

MicroRNAs Expressed during Viral Infection: Biomarker Potential and Therapeutic Considerations



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ABSTRACT: MicroRNAs (miRNAs) are short sequences of noncoding single-stranded RNAs that exhibit inhibitory effects on complementary target mRNAs. Recently, it has been discovered that certain viruses express their own miRNAs, while other viruses activate the transcription of cellular miRNAs for their own benefit. This review summarizes the viral and/or cellular miRNAs that are transcribed during infection, with a focus on the biomarker and therapeutic potential of miRNAs (or their antagonists). Several human viruses of clinical importance are discussed, namely, herpesviruses, polyomaviruses, hepatitis B virus, hepatitis C virus, human papillomavirus, and human immunodeficiency virus.

KEYWORDS: microRNA, biomarker, virus, herpesvirus, hepatitis

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Introduction

Several types of small noncoding RNAs have been discovered that affect a multitude of biological pathways within the cell. One such class includes microRNAs (miRNAs), short sequences of noncoding single-stranded RNAs that exhibit inhibitory effects on complementary target mRNAs. Research on miRNAs can provide insights into the development, treatment, and monitoring of diseases, including viral diseases. This review aims to provide an overview of recent research characterizing the role of miRNAs during viral infection. We focus on several viruses of clinical importance in order to assess the potential of host- and virus-derived miRNAs as therapeutic targets or biomarkers of disease.

A *biomarker* is an objectively measured indicator that reflects the presence or progression of a particular condition. In addition, biomarkers can be used to monitor the efficacy of treatments for a disease. The discovery and validation of novel biomarkers reduce the time and cost associated with drug development and therefore increase the success rate of translating experimental drugs into clinical therapeutics.¹ They are a valuable tool to improve animal models of disease, monitor drug candidate safety and efficacy, and detect changes in the pathological state of a disease. Biological fluids from the local site of pathology, known as proximal fluids, often provide a more accurate assessment of the pathological state. Plasma is often used for biomarker assessment, due to the ease in obtaining it, and thus, any proximal biomarkers that spill over into

the bloodstream may prove to be effective biomarkers that are more easily accessible than proximal fluids.

Biogenesis of miRNAs

Several excellent reviews are available that explain the details of miRNA biogenesis.^{2,3} Most miRNAs are transcribed through the actions of RNA polymerase II from templates found within introns of protein-coding genes or directly from independent genes.^{3,4} In the cytoplasm, the miRNA guide strand remains associated with Argonaute (Ago) within the RNA-induced silencing complex (RISC), while the complementary strand, referred to as the miRNA* (star strand) or passenger strand, is degraded. Unlike most cellular miRNAs, certain viral miRNAs can be derived from both strands of the double-stranded miRNA molecule, leading to the convention of naming the strands with -5p or -3p suffixes.

The guide miRNA is the primary mechanism for targeting the RISC complex to mRNAs. Although miRNAs are ~22 nucleotides in length, the mRNA target is generally recognized through complementary base pairing of the seed sequence comprising nucleotides 2–7 at the 5' end of the miRNA strand.⁵ Consequently, one miRNA:RISC complex can silence hundreds of mRNAs with complementarity to the same seed sequence, regardless of their translational products. Once bound by the RISC, miRNAs usually target the 3'-untranslated region (UTR) of mRNAs,⁶ resulting in the repression of translation and/or degradation of the target



mRNA.^{3,7} It is now well established that miRNAs influence an extensive number of biological pathways in this manner through regulation of protein-coding genes.

Techniques for miRNA Identification and Verification

Host- and virus-derived miRNAs can be identified computationally using bioinformatics or through functional screening assays. A variety of bioinformatics programs are now available that predict miRNAs (Table 1). Although bioinformatics has been extremely valuable in bringing together possible sets of miRNAs and their targets, the advent of relatively inexpensive next-generation sequencing (deep sequencing) platforms and high-density oligonucleotide arrays has simplified the functional screening of viral miRNAs. Most viral miRNAs were initially identified through a modified rapid amplification of cDNA ends protocol. Briefly, polyacrylamide gel-purified small RNAs were modified with 5' or 3' oligonucleotides that functioned as primers for PCR after reverse transcription and the amplified segments were cloned and sequenced. Next-generation sequencing techniques have since then replaced the time-consuming process of cloning and can process millions of sequence reads in parallel. An elegant use of this technique to identify biologically relevant miRNAs is termed **high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP)**. In this process, ultraviolet (UV) irradiation is used to covalently crosslink Ago-associated miRNAs and target mRNAs, which are then immunoprecipitated and sequenced.⁸ This procedure allows for the identification of miRNAs and their cognate mRNA recognition elements (MREs) that are directly associated with RISC from cell lines or tissue samples.⁹ **Photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP)** is a related technique that uses a photoactivatable nucleoside analog, most often 4-thiouridine, which is randomly incorporated into nascent RNAs.¹⁰ The stable crosslinking of PAR-CLIP results in increased purification of bound miRNA/mRNA duplexes. However, this technique cannot be performed on primary

tissue samples. Both procedures enhance the precision of purified biologically relevant miRNAs and their targets that can then be confirmed *in vitro*.

Following their identification through bioinformatics or high-throughput techniques, putative miRNAs and their targets must be experimentally validated. Luciferase reporter assays are commonly used to verify direct binding of a miRNA to a particular mRNA sequence. The 3'-UTR region predicted to interact with a miRNA target is placed downstream of the luciferase gene in a reporter construct. The vector is then transfected into cells alongside an miRNA expression vector or a control vector, and luciferase expression is reduced if the miRNA targets the MRE. Site directed mutagenesis or target protector nucleotides can also be used to verify MRE sequence complementarity to the miRNA. Antisense oligonucleotides (molecular sponges) show specificity of a particular miRNA seed sequence for an MRE by complementary binding to the miRNA sequence.¹¹ Viral miRNA deletion mutants are also used to verify the biological function of individual or clusters of miRNAs.

Biomarker and Therapeutic Potential of miRNAs During Viral Infection

The majority of known virally encoded miRNAs are found within DNA viruses that replicate in the nucleus. The first viral miRNAs discovered were found to be expressed by Epstein-Barr virus (EBV).¹² Since then, the great majority of miRNAs have been discovered in herpesviruses. A few viral miRNAs have also been reported in other human DNA virus families, including polyomaviruses (described below) and possibly adenoviruses (in very low abundance from virus-associated RNAs). In addition, miRNAs have been reported in DNA viral families that do not infect humans, namely, ascoviruses (*Heliothis virescens* ascovirus), baculoviruses (*Bombyx mori* nuclear polyhedrosis virus), and nimaviruses (white spot syndrome virus). Notably, miRNAs from human papillomaviruses (HPVs) have yet to be definitively discovered.

Retroviruses possess a replication stage that involves nuclear DNA as a result of reverse transcription, and miRNAs

Table 1. Approaches used by miRNA target prediction software tools.

	THERMODYNAMIC	EVOLUTIONARY	PROBABILISTIC	SEQUENCE-BASED	REFERENCE
miRmap	X	X	X	X	356
TargetScan		X		X	6
PITA	X				357
PicTar	X	X	X		358
miRanda	X				359
RNAhybrid	X				360
DIANA-microT	X	X			361
EIMMo		X	X		362
PACMIT	X		X		363

Note: Table modified from Charles E. Vejnár, Evgeny M. Zdobnov. miRmap: Comprehensive prediction of microRNA target repression strength. *Nucl Acids Res.* 2007;40(22):11673–11683, with permission of Oxford University Press under a CC BY 3.0 license.



have indeed been discovered in a few retroviruses, including avian leucosis virus subgroup J, African green monkey simian foamy virus, and bovine leukemia virus.^{13,14} As will be discussed later, miRNAs have been reported from the human immunodeficiency virus (HIV)-1 transactivation response (TAR) RNA of CD4 T cells,^{15–17} although other studies have failed to find biologically relevant viral miRNAs from HIV-1 or human T-lymphotropic virus-infected cells.^{18–20}

The existence of miRNAs in cytoplasmic RNA viruses has yet to be detected, and several reasons have been proposed as to why they are unlikely in cytoplasmic viruses. The nuclear location of Drosha and DGCR8, necessary for genesis of pre-miRNAs, is one of the leading explanations for this absence. Interestingly, Rouha et al engineered a nuclear-replicating RNA virus to contain a miRNA-precursor stem-loop sequence element within its RNA genome. They found that a functional miRNA was produced without affecting viral replication,²¹ implying the possible existence of Drosha-independent miRNA generation pathways within the cytoplasm.

Herpesviruses. Herpesviruses are large, double-stranded DNA viruses that infect a range of invertebrate and vertebrate animals. Nine human herpesviruses (HHVs) exist, possessing genomes of ~125–230 kb in length. The great majority of miRNAs reported thus far have been found in herpesviruses, and several of the herpesviruses each encode over 20 predicted miRNAs (many of which have not yet been shown to be biologically functional). As DNA viruses that replicate in the nucleus, herpesviruses have access to the nuclear proteins Drosha and DGCR8 that are necessary for the processing of primary miRNAs (pri-miRNAs). Several herpesviruses appear to have auto-regulatory miRNAs, including those that maintain latency, while others use miRNAs to modulate cellular responses during infection (Table 2).

Alphaherpesvirinae subfamily members: herpes simplex virus-1 (HSV-1), herpes simplex virus-2, and varicella zoster virus. Members of the *Alphaherpesvirinae* subfamily, herpes simplexvirus-1 (HSV-1) and herpes simplexvirus-2 (HSV-2), are the causative agents of cold sores and genital herpes,

Table 2. Notable herpesvirus-encoded miRNAs.

