al. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature*. 2008; 454:780–783. [PubMed: 18596690]
46. Barth S, et al. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. *Nucleic Acids Res*. 2008; 36:666–675. [PubMed: 18073197]
47. Abend JR, Uldrick T, Ziegelbauer JM. Regulation of tumor necrosis factor-like weak inducer of apoptosis receptor protein (TWEAKR) expression by Kaposi's sarcoma-associated herpesvirus microRNA prevents TWEAK-induced apoptosis and inflammatory cytokine expression. *J Virol*. 2010; 84:12139–12151. [PubMed: 20844036]
48. Abend JR, et al. Kaposi's Sarcoma-Associated Herpesvirus MicroRNAs Target IRAK1 and MYD88, Two Components of the Toll-Like Receptor/Interleukin-1R Signaling Cascade, To Reduce Inflammatory-Cytokine Expression. *J Virol*. 2012; 86:11663–11674. [PubMed: 22896623]

49. Lei X, et al. A Kaposi's Sarcoma-Associated Herpesvirus MicroRNA and Its Variants Target the Transforming Growth Factor beta Pathway To Promote Cell Survival. *J Virol.* 2012; 86:11698–11711. [PubMed: 22915806]
50. Suffert G, et al. Kaposi's sarcoma herpesvirus microRNAs target caspase 3 and regulate apoptosis. *PLoS Pathog.* 2011; 7:e1002405. [PubMed: 22174674]
51. Gottwein E, Cullen BR. A human herpesvirus microRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest. *J Virol.* 2010; 84:5229–5237. [PubMed: 20219912]
52. Kim S, et al. Human cytomegalovirus microRNA miR-US4-1 inhibits CD8(+) T cell responses by targeting the aminopeptidase ERAP1. *Nat Immunol.* 2011; 12:984–991. [PubMed: 21892175]
53. Kim Y, et al. Human cytomegalovirus clinical strain-specific microRNA miR-UL148D targets the human chemokine RANTES during infection. *PLoS Pathog.* 2012; 8:e1002577. [PubMed: 22412377]
54. Pasquinelli AE, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature.* 2000; 408:86–89. [PubMed: 11081512]
55. Kincaid RP, Burke JM, Sullivan CS. An RNA virus microRNA that mimics a B-cell oncomiR. *Proc Natl Acad Sci U S A.* 2012
56. Thai TH, et al. Regulation of the germinal center response by microRNA-155. *Science.* 2007; 316:604–608. [PubMed: 17463289]
57. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci U S A.* 2007; 104:1604–1609. [PubMed: 17242365]
58. O'Connell RM, et al. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. *J Exp Med.* 2008; 205:585–594. [PubMed: 18299402]
59. Eis PS, et al. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc Natl Acad Sci U S A.* 2005; 102:3627–3632. [PubMed: 15738415]
60. Kluiver J, et al. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J Pathol.* 2005; 207:243–249. [PubMed: 16041695]
61. Gottwein E, et al. A viral microRNA functions as an ortholog of cellular miR-155. *Nature.* 2007; 450:1096–1099. [PubMed: 18075594]
62. Skalsky RL, et al. Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. *J Virol.* 2007; 81:12836–12845. [PubMed: 17881434] These two papers^{61, 62} reported the first example of a viral miRNA mimic of a cellular miRNA.
63. Boss IW, et al. A KSHV encoded ortholog of miR-155 induces human splenic B-cell expansion in NOD/LtSz-scid IL2R{gamma}null mice. *J Virol.* 2011
64. Zhao Y, et al. Critical role of the virus-encoded microRNA-155 ortholog in the induction of Marek's disease lymphomas. *PLoS Pathog.* 2011; 7:e1001305. [PubMed: 21383974] This is the first report documenting a critical role for a viral miRNA in cell transformation *in vivo*.
65. Ho BC, et al. Enterovirus-induced miR-141 contributes to shutoff of host protein translation by targeting the translation initiation factor eIF4E. *Cell Host Microbe.* 2011; 9:58–69. [PubMed: 21238947]
66. Yin Q, et al. MicroRNA-155 is an Epstein-Barr Virus induced gene that modulates Epstein Barr virus regulated gene expression pathways. *J Virol.* 2008; 82:5295–5306. [PubMed: 18367535]
67. Forte E, et al. The Epstein-Barr virus (EBV)-induced tumor suppressor microRNA MiR-34a is growth promoting in EBV-infected B cells. *J Virol.* 2012; 86:6889–6898. [PubMed: 22496226]
68. Lu F, et al. Epstein-Barr virus-induced miR-155 attenuates NF-kappaB signaling and stabilizes latent virus persistence. *J Virol.* 2008; 82:10436–10443. [PubMed: 18753206]
69. Gatto G, et al. Epstein-Barr virus latent membrane protein 1 trans-activates miR-155 transcription through the NF-kappaB pathway. *Nucleic Acids Res.* 2008; 36:6608–6619. [PubMed: 18940871]
70. Linnstaedt SD, Gottwein E, Skalsky RL, Luftig MA, Cullen BR. Virally induced cellular microRNA miR-155 plays a key role in B-cell immortalization by Epstein-Barr virus. *J Virol.* 2010; 84:11670–11678. [PubMed: 20844043]

71. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science*. 2005; 309:1577–1581. [PubMed: 16141076]
This is the first report showing that a cellular miRNA is critical for virus replication.
72. Machlin ES, Sarnow P, Sagan SM. Masking the 5' terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. *Proc Natl Acad Sci U S A*. 2011; 108:3193–3198. [PubMed: 21220300]
73. Cazalla D, Yario T, Steitz JA. Down-regulation of a host microRNA by a Herpesvirus saimiri noncoding RNA. *Science*. 2010; 328:1563–1566. [PubMed: 20558719]
74. Buck AH, et al. Post-transcriptional regulation of miR-27 in murine cytomegalovirus infection. *RNA*. 2010; 16:307–315. [PubMed: 20047990]
75. Andersson MG, et al. Suppression of RNA interference by adenovirus virus-associated RNA. *J Virol*. 2005; 79:9556–9565. [PubMed: 16014917]
76. Lu S, Cullen BR. Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and microRNA biogenesis. *J Virol*. 2004; 78:12868–12876. [PubMed: 15542639]
77. Kelly EJ, Hadac EM, Greiner S, Russell SJ. Engineering microRNA responsiveness to decrease virus pathogenicity. *Nat Med*. 2008; 14:1278–1283. [PubMed: 18953352]
78. Ylösmäki E, et al. Generation of a conditionally replicating adenovirus based on targeted destruction of E1A mRNA by a cell type-specific MicroRNA. *J Virol*. 2008; 82:11009–11015. [PubMed: 18799589]
79. Barnes D, Kunitomi M, Vignuzzi M, Saksela K, Andino R. Harnessing endogenous miRNAs to control virus tissue tropism as a strategy for developing attenuated virus vaccines. *Cell Host Microbe*. 2008; 4:239–248. [PubMed: 18779050]
80. Otsuka M, et al. Hypersusceptibility to vesicular stomatitis virus infection in Dicer1-deficient mice is due to impaired miR24 and miR93 expression. *Immunity*. 2007; 27:123–134. [PubMed: 17613256]
81. Davis M, Sagan SM, Pezacki JP, Evans DJ, Simmonds P. Bioinformatic and physical characterizations of genome-scale ordered RNA structure in mammalian RNA viruses. *J Virol*. 2008; 82:11824–11836. [PubMed: 18799591]
82. Watts JM, et al. Architecture and secondary structure of an entire HIV-1 RNA genome. *Nature*. 2009; 460:711–716. [PubMed: 19661910]
83. Mathonnet G, et al. MicroRNA inhibition of translation initiation in vitro by targeting the capbinding complex eIF4F. *Science*. 2007; 317:1764–1767. [PubMed: 17656684]
84. Humphreys DT, Westman BJ, Martin DI, Preiss T. MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc Natl Acad Sci U S A*. 2005; 102:16961–16966. [PubMed: 16287976]
85. Lecellier CH, et al. A cellular microRNA mediates antiviral defense in human cells. *Science*. 2005; 308:557–560. [PubMed: 15845854]
86. Jurak I, et al. Numerous conserved and divergent microRNAs expressed by herpes simplex viruses 1 and 2. *J Virol*. 2010; 84:4659–4672. [PubMed: 20181707]
87. Tang S, Patel A, Krause PR. Novel less-abundant viral microRNAs encoded by herpes simplex virus 2 latency-associated transcript and their roles in regulating ICP34.5 and ICP0 mRNAs. *J Virol*. 2009; 83:1433–1442. [PubMed: 19019961]
88. Umbach JL, Nagel MA, Cohrs RJ, Gilden DH, Cullen BR. Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. *J Virol*. 2009; 83:10677–10683. [PubMed: 19656888]
89. Tuddenham L, Jung JS, Chane-Woon-Ming B, Dolken L, Pfeffer S. Small RNA deep sequencing identifies microRNAs and other small noncoding RNAs from human herpesvirus 6B. *J Virol*. 2012; 86:1638–1649. [PubMed: 22114334]

Figure 1. Schematic representation of the genome structure of the human herpesviruses EBV, KSHV and HCMV

This figure provides an overview of the repeat elements and viral miRNAs present in these viruses.

Figure 2. Sequence alignment of miR-155 with viral mRNA mimics

Alignment of human (hs) and chicken (gga) miR-155 with the sequences of the viral miR-155 mimics KSHV miR-K11 and MDV-1 miR-M4.

Table 1

MicroRNAs encoded by human viruses

Virus Family	Virus	Number of pre-miRNAs	References
α -herpesviruses	HSV-1	16	45, 86
	HSV-2	17	86, 87
	VZV	0	88
β -herpesviruses	HCMV	12	28, 37
	HHV6	4	89
γ -herpesviruses	EBV	25	24, 31, 32
	KSHV	12	35, 28
Adenoviruses	Ad5	2	75
Polyomaviruses	JC, BK, MCPV	1	25

Table 2

Selected cellular innate immunity factors inhibited by EBV, KSHV or HCMV-encoded microRNAs

Cellular factor	Targeted by	Predicted phenotype
PUMA	EBV miR-BART5 and miR-BART19	Reduced apoptosis in response to DNA damage
BIM	EBV miR-BART4 and miR-BART15	Reduced apoptosis
MICB	HCMV miR-UL112 KSHV miR-K7 EBV miR-BART1, BART3, BART5 and BART9	Reduced killing by NK cells
TWEAKR	KSHV miR	Reduced apoptosis
IRAK1	KSHV miR	Reduced TLR signaling, weaker response to proinflammatory cytokines
MYD88	KSHV miR	Reduced TLR signaling, weaker response to pro-inflammatory cytokines
TGFBRII	KSHV miR	Prevention of TGF β -induced apoptosis
Caspase 3	KSHV miR-K1, miR-K3 and miR-K4	Inhibition of apoptosis induced by pro-inflammatory stimuli
p21	KSHV miR	Reduced cell cycle arrest after DNA damage
ERAP1	HCMV miR	Inhibition of CTL killing due to poor antigen presentation
RANTES	HCMV miR	Reduced recruitment of immune effector cells

See text for more detailed discussion of the potential effects exerted by these viral miRNAs, as well as relevant references.