HERPESVIRUS	miRNA	mRNA TARGET	REFERENCES
HSV-1	miR-H2	<i>ICP0</i> , a transactivator of IE, E, and L genes	31,32
	miR-H4	<i>ICP34.5</i> , a neurovirulence factor and immune inhibitor	33
	miR-H6	<i>ICP4</i> , a transactivator of E and L genes	31
HSV-2	miR-H2	HSV1-miR-H2 ortholog; represses <i>ICP0</i>	32
	miR-H3	<i>ICP34.5</i>	32,38
	miR-H4	<i>ICP34.5</i>	32,38
VZV	None reported		
EBV	miR-BART2	<i>BALF5</i> , the viral DNA polymerase	12,54
	miR-BART3, -BART5, -BART16, -BART17, -BART19, -BART20	<i>LMP1</i> , a viral gene that promotes cell survival	55–57
	miR-BART5, -BART19	Cellular <i>PUMA</i> , a pro-apoptotic gene	55,59
	miR-BART2	Cellular <i>MICB</i> , a stress-induced NK cell ligand	55,63
	miR-BART18	Cellular <i>CBP</i> , involved in type 1 IFN production	65
HCMV	miR-UL112	Targets HCMV genes IE1, IE72, UL112/113, UL114, UL120/121	94–96
	miR-UL112	Cellular <i>MICB</i> , a stress-induced NK cell ligand	63,101
	miR-UL148D	Cellular <i>RANTES</i> , a T cell-attracting chemokine	102
HHV-6B	miR-Ro6–1	Antisense to B3 IE ORF; function unknown	104
	miR-Ro6–2	Antisense to B2 IE ORF; function unknown	104
	miR-Ro6–3	Antisense to B1 IE ORF; function unknown	104
	miR-Ro6–4	Function unknown	104
HHV-7	None reported		
KSHV	miR-K12–9, miR-K12–7	RTA, the activator of the latent-lytic switch	72,73
	miR-K12–1	Cellular <i>p21</i> , involved in cell cycle arrest	82
	miR-K12–7	Cellular <i>MICB</i> , a stress-induced NK cell ligand	63
	miR-K12–12	Cellular <i>CBP</i> , involved in Type 1 IFN production	65

respectively. They establish productive (lytic) infection in epithelial cells and eventually infect sensory neurons, where they become latent and persist for the lifetime of the host.

The process of virion replication takes ~18–20 hours to complete and occurs in three stages corresponding to the expression of immediate early (IE; α), early (E; β), and late (L; γ) genes. During latency, there is little detectable transcription of IE, E, or L genes in infected neurons, even though it is known that they produce high levels of untranslated latency-associated transcripts (LATs). An unstable spliced 6.3 kb transcript and two stable LAT introns of 2 kb and 1.5 kb are spliced from an unstable 8.3 kb primary transcript.^{22–25} Although protein products of the LAT gene locus have not been discovered, the site has been associated with the repression of IE gene transcription and the maintenance of latency.^{26–28} More recently, miRNAs of both virus and host origin have been connected with maintaining HSV-1 or HSV-2 latency.

According to miRBase.org,²⁹ 18 virus-derived pre-miRNAs have been identified from HSV-1 that encode 27 mature miRNAs. Many of these have been identified using bioinformatics or sequencing analysis and have yet to be characterized in functional biological assays. The best characterized miRNAs are HSV1-miR-H1 through miR-H6. miR-H1 and miR-H6 are located in the LAT promoter, while miR-H2, miR-H3, miR-H4, and miR-H5 are derived from the primary LAT transcript (Fig. 1).³⁰

The targets of most HSV-1 miRNAs are still unknown, but those that have been characterized point to a role in

preventing viral reactivation from latency through regulation of *ICP0*, *ICP34.5*, and *ICP4* genes. miR-H2 is derived from the large LAT transcript and is antisense to *ICP0*. Transient-transfection assays showed that miR-H2 is able to downregulate the IE ICP0 protein,^{31,32} which functions as a transactivator of IE, E, and L genes and, consequently, promotes viral replication. Also derived from the large LAT transcript, miR-H4 (and possibly miR-H3) inhibits the expression of *ICP34.5*,³³ a neurovirulence factor and L gene product that induces transcription. Similarly, miR-H6 can repress the translation of the ICP4 protein,³¹ an IE protein transactivator that is essential for the maximal transcription of E and L genes. By suppressing the transcription of *ICP0*, *ICP4*, and *ICP34.5* genes, these LAT-derived or LAT-associated miRNAs may be a component of maintaining latency and suppressing active viral replication.

The earliest studies of HSV-1 miRNAs described their roles in maintaining latency. It is now known that many of these are also present and differentially expressed during productive infection.^{30,34,35} For example, miR-H1 and miR-H6 are more highly expressed than miR-H2, miR-H3, and miR-H4 during productive infection than latency.³⁰ The opposite is true during latency, implying that miRNA expression plays a role in controlling the ordered expression of viral genes. Flores et al took the analysis of HSV-1 miRNA a step further by examining which of the miRNAs are loaded into the RISC as an indication of miRNA biological function. Surprisingly, only nine HSV-1 miRNAs were found to be associated with

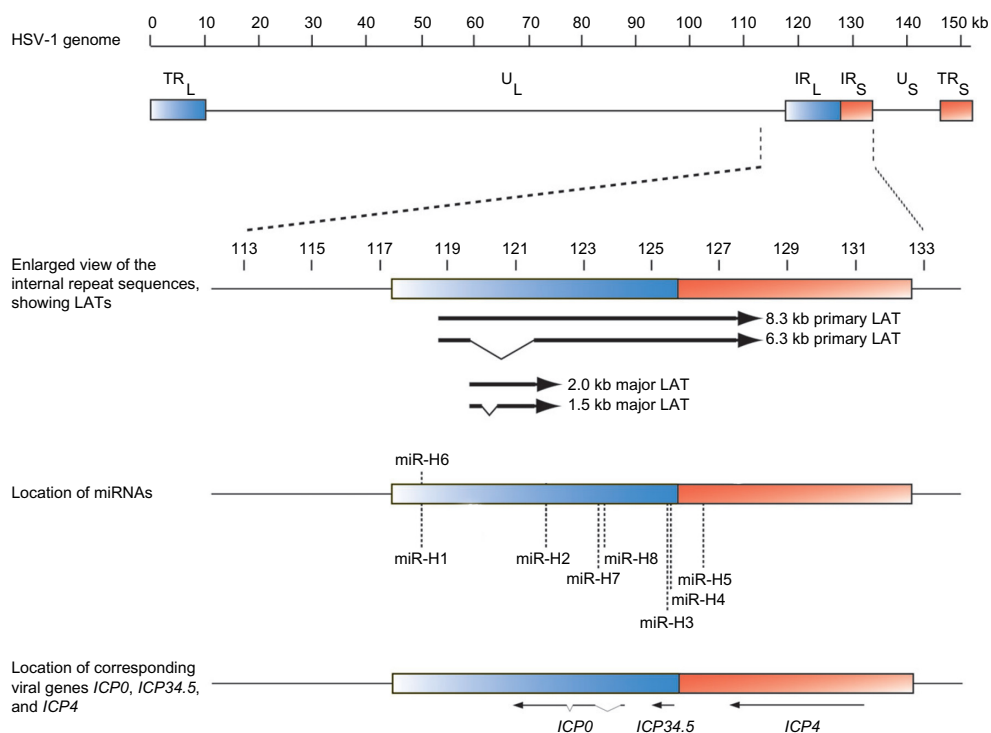


Figure 1. Genomic location of selected HSV-1 pre-miRNAs.

Note: Figure modified from Nicoll MP, Proença JT, Efstathiou S. The molecular basis of herpes simplex virus latency. *FEMS Microbiol Rev.* 2012;36:684–706 with permission of Oxford University Press on behalf of the Federation of European Microbiological Societies.⁴⁴⁶



the RISC. In addition, the miRNAs were found to associate with the RISC at differing rates,³³ suggesting that several may not be functional. A recent study by Belter et al shows that miRNAs in high concentration are able to form secondary structures resembling RNA aptamers,³⁶ molecules whose specific 3D shapes allow them to bind targets with high affinity in the absence of the RISC.

A recent report indicates that host-derived miRNAs may also play a role in promoting HSV-1 latency. Pan et al found that the neuron-expressed miR-138 downregulated the expression of ICP0. A mutant HSV-1 virus lacking an ICP0 mRNA site complementary to miR-138 showed a two-fold to fourfold decrease in ICP0 protein in neuronal cells.³⁷ Notably, the expression of ICP0 was unchanged in Vero cells infected with the mutant virus, further emphasizing that cell type is an important factor in determining the biological relevance of both viral and host miRNAs.

HSV-2 is the causative agent of genital herpes. Although viral miRNAs are not necessarily conserved between related viruses, HSV-2 shares ~85% homology with HSV-1 and has several miRNAs located at corresponding locations within its genome. HSV-2 miRNAs often share a high degree of homology with their HSV-1 counterparts when considering the seven-base pair seed regions of the miRNAs.³⁰ HSV-2 miRNAs have also been shown to exert similar regulation upon orthologous HSV-2 genes. For example, both HSV-2 miR-H2 (ie, miR-III) and HSV-1 miR-H2 are able to repress ICP0.³² Similarly, HSV-2 miR-H3 and miR-H4 (ie, miR-I and miR-II, respectively), which are found in high copy number in neurons, are complementary to and lead to the downregulation of ICP34.5.^{32,38} There are exceptions to this conservation that demonstrate that the relative expression of each miRNA is not necessarily the same between the two viruses and that they may differentially regulate the expression patterns of viral genes. Specifically, miR-H2 is the most highly expressed miRNA during HSV-1 infection, while miR-H3 is most abundant during HSV-2 infection.³⁹ Additionally, not all miRNAs are identical between the two viruses: HSV-2 lacks an ortholog of miR-H1 and the miR-H6 found in HSV-2 uses the 5' strand of the miRNA duplex, whereas HSV-1 miR-H6 uses the 3' strand.^{30,31} Interestingly, the seed sequence of the HSV-2 miR-H6 5' strand is identical to the seed sequence of the HSV-1 miR-H1, although the targets of either miRNA are currently unknown.³⁰

HSV-2 encodes a total of 18 virus-derived pre-miRNAs that derive 24 mature miRNAs. HSV-1 miR-H1, miR-H8, miR-H14, miR-H18, miR-H26, and miR-H27 have no known HSV-2 counterparts, while HSV-2 uniquely encodes miR-H9, miR-H10, and miR-H19 through miR-H25.

Varicella zoster virus (VZV; officially HHV-3) is the final member of the *Alphaherpesvirinae* subfamily and causes chickenpox (varicella) during primary infection and shingles (herpes zoster) upon reactivation. The latency program of VZV is different from that of HSV-1 and HSV-2. VZV

lacks the *LAT* locus and does not have an RNA transcript analogous to LAT. In addition, it expresses several proteins during latency,⁴⁰ which is not characteristic of HSV-1 and HSV-2. Deep sequencing of human trigeminal ganglia identified VZV genomic DNA but no VZV-specific miRNAs during latency.⁴¹ In support of this result, it was reported in the same study that miRNAs were not able to be recovered from the trigeminal ganglia of rhesus macaques infected with the related simian varicella virus, although they have been discovered in other animal varicelloviruses, bovine herpesvirus 1 and suid herpesvirus 1 (Aujeszky's disease virus/pseudorabies virus).⁴²⁻⁴⁴ It is worth noting, however, that the miRNAs from suid herpesvirus 1 map to the large latency transcript and are complementary to IE transactivators *EPO* and *EP180*,^{43,44} which are homologs of HSV-1 *ICP0* and *ICP4*.⁴⁵ It is thus possible that the miRNAs of Suid herpesvirus 1 share conserved function with miRNAs of HSV-1, but not those of VZV. Computational analyses have predicted that VZV does not encode any miRNAs,¹⁸ although the possibility of VZV miRNAs during productive infection has yet to be fully examined.

Gammaherpesvirinae subfamily members: *EBV* and *Kaposi's sarcoma-associated herpesvirus*. EBV (HHV-4) and KSHV (HHV-8) are the two herpesviruses within the *Gammaherpesvirinae* subfamily of herpesviruses. Both viruses productively infect and become latent within lymphocytes or lymphoid tissues, and both can induce transformation of infected cells that can lead to malignancies.

EBV causes 90% of the cases of mononucleosis in teenagers or adults. The virus is also associated with Burkitt's lymphoma (BL), Hodgkin's lymphoma, primary effusion lymphoma (PEL), nasopharyngeal carcinoma (NPC), and gastric carcinomas (GaCas).

EBV was the first virus demonstrated to express miRNAs.¹² There are two clusters in the EBV genome that encode 25 pre-miRNAs that ultimately produce 44 mature miRNAs. The BamHI fragment H rightward open reading frame 1 (BHRF1) cluster is found within the BHRF1 locus and encodes three miRNAs: miR-BHRF1-1, miR-BHRF1-2, and miR-BHRF1-3. miR-BHRF1-1 is located in the promoter of BHRF1, whereas miR-BHRF1-2 and miR-BHRF1-3 are encoded in the 3'-UTR region of the gene. The second cluster of EBV miRNAs is located within introns of the BamHI-A region rightward transcript (BART) locus. This cluster encodes miR-BART1 through miR-BART22, with the exception of miR-BART2, which is found downstream of the BART locus between *BILF1* and *BALF5* genes.^{46,47}

EBV establishes and maintains persistent infection through a series of transcription programs characterized by regulated viral gene expression,⁴⁸ and the expression of certain miRNAs correlates with the latency program of infected cells. The first transcription program, known as Latency 3 or the growth transcription program, occurs when EBV infects a naïve B cell. This causes the cell to differentiate into a lymphoblast

and proliferate. As with normally activated B lymphoblasts, the cell migrates to the lymph node germinal center follicle and continues to proliferate. It is at this point that the cell switches to the Latency 2 transcription program, also known as the default transcription program, which induces cell differentiation that causes the cell to leave as a resting memory B cell. In the periphery, the Latency 0 program initiates and protein translation ceases, except during the Latency 1 program, when the cell divides due to the expression of EBNA1. The process of productive viral replication and shedding into saliva is provoked by memory cells that return to the tonsil and undergo differentiation into antibody-producing plasma cells.⁴⁸

The BHRF1 miRNAs are highly expressed during the Latency 3 transcription program of infected BL and in lymphoblastoid cell lines (LCLs), which are generated through EBV infection of resting B cells.⁴⁹ Studies that created mutant viruses by introducing mutations into the BHRF1 miRNAs showed that these miRNAs are involved in contributing to B cell transformation by promoting cell-cycle progression and inhibiting apoptosis.^{50,51} In stark contrast, BHRF1 miRNAs were not detectable in NPC, PEL, or BL cell lines in Latency 1 or 2 transcription programs, whereas BART miRNAs were expressed to high levels.⁴⁹ In addition, NPC and GaCa epithelial tumors exhibited 13- and 8-fold higher expression, respectively, of BART miRNAs compared to LCLs.⁵² Taken together, this suggests that BART miRNAs appear to be preferentially expressed in epithelial cells (such as NPCs), while they are moderately expressed in B cells and dispensable for *in vitro* EBV-induced transformation.⁵³

Several viral and host mRNA targets of EBV miRNAs have been identified. miR-BART2 is complementary to *BALF5* and downregulates the expression of this viral DNA polymerase,^{12,54} and miR-BART10 targets the 3'-UTR of *BHRF1* to inhibit apoptosis.⁵⁵ Several EBV miRNAs (miR-BART3, miR-BART5, miR-BART16, miR-BART17, miR-BART19, and miR-BART20) have been reported to downregulate latent membrane protein 1 (LMP1), an EBV integral membrane protein that functions as an oncogene by promoting cell survival and preventing apoptosis.⁵⁵⁻⁵⁷ Although it is counterintuitive that EBV miRNAs would negatively regulate LMP1, high levels of LMP1 can actually promote apoptosis,⁵⁸ and so tight control of the gene is necessary. It is also of interest to note that *BZLF1* and *BRLF1*, two EBV genes associated with the switch from latency to lytic infection, are not targeted in BL cell lines by these EBV miRNAs.⁵⁵ This indicates that these miRNAs are not directly responsible for regulating the latent-lytic switch, unlike HSV-1 and HSV-2 miRNAs.

Also, EBV miRNAs can target host mRNAs to prevent apoptosis. Gene ontology analysis of the host mRNA targets of the 12 most abundant EBV miRNAs indicated that 132 apoptosis-associated host genes may be targeted by EBV miRNAs.⁵⁵ Notably, the p53 upregulated mediator of apoptosis (PUMA), a pro-apoptotic gene induced by p53, has been shown to be targeted by miR-BART5 and miR-BART19.^{55,59}

Also related to promoting apoptosis, the BCL2 family member *BCL2L11* (BIM) is targeted by miR-BART4 and miR-BART15,⁶⁰ and possibly several miRNAs together,⁶¹ to orchestrate the downregulation of this target. EBV-infected GaCa cell lines exhibited reduced apoptosis due to the interaction of miR-BART4 with *BID*, another Bcl-2 family member involved in regulating apoptosis.⁶² Again, differential expression of these miRNAs may occur depending upon the type of cell (B cell versus epithelial cell) or latency program.⁴⁷

EBV miRNAs have also been implicated in evasion of NK cells through miR-BART2-mediated downregulation of MHC Class I Polypeptide-Related Sequence B (MICB), a stress-induced NK cell ligand, in epithelial cell lines.^{55,63} In addition, the T cell-attracting chemokine CXCL11, produced by B cells during EBV infection, is downregulated by EBV miR-BHRF1-3.^{12,64} EBV miR-BART18 decreases the histone acetylase cyclic AMP-responsive element-binding protein (CBP),⁶⁵ which along with p300 associates with IRF3 and IRF7 to induce the transcription of Type 1 interferon (IFN) genes. Blocking miR-BART18 in Akata A.15 cells, an EBV+ BL cell line, led to increased Type 1 IFN signaling, as measured in terms of *ISG-15* expression using Northern blot.⁶⁵ As Type 1 IFN is important in inducing an antiviral state and activating NK and T cells, miRNA-mediated downregulation of the expression of this cytokine would be expected to greatly benefit the virus. Taken together, this work shows that in addition to preventing apoptosis of infected cells, viral miRNAs may also function to prevent host immune effects without the use of immunogenic viral proteins. All herpesviruses still contain a large cohort of protein-encoding genes that interfere with host immune responses, which raises the question of the relative *in vivo* significance of viral miRNAs versus proteins. Infection of mouse models with homologous miRNA-deficient herpesvirus strains will likely provide important context within an *in vivo* system.

The other human gammaherpesvirus is Kaposi's sarcoma-associated herpesvirus (KSHV) (HHV-8). The greatest risk of infection with KSHV is for immunocompromised individuals, who more frequently develop Kaposi's sarcoma. Like EBV, KSHV infection is also associated with PEL and multicentric Castleman disease.⁶⁶

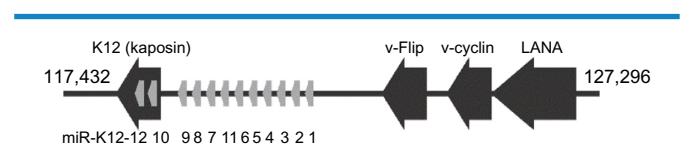


Figure 2. Genomic location of KSHV pre-miRNAs. KSHV pre-miRNAs cluster within the KLAR, which also contains genes for LANA, v-Cyclin, v-FLIP, and Kaposin. All the miRNAs map to the K12 locus: miR-K12-1 through miR-K12-9 and miR-K12-11 are encoded within the K12 intron, while miR-K12-10 and miR-K12-12 map to a K12 open reading frame and the 3'-UTR, respectively.

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According to miRBase.org,²⁹ 13 pre-miRNAs and 25 miRNAs are found within the KSHV genome. The miRNAs are all located as a cluster within the KSHV latency-associated region (KLAR), which encodes four genes expressed during latency and lytic infection: latency-associated nuclear antigen (LANA), v-Cyclin, v-FLIP, and Kaposin (K12). All the miRNAs map to the K12 locus: miR-K12-1 through miR-K12-9 and miR-K12-11 are encoded within the K12 intron, while miR-K12-10 and miR-K12-12 map to a K12 open reading frame and the 3'-UTR, respectively (Fig. 2).^{18,67,68} Although the relative expression levels can vary in different cell types, the 10 miRNAs encoded on the K12 intron are generally expressed as a group in latently infected cells.^{9,69,70} In contrast, miR-K12-10 and miR-K12-12 are expressed alongside the K12 gene during lytic infection.⁷¹

The cluster of KSHV miRNAs is expressed alongside the KLAR genes during latency, and similar to HSV-1 and HSV-2 miRNAs, it has been shown to play a role in preventing reactivation to the lytic cycle. The expression of the KSHV replication and transcription activator (RTA), the master regulator of the latent-lytic switch, is negatively regulated by miR-K12-9 and miR-K12-7.^{72,73} Similarly, miR-K12-3 and miR-K12-11 target cellular activators of RTA.⁷⁴ Inhibiting or eliminating these KSHV miRNAs (or a cluster including these miRNAs) induced elevated lytic gene expression and spontaneous lytic reactivation in fibroblasts, endothelial cells, and PEL cells.⁷²⁻⁷⁵ This further emphasizes that several KSHV miRNAs are involved in the maintenance of latency in both lymphoid and non-lymphoid cells. It is also interesting to note that host-encoded miR-498 and miR-320d have also been shown to target RTA in a PEL cell line,⁷⁶ and 5 of the 99 cellular miRNAs induced by ectopic expression of HIV *nef* have putative binding sites in the 3'-UTR of the RTA gene.⁷⁷

Recent work by McClure et al and Bai et al mapped the 3'-UTRs of KSHV genes and discovered that these regions are important in the negative regulation of KSHV genes.^{78,79} A total of 28 potential KSHV gene targets of known KSHV miRNAs were identified that correspond to all stages of viral replication,⁷⁹ indicating that many miRNA targets have yet to be investigated.

In addition to viral transcripts, KSHV miRNAs target many host mRNAs. Notably, KSHV miRNAs are thought to target >1000 putative host genes,^{9,70} although not necessarily directly.⁸⁰ Several classes of cellular genes consistently targeted include apoptosis, angiogenesis, cellular metabolism, lymphocyte activation, and immune modulation genes.^{9,80,81} For example, several KSHV miRNAs target cellular thrombospondin 1, which is thought to negatively regulate angiogenesis and proliferation.⁸¹ KSHV miR-K12-K1 binds the 3'-UTR of *CDKN1A* (p21) to inhibit p53-induced cell-cycle arrest in PEL B cells.⁸² Similar to EBV, *MICB* is also targeted by miR-K12-K7, a KSHV miRNA.⁶³ Haecker et al used Ago HITS-CLIP to identify a collection of cellular pathways targeted by KSHV miRNAs. Identified genes included 42 involved in apoptosis,

11 in cellular metabolism (glycolysis), 13 in lymphocyte activation (in BCBL-1 cells), and 21 in mitosis (in BC-3 cells).⁹ This emphasizes that this complex virus is capable of affecting multiple host signaling pathways through the effects of numerous viral miRNAs.

As a herpesvirus, KSHV encodes several viral orthologs of host genes, including *IL6*, *CFLAR* (FLIP), *CCND*, and *IRF3*. Similarly, the virus also encodes orthologs of host miRNAs. KSHV expresses miR-K12-11, an ortholog of the human miR-155, during latent infection.⁸³ Host miR-155 is involved in the clonal expansion of lymphocytes following antigenic stimulation,⁸⁴ and miR-155 transgenic mice have been shown to develop B cell lymphomas,⁸⁵ lending support to the hypothesis that miR-K12-11 may assist in the induction of oncogenic processes within cells. Notably, although the related gammaherpesvirus EBV does not encode its own ortholog of miR-155, this cellular miRNA is the most abundant miRNA (of cell or virus origin) induced by EBV infection of LCLs.⁸⁶ KSHV miR-K12-10a and miR-K12-3 (including the SNP-containing miR-K12-3+1) also act as viral orthologs of cellular miR-142-3p and miR-23, respectively.^{70,87} The viral and cellular miRNAs can show mutually exclusive expression in different cell types; however, miR-23 is highly detected in endothelial cells, while miR-K12-3 is expressed in PEL cell lines.⁸⁷ Similarly, cellular miR-155 is highly expressed in infected endothelial cells, while miR-K12-11 is expressed in PEL cell lines.⁸⁸

In addition to encoding homologs of cellular miRNAs and its own miRNAs that target host genes, KSHV also expresses proteins that induce several infection-promoting cellular miRNAs. For example, knocking down cellular miR-21 and miR-31 prevented KSHV K15M-mediated motility of PEL cells,⁸⁹ and cellular miR-132 regulates interferon-stimulated genes in KSHV-infected lymphatic endothelial cells.⁹⁰ It is not surprising that several herpesviruses use miRNAs to interfere with Type 1 IFN signaling; miR-K12-12 also appears to target CBP, which is involved in the activation of Type 1 IFN genes.⁶⁵

Betaherpesvirinae subfamily members: human cytomegalovirus, HHV-6A, HHV-6B, and HHV-7. Human cytomegalovirus (HCMV; officially HHV-5), HHV-6A, HHV-6B, and HHV7 establish latency in leukocytes and are characterized by slower replication than the other herpesviruses. Healthy individuals generally do not display symptoms when infected with HCMV, although 10–20% of infectious mononucleosis cases are attributed to the virus. The virus can reactivate and cause life-threatening disease in immunocompromised individuals, and HCMV is the most prevalent congenital infection in industrialized countries.

According to miRBase.org,²⁹ 15 pre-miRNAs encode 26 mature HCMV miRNAs. Unlike KSHV or EBV, the HCMV miRNAs are scattered throughout the viral genome. They can be found on both strands, present in 3'-UTRs or within intergenic regions.⁹¹



HCMV miRNAs target both viral and host genes. Most HCMV miRNAs correlate with the expression of lytic genes.^{92,93} miR-UL112-1 targets several HCMV genes, including IE1, IE72, UL112/113, UL114, and UL120/121.⁹⁴⁻⁹⁶ The expression of UL138, a viral protein that contributes to latency,⁹⁷ is decreased 46% in miR-UL36-transfected HEK293 cells,⁹⁸ suggesting that this miRNA may be involved in maintaining productive infection. However, no HCMV miRNA has been found to be essential for replication.

Although the majority of its miRNAs have been characterized during lytic infection, HCMV may also encode a subset of miRNAs that self-regulate to maintain a latent state. As mentioned above, miR-112-1 has been shown to downregulate IE72, an abundant HCMV transactivator necessary for induction of E and L gene expression.⁹⁵ The HCMV IE1 and IE2 transactivators are central to reactivation from latency,⁹⁹ and HCMV miR-112-1 binds to the 3'-UTR of IE1 and reduces reporter expression in transient-transfection assays of HEK293T cells.⁹⁶ In support of this, miR-US33 transfection of human embryo lung fibroblast cells reduced infectious viral titers and IE1/IE2 proteins at a multiplicity of infection of 0.01 (although not 0.1, 1, or 5).⁹³ Thus, although few HCMV miRNAs are found to be expressed during latency, several putative miRNAs may have a significant effect upon the suppression of required IE genes.

In addition to targeting viral genes, HCMV also encodes miRNAs that target cellular genes. For example, miR-US25-1 and miR-US25-2 target several cellular targets, many of which are associated with cell-cycle control, and infection with an miR-US25-1 knockout virus resulted in increased cyclin E2 expression in human primary fibroblast cells.¹⁰⁰ Like other herpesvirus miRNAs, HCMV also encodes miRNAs that target cellular genes in an effort to evade host immune responses. Notably, HCMV miR-UL112 was the first miRNA shown to inhibit expression of MICB. It also acts in concert with the HCMV-encoded UL16 protein to inhibit expression of MICB when UL16 protein levels are reduced.¹⁰¹ CCL5, a chemokine that attracts T cells, is downregulated in human foreskin fibroblasts by HCMV miR-UL148D,¹⁰² while MHC Class I peptide loading is inhibited by the miR-US4-1-mediated downregulation of endoplasmic reticulum (ER) aminopeptidase 1 (ERAP1).¹⁰³ Taken together, HCMV encodes several miRNAs that function to regulate host responses.

Whereas HHV-6A is an orphan virus, HHV-6B and HHV-7 cause roseola infantum and are associated with febrile seizures of children below two years of age. Deep sequencing has identified four miRNAs, termed hhv6b-miR-Ro6-1 through miR-Ro6-4, encoded within the left and right direct repeat regions of HHV-6B.¹⁰⁴ Hhv6b-miR-Ro6-3, miR-Ro6-2, and miR-Ro6-1 are antisense to the predicted B1, B2, and B3 open reading frames (ORFs), respectively, that are thought to encode IE genes.¹⁰⁵ Future studies will determine if these HHV-6 miRNAs are involved in regulating early lytic

infection or maintaining latency. Thus far, no viral miRNAs have been identified for HHV-7.

Biomarker and therapeutic potential with respect to herpesvirus miRNAs. Herpesviruses encode proteins that subvert host immune responses, and it is clear that the viruses also encode several miRNAs that interfere with innate and adaptive antiviral mechanisms. Type 1 IFN signaling activates a variety of cellular antiviral pathways and is a potent inducer of NK cell activation and cytotoxicity.¹⁰⁶ Not surprisingly, HCMV, EBV, and KSHV encode miRNAs that target Type 1 IFN signaling,⁶⁵ and these viruses also encode miRNAs that downregulate MICB.^{55,63,101} Blocking miR-BART18 in an EBV+ BL cell line resulted in increased Type 1 IFN signaling,⁶⁵ highlighting that efforts to inhibit herpesvirus miRNAs could be fruitful in reestablishing IFN-induced antiviral pathways. Likewise, inhibition of viral miRNAs may be useful to reverse IFN refractoriness of EBV+ or KSHV+ tumor cells⁶⁵ or increase the efficacy of interferon therapy for herpesvirus conditions, such as HSV epithelial keratitis.¹⁰⁷ Conversely, these herpesvirus miRNAs could assist in the regulation of aberrant Type 1 IFN responses in systemic lupus erythematosus and other diseases.¹⁰⁸

Adaptive immune responses are also circumvented through herpesvirus miRNAs, either directly or indirectly. Inhibition of Type 1 IFN signaling through miRNAs mentioned above can negatively regulate antigen-presenting cell (APC) and CD8 T cell responses.^{109,110} In addition, T cell-attracting chemokines CXCL11 and CCL5 are targeted by EBV and HCMV miRNAs, respectively,^{12,64,102} and HCMV miR-US4-1 downregulates ERAP1 to inhibit MHC Class I peptide loading in the ER.¹⁰³ Correspondingly, inhibition of herpesvirus miRNAs during infection would be expected to modulate APC and T cell responses.

Herpesvirus miRNAs have huge potential as biomarkers of diagnosis and disease progression. As an example, HCMV is the most common infection of patients after organ transplantation and can drastically affect morbidity, mortality, and organ rejection. Diagnostic results assist in monitoring of infection following transplantation and guide decisions to give preemptive therapy.¹¹¹ The current standards to test for acute infection include serology for IgM/IgG titers, an antigenemia assay to test for HCMV pp65 antigen, and quantitative nucleic acid testing (QNAT) using PCR. However, false-positive serology results can occur in patients with EBV or HHV-6 infections, and IgM antibodies take weeks to appear. This underscores the limitations of traditional serology to quickly assess new infection. Moreover, the pp65 antigenemia assay cannot be used in patients with neutropenia, and QNAT is extremely sensitive and faster than culture but does not differentiate between shedding of virus in the absence of active disease.¹¹¹ Certain HCMV miRNAs are expressed at different stages of viral replication and could therefore be used to further monitor primary infection or reactivation from latency. Samples could be easily obtained from biopsies, whole



blood or plasma, known areas of shedding such as saliva, or other fluids such as cerebrospinal fluid. In support of using herpesvirus miRNAs as biomarkers, Kawano et al found that certain EBV miRNAs were elevated in plasma of patients with chronic active EBV infection compared to control patients or patients with infectious mononucleosis.¹¹² A recent study by Zhang et al found that EBV miR-BART7 and miR-BART13 levels in plasma specimens produce a 90% predictive value for NPC. In addition, both miRNAs were downregulated following radiotherapy.¹¹³

Exosomes, which are lipid microvesicles released from cells, are thought to provide extra stability to their encapsulated miRNAs. Exosome miRNA can be separated from free miRNAs, virion-associated miRNAs, or genomic nucleic acid.¹¹⁴ miRNAs have been detected within exosomes in plasma from human patients with KSHV and from EBV+ LCL and NPC cell lines.^{69,114–116} Exosome-associated miRNAs in plasma could provide more stable samples for analysis, leading to better diagnostics to monitor active infection or herpesvirus-associated malignancies.

Although some herpesviruses exhibit conservation in their miRNA seed regions, diagnostic miRNA microarrays could provide a means to differentiate between herpesvirus infections that cause similar conditions, such as roseola infantum caused by HHV-6B or HHV-7. The plausibility of this idea is supported by a study by Marshall et al that showed that the majority of KSHV-encoded miRNAs were highly conserved within clinical isolates.⁶⁹ Because reactivation from latency is a critical step in the replication of herpesviruses, comparison of lytic versus latent miRNAs may also be useful in diagnosis, monitoring, and treatment. Although excellent work has been done to elucidate the biological functions of the miRNAs present during herpesvirus infection, additional serum profiling studies from infected individuals need to be performed in order to have a more thorough understanding of which viral and cellular miRNAs are present during the different stages of herpesvirus infection.

Polyomaviruses. Another family of double-stranded DNA viruses that replicate in the nucleus is the *Polyomaviridae*. The family is divided into three genera, two of which infect mammalian hosts while the third infects avian hosts. A curiosity of the polyomaviruses lies in the fact that humans exhibit subclinical persistent infection at very high rates in the population, with primary infection occurring at a young age. It is unclear whether or not there is a true latent stage, but

asymptomatic individuals routinely shed polyomavirus particles in their blood and urine.

Infections of humans with polyomaviruses can result in serious diseases, although it is usually quite rare. For example, infection with Merkel cell polyomavirus (MCPyV) can lead to Merkel cell carcinoma¹¹⁷ and BK polyomavirus (BKPyV) can cause polyomavirus allograft nephritis, polyomavirus hemorrhagic cystitis, and bladder cancer in some cases.^{118–125} Additionally, JC polyomavirus (JCPyV) can cause progressive multifocal leukoencephalopathy (PML) and has been associated with cases of colorectal cancer,^{126,127} while trichodysplasia spinulosa-associated polyomavirus can cause trichodysplasia.¹²⁸

As nuclear DNA viruses, polyomaviruses are good candidates to encode miRNAs. Unlike the herpesviruses that each encodes a handful of miRNAs, most or all polyomaviruses encode a single pre-miRNA within the large T antigen (LTag) gene (Table 3). Although the exact location of the pre-miRNA varies, it is always oriented in the opposite direction of the LTag and is transcribed as part of late transcription events. Two mature miRNAs are produced from the pre-miRNA that downregulate the expression of the LTag, which is necessary for the early/middle phase of the infection cycle. Therefore, the main role of the polyomavirus miRNAs appears to be in helping the transition from the early to late phase of the infection cycle.

Cellular targets of polyomavirus miRNAs have also been identified and appear to target immune responses. Both JCPyV and BKPyV miRNAs bind to the 3'-UTR and reduce translation of UL16-binding protein 3 (*ULBP3*) mRNA, a stress-induced ligand of NK cells, in an effort to evade an NK response against the virus.^{129,130} Cytotoxic T cell responses against SV40, specifically cell lysis and IFN- γ production, are reduced indirectly through viral miRNA-induced downregulation of LTag and small T antigen.¹³¹ Other host targets have been predicted in SV40 and MCPyV, but are yet to be shown experimentally.^{132,133}

The 5' and 3' miRNAs encoded by polyomaviruses appear to have potential as biomarkers of infection and are detectable in plasma, urine, brain tissue, colon tissue, and feces.^{134–136} JCPyV miRNA was isolated from both plasma and urine in healthy asymptomatic individuals using qRT-PCR, which was more sensitive in detecting the presence of the virus than traditional serological methods.¹³⁶ JCPyV miRNA has been found in postmortem brain tissues of PML

Table 3. Summary of human polyomavirus-encoded miRNAs.

VIRUS	miRNA	miRBASE NO.	GENOMIC POSITION	TARGET	REFERENCES
JCPyV	jcv-mir-J1	MI0009980	3' end of LTag	LTag, ULBP3	130,134
BKPyV	bkv-mir-B1	MI0009981	3' end of LTag	LTag, ULBP3	130,134
MCPyV	mcv-mir-M1	MI0010647	Middle of LTag	LTag	133,138



patients,¹³⁴ and miR-J1-5p was also detectable in colon tissue and feces of healthy subjects.¹³⁵ Interestingly, miRNA expression was lower in diseased versus normal adjacent tissue and inversely correlated with expression of LTA_g, possibly supporting its role in downregulating expression of the protein.¹³⁵ Currently, serology for JCV proteins is most commonly used to indicate infection, but together with the apparent stability of the miRNA,¹³⁴ the ease in obtaining polyomavirus miRNAs from patient samples indicates that they may be powerful biomarkers for detecting asymptomatic forms of polyomavirus infections. On the other hand, using host-encoded miRNAs that are induced by PyV infection as potential biomarkers may not be as fruitful: in a miRNA profiling study, Lagatie et al found that there was no significant difference in host-encoded miRNAs between individuals with or without JCPyV.¹³⁷

Using polyomavirus miRNAs or their respective antagonists (complementary oligonucleotides used to silence miRNAs) as therapeutics will most likely be difficult. A major problem lies in the fact that most individuals are infected asymptotically by PyVs and never develop serious health consequences. Later, when the virus is assumed to be in a latent form of infection, the miRNA is not being expressed and thus there is no target to antagonize. In cases where tumorigenesis has occurred and a proviral form exists, the virus is likely to be expressing only E genes,¹³⁸ which does not include the miRNA. Since tumorigenic activity is believed to largely come from the action of LTA_g,¹³⁹ it is interesting to speculate that the PyV miRNA could serve as a therapeutic in malignant cells to theoretically reduce the amount of LTA_g expressed. However, this is also likely to induce the lytic cycle, which may have its own unintended consequences.

Hepatitis B virus. The hepatitis B virus (HBV) is a small, enveloped DNA virus belonging to the *Hepadnaviridae* family. The primary cellular target of infection by HBV is human hepatocytes. It is estimated that as many as 400 million people may be chronically infected worldwide^{140,141} with up to 1 million annual deaths, as a result. Not only can chronic HBV infection lead to hepatitis or cirrhosis but also is the cause of up to 80% of the cases of hepatocellular carcinoma (HCC), the fifth most commonly diagnosed cancer and the second most common cause of cancer death in the world.¹⁴²

As a DNA virus that enters the nucleus, HBV is a good candidate to encode miRNAs. Nevertheless, no HBV miRNAs have been verified experimentally, and only one has been identified through computational methods.¹⁴³ On the other hand, there are a large number of host-encoded miRNAs described that interact with HBV and are differentially expressed during the course of infection (Table 4). Additionally, miRNAs have been identified that are specifically involved in the progression of HBV-related diseases.¹⁴⁴ Thus, cellular miRNAs, rather than viral miRNAs, may have the potential to become powerful biomarkers of HBV pathology.

The most studied miRNA involved in the course of HBV infection is perhaps miR-122. It represents 50–70% of the

miRNAs found in normal liver cells^{145–147} and plays a role in maintaining homeostasis, as well as regulating metabolic pathways involving lipid and cholesterol metabolism.^{148,149} miR-122 has been shown to downregulate cyclin G1 and heme oxygenase 1 (HMOX1), resulting in inhibition of HBV replication.^{150–152} Additionally, Chen et al found that miR-122 has viral mRNA target sequences located in the HBV coding region of the reverse transcriptase and the 3'-UTR of the HBcAg core protein.¹⁵³ The virus counters with production of X protein, which binds peroxisome proliferator-activated receptor gamma to inhibit the transcription of miR-122.¹⁵⁴ In addition, all four HBV mRNAs have complementary sites for miR-122 that act as sponges to sequester endogenous copies of the miRNA.¹⁵⁵

Not surprisingly, miR-122 is downregulated in a number of HBV-associated hepatic cancers.¹⁵⁶ Fan et al showed that miR-122 negatively regulates tumor-promoting N-myc downstream-regulated gene 3, which is upregulated in HCC+ patient liver samples compared to normal adjacent tissue.¹⁵⁷ The biological importance of miR-122 was further emphasized when the malignant phenotype of an HBV-related HCC cell line could be reversed by transient transfection of miR-122 into the cells.¹⁵⁷ Inhibition of miR-122 also leads to the increased expression of pituitary tumor transforming gene binding factor, resulting in liver cancer cell proliferation, invasion, and tumor growth.¹⁵⁸

Other cellular miRNAs are targeted in a similar manner by HBV. Two well-known tumor suppressor miRNAs, miR-15a and miR-16-1, are found in multiple human cancers and are decreased in HBV-infected cells by HBV X protein.^{159–161} As occurs with miR-122, HBV mRNAs have a site complementary to miR-15a and miR-16-1 and act as sponges to sequester the miRNAs. As a result, the oncogene Bcl-2, a regulatory target of miR-15a/16-1, was increased significantly in HBV-transfected cells.¹⁶² Viral targeting of these miRNAs, specifically miR-15a, likely evolved in response to the ability of the host miRNA to target HBp and HBx transcripts to suppress HBV infection.¹⁵⁹

Several other cellular miRNAs are induced by infection, although not all result in a benefit to the host. HBsAg expression and HBV proliferation are directly suppressed by miR-199a-3p and miR-210,¹⁶³ and miR-125a-5p downregulates the expression of HBsAg.¹⁶⁴ On the other hand, Zhang et al showed that miR-1 indirectly enhances HBV replication by targeting histone deacetylase 4 and E2F transcription factor 5, the activation of which arrests the cell cycle and reverses the cancer phenotype.¹⁶⁵ In support of this, it has been found that miR-1 is epigenetically regulated and found in lower levels in HCC cells.¹⁶⁶ Paradoxically, the HBV HBx protein promotes the expression of cellular miR-148a, which enhances tumorigenesis and promotes cell proliferation, cell migration, and anchorage-independent growth of HepG2 and Hep3B cells.^{167,168} Further research is needed to discern the relationship between tumorigenic and antitumorigenic miRNAs during HBV infection.

Traditional detection methods and notable miRNA biomarkers of HBV infection. Alanine aminotransferase (ALT) and

**Table 4.** Host miRNAs involved in HBV-related health conditions.

miRNA	TARGET	EFFECT	REFERENCES
miR-1	FXRA, HDAC4, MET	Increase HBV transcription	165,166
miR-15a/16	HBV RNA, BCL2, CCND1 (host), NCOR2	HBV RNA is sponged; prevents apoptosis	159,161,162,364
miR-17/92 cluster	HBV RNA, E2F1, E2F3	Promotes HCC proliferation	365,366
miR-18a	ESR1	Promotes HCC proliferation	367
miR-21	PTEN, PDCD4		366,368,369
miR-22	HDAC4, ESR1, CDKN1 A	Inhibits HBV replication	370–372
miR-23b	PLAU, MET		373
miR-26a	ESR1, IL6, CCND2, CCNE2		374,375
miR-29a	PTEN		376
miR-29c	TNFAIP3	Tumor suppressor	377
miR-34a	CCL22, MET	Inhibits HBV	378
miR-99a	IGF1R, MTOR		379
miR-101	DNMT3A, FOS, MCL1, EZH2		380–384
miR-122	CCNG1, HMOX1, HBV mRNA	Inhibits HBV replication and translation; increased HBV core stability	153,154,156,160,161
miR-125a-5p	HBV S gene, ERBB2	Reduced expression of HBsAg; inhibits cell proliferation	164,385–387
miR-141	PPARA	Inhibits HBV replication	388
miR-143	FNDC3B		389
miR-145	HDAC2, ADAM17		390,391
miR-146a	STAT1	Promotes HBV infection	392
miR-148a	DNMT1, HPIP, MET	Loss of repression of HBV transcription	168,240,393
miR-152	DNMT1	Loss of repression of HBV transcription	240,394
miR-155	CEBPB, SOCS1, SOX6	Decreases HBV transcription; increases host immune response	395,396
miR-199-3p	PAK4, MTOR, MET, HBsAg mRNA	Inhibits HBV replication	163,397–399
miR-199a	HBV S region	Inhibits HBV replication	163
miR-205	HBx mRNA	Inhibits HBV	400
miR-210	AIFM3, HBsAg mRNA, HBV Pre-S1 region	Reduces HBsAg; inhibits HBV replication	163
miR-222	PPP2R2 A		401
miR-224	API5, ARHGAP9, ARHGAP12, SMAD4	Inhibits apoptosis; promotes metastasis	402–404
miR-372	PRKACB, NF1B	Decreases HBV transcription; increases HBV expression	405,406
miR-501	HBXIP	Promotes HBV replication	407
miR-548	IFNL1	Promotes HBV infection	408
miR-602	RASSF1 A	Oncogenic	409
let-7 family	STAT3, COL1A2, MYC, NGF, BCL2L1, RAS, HMGA2	Increases cell proliferation	364,410,411

aspartate aminotransferase (AST) are the most commonly used biomarkers to assess liver damage,¹⁶⁹ although levels can be altered through conditions unrelated to the liver.^{170,171} A more definitive diagnosis can be obtained by liver biopsy, but biopsy is costly, inconvenient, and may not be accessible to all individuals.¹⁷² Furthermore, complications such as pain, mental and physical anguish, bleeding, and even death are possible.^{172–174} Another problem with liver biopsy lies in the

sampling nature of the biopsy itself. Typically, liver samples taken at biopsy represent 1/50,000th of the total liver, which could result in random sampling error.¹⁷² While ultrasound is a tempting noninvasive alternative and good for assessing late stage liver damage, it is not very useful in assessing earlier stages of disease.¹⁷⁵

For specifically identifying HBV as a causative agent of liver damage, current serological methods consist of antibody



Table 5. Host miRNAs reported to be differentially expressed in serum/plasma during HBV infection.

miRNA UPREGULATED	miRNA DOWN-REGULATED	REFERENCES
miR-10a, miR-23a/b, miR-99a, miR-122, miR-150, miR-223, miR-342-3p, miR-375, miR-423, miR-572, miR-575, miR-638	miR-15a, miR-16-1, miR-21, miR-744	177,178,180, 182,183, 412–414

tests for HBsAg and HBeAg antigens. PCR assays may also be used for direct determination of HBV genomic DNA in serum. For identification of HBV in tissues, detection of HBsAg and HBeAg by immunohistochemical staining or HBV DNA by Southern hybridization, in situ hybridization, or PCR is performed.¹⁷⁶

Considering the above challenges, miRNAs have emerged as an alternative biomarker method with the possibility of monitoring the progression of disease in individuals. Multiple studies have already shown that noninvasive testing for miRNAs has the potential to be used as biomarkers of HBV infection and HBV-positive HCC.^{177,178} A number of specific, differentially expressed host miRNAs have been identified in the last decade; a partial list is shown in Table 5.

One of the most widely used miRNA biomarkers for HBV is miR-122, not surprising due to it being one of the most thoroughly studied miRNAs in HBV infection and liver disease. Waidmann et al found that the serum levels of miR-122 were effective as a biomarker in HBV-infected patients as they discriminated infected from healthy subjects, discriminated inactive carrier patients with high or low levels of HBsAg, and correlated with the levels of ALT, HBV genomic DNA, and HBsAg.¹⁷⁹ It has also been shown that both miR-122 and miR-18a are released in the blood and could be used for HBV-related HCC screening.^{160,179,180} An miRNA profiling study on HBV and hepatitis C virus (HCV) found that miR-122 was significantly upregulated in serum of patients with both viruses, whereas elevated miR-22, miR-99, and miR-125b levels were more characteristic of chronic HBV infection and may be useful in discriminating between infection with the two viruses.¹⁸¹

Other panels of miRNAs have also been found to be differentially expressed between healthy and chronic HBV patients. Zhang et al identified 34 miRNAs dysregulated in chronic hepatitis B patients, with miR-122, miR-572, miR-575, and miR-638 upregulated and miR-744 downregulated significantly.¹⁸² A study examining miRNAs in the plasma of chronically infected children identified 16 miRNAs to be upregulated in HBeAg+ compared to children with HBeAg-: miR-99a, miR-100, miR-122, miR-122*, miR-125b, miR-192, miR-192*, miR-193b, miR-194, miR-215, miR-365, miR-455-5p, miR-455-3p, miR-483-3p, miR-885-5p, and miR-1247.¹⁸³ Another miRNA serum profiling study also identified miR-99a-5p, miR-122-5p, and miR-192-5p as

significantly overexpressed between chronic hepatitis B adult patients and inactive carriers. Using an MiR-B-Index that normalized these three miRNAs to internal control miRNAs (miR-126, miR-320a, and miR-335), the same study showed that the serum miRNA profile of patients responding to pegylated (PEG)-IFN alpha resembled that of inactive carriers, while nonresponders and relapsers matched baseline measurements of patients with chronic hepatitis B.¹⁸⁴ This comprehensive study illustrated that miRNAs could be useful not only in assessing disease status (inactive carriers vs. chronic hepatitis B patients) but also in identifying responders to certain treatments, such as PEG-IFN. The study also emphasized that although a single biomarker is desirable for simplicity and financial reasons, panels of biomarkers can also be effective in determining disease state and response to treatment. Further support for biomarker panels is provided by several studies.^{177,185,186} Notably, Li et al used a panel of 13 miRNAs to distinguish control patients from those with HBV infection (miR-10a, miR-223, miR-375, and miR-423), control patients from those with HBV-HCC (miR-23a, miR-23b, miR-92a, miR-342-3p, miR-375, and miR-423), patients with HBV from those with HCV infection (miR-92a up in HCV and miR-375 up in HBV), and patients with chronic HBV from those with HBV-positive HCC (miR-19a and miR-125b).¹⁷⁷

Liver injury can produce similar biomarker responses, regardless of the causative agent. An interesting miRNA profiling study by Ura et al examined the expression of 188 miRNAs from HBV-HCC, HCV-HCC, and normal patients. They identified 19 miRNAs that were differentially expressed between HBV and HCV.¹⁸⁷ In all, 31 miRNAs were associated with liver disease, regardless of the virus, but 6 were specific for HBV and 13 for HCV. Notably, miR-105, miR-134, and miR-211 were over fourfold upregulated in HBV and miR-34c was over fourfold increased in HCV.¹⁸⁷ However, other studies have produced conflicting results,^{188,189} and thus, the miRNAs identified are likely to need further validation.

Role of miRNAs as therapeutics against HBV. The potential that miRNAs hold to serve as therapeutic agents against HBV has long been recognized,^{190–193} and a number of studies have attempted to employ miRNA to combat HBV *in vivo*. The first *in vivo* result of antiviral RNAi activity used short hairpin RNAs (shRNAs), essentially, the pre-miRNA hairpin structure, in a study that targeted a mouse model of HBV infection using hydrodynamic delivery of HBV gene and shRNA vectors.¹⁹⁴ Several other early studies also demonstrated inhibition of HBV by RNAi.^{195–201} The use of 2'-OH-modified siRNAs was another approach that enhanced stability and inhibited infection in an *in vivo* mouse model of HBV replication.^{202,203} shRNAs were utilized in early studies,^{204–209} but the RNA polymerase III-delivery method commonly used presented toxicity problems to the host.²¹⁰ Thus, modified RNA polymerase II-dependent systems encoding pri-miRNAs were used that were expressed at lower levels due to the requirement for Drosha cleavage. These were not toxic and did not interfere



with the endogenous miRNA production necessary for proper cell function.^{201,211–214}

Multimeric miRNA expression cassettes have been used to effectively target multiple sites within the virus genome using mouse models of HBV infection,²¹⁴ and a common delivery method for the miRNA therapeutic uses adeno-associated virus (AAV) or lentivirus systems.^{215,216} AAV-delivered miR-26a resulted in cell-cycle arrest *in vitro* through direct targeting of cyclins D2 and E2 in HCC cells. In addition, systemic intravenous administration of the AAV-miR-26a led to reduced HCC cell proliferation and tumor-specific apoptosis.²¹⁷ In an HBV-HCC model, tumor growth was significantly decreased after lentiviral transduction of miRNA targeting HBsAg into HBV+ HepG2.2.15 cells before injection into nude mice.²¹⁸ These and other experiments provide proof of concept that miRNAs downregulated in cancers could be reconstituted to normal levels to reduce tumor growth and that viral genes involved in tumor progression could be targeted by engineered miRNAs.

Hepatitis C virus. The HCV is a small, enveloped, positive-strand RNA virus belonging to the *Flaviviridae* family. There are an estimated 170 million people infected worldwide, with up to 500,000 deaths annually as a result. The primary tropism of the virus is for human hepatocytes. Approximately 80% of those initially infected are asymptomatic, but only 15–25% of initial infections are cleared by the host immune

system. The remainder results in chronic infections, 15–30% of which will eventually progress to cirrhosis in ~20 years. HCV chronic infection is also a significant cause of HCC.¹⁴²

As a cytoplasmic RNA virus, HCV is unable to encode miRNAs using traditional nuclear processing machinery, and no HCV-encoded miRNAs have yet been described. Additionally, encoding a miRNA within its genome would be a risky strategy for an RNA virus, as the miRNA processing cell machinery might target the genome for cleavage. HCV does encode suppressor of RNAi silencing proteins via the HCV core protein and envelope protein E2,^{219,220} and a plethora of host-encoded miRNAs have been described that interact with HCV and are differentially expressed during the course of an infection. In fact, as the disease progresses from initial infection all the way to HCC, corresponding changes in miRNA levels have been detected in profiling studies.^{187–189,221–223} If a reproducible pattern of specific host miRNAs could be documented as disease progression occurs, it could identify miRNAs as powerful biomarkers of HCV pathology. Table 6 provides a partial list of miRNAs shown to be differentially expressed or found to interact with HCV during infection.

Like HBV, most of the attention regarding miRNAs and HCV has centered around miR-122, which, as mentioned above, represents 50–70% of the miRNAs found in normal liver cells.^{145–147} It is important to note that while miR-122

Table 6. miRNAs involved in HCV infection.

miRNA	TARGET	EFFECT	REFERENCES
miR-21	SMAD7	Increases fibrogenesis	415
miR-26a	CCND2, CCNE2	Inhibits cell proliferation	217
miR-27a	RXRA, ABCA1	Reduces production of HCV virions	416
miR-29	Unknown	Loss leads to fibrosis	417
miR-30d	GNAI1	Promotes metastasis	418
miR-101	MCL1, FOS	Promotes apoptosis	383
miR-122	5' UTR of HCV genome, XRN1, AGO2, CCNG1, SOCS3 promoter, ADAM17	Promotes replication, protects HCV 5' UTR, stabilizes HCV 5' UTR, promotes HCV replication, inhibits metastasis	155,225,226,228,229,419–421
miR-124	ROCK2, EZH2, SMYD3	Tumor suppressor	422,423
miR-130a	IFITM1	Increases HCV replication	424
miR-139	ROCK2	Inhibits metastasis	425
miR-141	DLC1	Increases HCV replication	256
miR-155	APC	Promotes proliferation and tumorigenesis	426
miR-194	CD81	Inhibits HCV entry	427
miR-196 family	HCV NS5A, BACH1	Inhibits HCV replication and RNA/protein expression	237,238
miR-199a	HCV 5' UTR IRES	Inhibits HCV replication	236
miR-221	CDKN1B, CDKN1C, BMF	Promotes cell proliferation, inhibits apoptosis	428,429
miR-448	HCV core region	Inhibits HCV replication	238
miR-449a	NOTCH1	Promotes inflammation	430
let-7b	HCV NS5B and 5' UTR	Inhibits HCV infection	431
let-7g	MYC, CDKN2A, COL1A2	Inhibits proliferation, suppresses metastasis	410, 432

**Table 7.** Host miRNAs upregulated during HCV infection.

COMPARTMENT	miRNA UPREGULATED	REFERENCES
Serum	miR-20a, miR-92a, miR-122, miR-134a, miR-320c, miR-483-5p	181,433,434
Infected cell lines	miR-192, miR-193b, miR-194, miR-215, miR-585, miR-768-5p	222,435,436

is an inhibitor of infection by HBV,^{150,151,153,157} it *aids* in the infection of host hepatocytes by HCV. During HCV infection, miR-122 is upregulated and protects the HCV genome by binding to and masking the 5'-UTR, protecting it from degradation, and promoting overall replication.^{224–226} While bound to the HCV 5'-UTR, miR-122 can also recruit Ago2 to its 5' end, which further stabilizes the genome and stimulates translation of HCV proteins.^{227–229} Furthermore, miR-122 protects the 5' end from degradation by Xrn1 exonuclease.¹⁵⁵ The net result is an increase in HCV replication and translation of HCV proteins.

Traditional detection methods and putative biomarkers of HCV infection. The initial testing for HCV is usually done by enzyme immunoassay to detect host antibodies directed against HCV.²³⁰ Liver damage associated with HCV is also screened for using ALT and AST enzyme tests.^{230,231} Positive results in initial screenings are usually confirmed via molecular testing to detect the presence of HCV RNA, sometimes followed by patient biopsy to assess the extent of liver damage, if necessary.²³⁰ As described above for HBV, liver biopsies are not without serious drawbacks.^{173,174}

Algorithms are also used that include a variety of additional criteria and biomarkers such as age, sex, levels of other liver enzymes, or platelet numbers. Examples include Firbo-Test and HepaScore.^{232,233} Unfortunately, many results fall in the *indeterminate* category or do not represent liver-specific injury. Several studies have suggested that miRNAs may be of great utility in differentiating liver diseases, and one study in particular showed miRNAs to be more discriminating than AST/ALT while differentiating between liver and muscle injury.²³⁴

Compared to standard diagnostic methods, the potential advantages of using miRNAs as HCV biomarkers include the noninvasive nature of retrieval, generation of fairly rapid results, and possible lower costs compared to other methods. Profiling studies are often used as the first step in identifying candidate miRNAs that could serve as biomarkers, and there are already several studies that compare HCV-related disease

states and healthy individuals.^{187–189,221–223,235} These studies have begun to reveal the potential of miRNAs to be a useful tool for diagnosing HCV-related disease as well as monitoring the progression or regression of the disease.

Several miRNAs are differentially regulated as a result of HCV or HCV-induced conditions (Tables 7 and 8). Notably, studies have demonstrated the utility of using miR-122 to identify HCV, HCC, and hepatocyte injury,^{181,234} and this miRNA is also extensively used to identify HBV status.^{160,177,179} Other host miRNAs directly target HCV genomic sequences: miR-199a reduces HCV replication by targeting the 5'-UTR of the genome internal ribosome entry site (IRES) region.²³⁶ Additionally, the expression of miRNA-196b appears to be upregulated by the IFN pathway and can thus inhibit HCV by directly targeting NS5A or by indirectly targeting BACH1 and HMOX1.^{237–239} However, there have been conflicting results reporting whether it is upregulated or downregulated during infection,^{239–241} possibly making its implementation as a biomarker difficult. As a way of effectively distinguishing HCV versus HBV infection, miR-92a and miR-375 are more highly upregulated in HCV+ or HBV+ patients, respectively. miR-10a and miR-125b are not upregulated in HCV+ but highly upregulated in HBV+ individuals,¹⁷⁷ although miR-10a is upregulated in HCV-HCC cases.²²³ Several biomarkers overlap in these and other non-viral liver conditions, emphasizing the importance of biomarker panels to properly differentiate these diseases.

miRNAs as therapeutics for HCV. Current therapeutics for HCV include the use of PEG-IFN and ribavirin,^{242–244} and more recently, sofosbuvir, ledipasvir, and boceprevir have been used effectively.^{245–248} While many therapies are successful, there are still a significant number of patients in whom the virus is not fully cleared.^{249,250} Additionally, therapies are costly, not easily accessible to all infected individuals, and can have severe side effects.^{251,252}

Recently, miR-122 has emerged as a promising candidate in the implementation of miRNAs as therapeutics. The observation that this abundant liver miRNA is upregulated

Table 8. Host miRNAs differentially expressed in liver tissue biopsies during HCV infection, HCC, or HCV-associated HCC.

CONDITION	miRNA UPREGULATED	miRNA DOWNREGULATED	REFERENCES
HCV	miR-34c, miR-130a, miR-141, miR-155	miR-29, miR-449a	187,256,417,424,426,430
HCC	miR-181, miR-199a, miR-221, miR-301	miR-29, miR-101, miR-139, let-7g	188,383,410,425,429,437,438
HCV-HCC	miR-10a, miR-21, miR-27a, miR-100, miR-122, miR-155	miR-122, miR-124, miR-145, miR-198	188,223,224,254,364,415,416,423,426



and promotes replication during HCV infection made it an obvious choice to antagonize therapeutically. A small effector molecule against miR-122 inhibited HCV replication in liver cells,²⁵³ and Lanford et al used an antisense locked nucleic acid (LNA; an oligonucleotide with greater strength of binding due to chemical modification) derivative of miR-122 as a therapeutic in HCV-infected chimpanzees. A sharp reduction in HCV RNA and improved liver histology was observed without significant side effects or detectable resistance from the virus.²⁵⁴ Phase 1 clinical trials of miravirsen, an LNA that targets miR-122, were completed in 2009 by sponsor Santaris Pharma. In Phase 2a studies of null responders to PEG-IFN with HCV genotype 1 infection, miravirsen reduced mean HCV RNA levels up to three orders of magnitude with no drug-associated severe or serious adverse events.²⁵⁵ Notably, HCV RNA levels increased following cessation of the drug. Long-term administration studies are ongoing (ClinicalTrials.gov identifiers NCT01727934 and NCT02031133). These results will possibly usher in a new era of miRNA-based biologics.

While the results using miR-122 are promising, there are still several other miRNAs worth considering to this end. Pedersen et al described mimics of five miRNAs (miR-196, miR-296, miR-351, miR-431, and miR-448) that reduced HCV replication and infection when transfected into HCV+ cells.²³⁸ Antagomir-mediated knockdown of miR-141 effectively inhibited HCV replication in infected hepatocytes.²⁵⁶ Moreover, the mimics of three miRNAs, miR-196b, miR-199a-3p, and miR-29, have been expected to function in anti-HCV therapy.²⁵⁷

An interesting related approach was considered by Yang et al who created artificial miRNAs based on five preselected targets on the HCV genome. When introduced into cells, they dramatically reduced the replication of cell culture-propagated HCV without causing hepatocellular toxicity.²⁵⁸

This may suggest that knowledge of the viral target sequence may be just as valuable, if not more so, than targeting naturally occurring miRNAs. Since artificial miRNAs and their derivatives are relatively easy to create and introduce into a host, this could help alleviate the need for extensive profiling studies to identify aberrantly expressed miRNAs as targets.

Human papillomavirus. HPV is a small, non-enveloped, double-stranded DNA virus that infects epithelial cells. Over 120 different types have been identified. While most infect cutaneous epithelium, ~40 types infect mucosal epithelium, some of which are associated with oncogenesis. Types 16 and 18 together account for ~70% of cervical cancer cases. HPV is also associated with less common cancers, including cancer of the anus, penis, vulva, and vagina. In the United States, HPV is the most common sexually transmitted disease. The virus is also associated with the vast majority of head and neck cancers in nonsmokers.²⁵⁹

The majority of HPV infections are eventually cleared from the host, but a small proportion result in persistent infections that can progress to cervical intraepithelial neoplasias (CINs). High-grade CINs (CIN2 or CIN3) are considered a precursor to cervical cancer, which may occur within years or decades.²⁵⁹ As such, much effort has been invested to characterize the role of the virus in oncogenesis, particularly pertaining to the roles of viral oncoproteins E5, E6, and E7.

Recently, host miRNAs have also been implicated in the process. Several miRNAs have been identified as specifically targeted by E5, E6, or E7 (Table 9), and their subsequent cellular effects in many cases have been characterized. E6 targets p53,^{260,261} a transcription factor that is thought to be used for transcription of many cellular miRNAs.^{262,263} As a result, many miRNAs may be drastically underexpressed and might lead to adverse cellular effects. miR-23b, miR-34a, miR-203, and miR-218 are all found to be downregulated

Table 9. HPV proteins known to alter host miRNAs.

HPV PROTEIN	miRNA TARGET	EFFECT (UP/DOWN)	POTENTIAL CELLULAR EFFECT	REFERENCES
E5	miR-146a	Up	Suppression of immune response, increased cell proliferation	291
	miR-203	Down	Deregulation of p63–increased cell proliferation	291
	miR-324-5p	Down	Increased N-Cadherin and E-Cadherin	291
E6	miR-23b	Down	Increase of uPA–induces migration of human cervical cancer cells	264
	miR-34a	Down	Increased cell proliferation and transformation	265–267,327
	miR-203	Down	Increased cell proliferation	268
	miR-218	Down	Deregulation of LAMB3–increased cell migration and tumorigenicity	269
	miR-15a/16	Up	pRB degradation, altering c-myc, PPAR, c-myc levels	326
	miR-15b	Up	Reduced Cyclin E1–inhibits proliferation	288
	miR-203	Down	Blocks MAPK/PKC; increases ΔNp63 activity–increases proliferation	290,439
	miR-205	Up	Decreases Akt pathway and Cyclin D1 levels–decreased proliferation	289
E6/E7	miR-21	Up	Targets CCL20–enhances tumorigenesis	440
	miR-24	Up	Decreases p27–increases proliferation	289



in E6-expressing cells,^{264–269} which is particularly noteworthy because of the association of these miRNAs with oncogenic processes, in part through direct or indirect effects of p53 inhibition.

Although all the above miRNAs have been implicated in cervical cancer, they have also been reported as tumor suppressors in a variety of other cancers: miR-23b in prostate, bladder, and breast cancers^{270–272}; miR-34a in neuroblastomas, HCC, and ovarian, colon, pancreatic, and bladder cancers^{266,273–276}; miR-203 in lung, prostate, and esophageal cancer, as well as leukemia and glioma^{277–279}; and miR-218 in prostate, colon, gastric, and bladder cancer.^{280–282}

E7 degrades the tumor suppressor protein pRB, which releases E2F from the pRB–E2F complex,²⁸³ freeing E2F to activate transcription. The promoter regions of several miRNAs contain E2F binding sites,^{280,284,285} which could possibly lead to adverse effects on the cell if oncogenic miRNAs are transactivated. In contrast to E6-induced miRNAs, most of the miRNAs shown to be dysregulated through the action of E7 are upregulated, such as miR-15b/16-1, miR-24, and miR-205.^{286–289} The downregulation of miR-203 has been credited to the indirect blocking of the MAPK/PKC pathway by E7.²⁹⁰

Less is known about the actions of E5 on cellular miRNAs. A recent study found that several miRNAs were altered

in HPV16 E5-expressing HaCaT cells.²⁹¹ Notably, the authors of that study found that miR-146a was induced, while miR-203 and miR-324-5p were repressed. miR-146a and miR-203 had previously been shown to be dysregulated in the same manner in cervical cancer tissues or HPV+ cell lines.^{290,292–300}

miRNAs as potential biomarkers of HPV infection and progression. It is suspected that the expression of many miRNAs may be altered during the course of HPV infection, possibly contributing to oncogenesis. Several studies have examined miRNAs dysregulated in cervical cancer or head and neck squamous cell carcinoma (HNSCC), two cancers that are likely to be the result of HPV infection. This section of our review focuses on those fewer studies that specifically addressed HPV+ cell lines or HPV+ cancer cells/tissues for the purposes of identifying HPV-specific biomarkers (Table 10), pointing out when individual miRNAs have also been identified in cancerous cells or tissues with a possible contribution by HPV.

miR-21 has been implicated as an oncogenic miRNA for many cancers.³⁰¹ Indeed, almost all profiling studies to date of cervical and head and neck cancers have found miR-21 to be overexpressed in the cancerous state,^{292,293,296,300,302–314} and miR-21 has been shown to be upregulated in HPV-related cancers as well.^{308,315} Lajer et al compared HPV+ and HPV–

Table 10. Selected miRNAs identified in HPV-associated conditions.

CHANGE	miRNA	DISEASE/SOURCE	REFERENCES
Upregulated	miR-15b	HPV+ cervical tissue	286
	miR-16	HPV+ cervical tissue	286
	miR-21	HPV+ TSCC tissue	315
	miR-31	OSCC tissue (HPV±), HPV+ cervical cell lines	269,318
	miR-34c	HPV+ cervical cell lines	269
	miR-146a	HPV16 E5-transfected keratinocytes	291
	miR-181c	HPV+ keratinocytes	441
	miR-200c	HPV+ cervical cell lines	269
	miR-203	HPV+ cervical cell lines	269
	miR-205	HPV+ cervical tissue	286
	miR-363	HPV+ HNSCC cell lines, PSCC tissue (HPV±), HPV+ TSCC tissue	315,318,319
	miR-497	HPV+ HNSCC cell lines	319
	Downregulated	miR-31	HPV+ TSCC tissue
miR-34a		HPV+ cell lines, HPV+ cervical tissues	275,326,327
miR-127-3p		PSCC tissue (HPV ±), HPV+ cervical tissue	318,442
miR-143		HPV+ cervical tissue, HPV+ cervical cell lines	269,286
miR-145		HPV+ cervical tissue, HPV+ cervical cell lines	269,286
miR-155		HPV+ HNSCC cell lines	319
miR-181a		HPV+ HNSCC cell lines	319
miR-203		HPV16 E5-, E6-, or E7-transfected keratinocytes	290,291
miR-218		HPV+ cervical cell lines, HPV+ HNSCC cell lines, HPV+ cervical tissue	269,319,442,443
miR-324-5p		HPV16 E5-transfected keratinocytes	291
miR-375		OSCC tissue (HPV±)	318



patients with tonsillar squamous cell carcinoma (TSCC) and found miR-21 to be upregulated in HPV+ patients. Additionally, miR-21 was upregulated in HPV+ or HPV- HNSCC as well as HPV+ cervical squamous cell carcinoma (CSCC),³¹⁵ implying that it may serve as an HPV-specific biomarker as well as a general cancer biomarker. The fact that miR-21 overexpression is detectable in plasma makes it even more appealing as a biomarker.³⁰⁸

Like miR-21, miR-31 is detectable in saliva³¹⁶ and has been implicated as an oncogenic miRNA.³¹⁷ Several oral cancer tissue profiling studies have found miR-31 to be upregulated,^{293,304,309,310} and it has also been documented to be upregulated in HPV+ cancers. A study comparing HPV+ oral squamous cell carcinoma (OSCC) patients to HPV- control patients found that miR-31 was the most upregulated miRNA of OSCC biopsies.³¹⁸ Interestingly, a follow-up study by the same group comparing miRNA expression in HPV+ or HPV- TSCC tissue revealed that miR-31 was one of the most downregulated miRNAs in HPV+ TSCC,³¹⁵ further indicating the potential utility of the miRNA in the differential diagnosis of HPV+ carcinomas.

Other interesting miRNAs were revealed in this study of HPV+ TSCC, including the upregulation of miR-363 and downregulation of miR-375. In accordance with these results, miR-363 has also been shown to be upregulated in both HPV+ and HPV- pharyngeal squamous cell carcinoma (PSCC) and in HPV16+ HNSCC cell lines.^{318,319} miR-375 has been found to be significantly downregulated in HNSCC and OSCC.^{300,302,311,320}

miR-155 is another miRNA that has been found to be upregulated in several oral cancer profiling studies^{304,310-312,321}; however, it has been documented as being downregulated when comparing HPV- and HPV+ cell lines or oral cancer tissues with control tissues.^{294,319} It is possible that this miRNA may behave differently in tissue culture as compared to actual tissue. This was the case for miR-181a, which was found to be downregulated between HPV- versus HPV+ tissue culture,³¹⁹ despite that several profiling studies using cancerous tissues have identified miR-181a as upregulated.^{294,302,304,321} Nonetheless, the consistent appearance of miR-155 in HPV-related conditions is compelling and worth further investigation.

Similarly, miR-203 has been shown to be upregulated or downregulated in different studies. miRNA was downregulated in keratinocyte cell lines expressing HPV oncoproteins,^{290,291} which is further supported by several profiling studies that used tissues from HNSCC or CSCC.^{294,296-300,306} Martinez et al, however, found miR-203 to be upregulated when comparing HPV+ and HPV- keratinocytes.²⁶⁹ Nonetheless, the repeated appearance of miR-203 in HPV-related conditions warrants its inclusion in further studies as a potential biomarker. It is also noteworthy that it has been detected in plasma in patients with laryngeal squamous cell carcinoma (LSCC).²⁹⁹

Other notably upregulated miRNAs are miR-200c and miR-205. When 28-fold increased, miR-200c was found to be

the most overexpressed miRNA in HPV16+ cell lines compared to HPV- cell lines.²⁶⁹ miR-205 is known to be induced via E7 and has been shown to be upregulated in HPV+ cervical cells²⁸⁶ and in several oral cancer profiling studies, as well.^{304,322-324} However, one study has indicated that miR-205 is downregulated in HNSCC and is significantly associated with poor survival.³⁰³

In addition to the upregulated miRNAs mentioned above, several miRNAs have been shown to be downregulated during HPV infection. The expression of miR-143 and miR-145 is reduced in HPV+ cervical cancer cell lines compared to HPV- cell lines.^{269,286} Both miRNAs are similarly downregulated in tissues from patients with cervical carcinomas, hypopharyngeal squamous cell carcinoma (HSCC), LSCC, or HNSCC.^{297,298,300,304,306,307,311,325} miR-34a is known to be negatively regulated by the HPV E6 protein, and its expression was downregulated in HPV16+ cervical cells and HPV+ cervical tissue.³²⁵⁻³²⁷ It has also been shown to be downregulated in OSCC.^{294,305}

One of the more promising HPV biomarkers may exist in miR-218, also regulated by the HPV E6 protein. It has been identified as significantly downregulated in multiple studies using HPV-infected cells.^{269,300,319} In fact, a study comparing HPV+ versus HPV- cervical cancer cell lines found miR-218 to be (the only) significantly underexpressed miRNA.²⁶⁹ It has been shown to be downregulated in HPV+ squamous cell carcinoma of the head and neck cell lines and in the HSCC tissue. Collectively, these studies identify miR-218 as a potential HPV-specific biomarker.

Human immunodeficiency virus. HIV is an enveloped lentivirus belonging to the *Retroviridae* family. There is much controversy concerning whether HIV actually encodes its own miRNA. As a nuclear RNA virus, HIV might reasonably avoid encoding miRNAs since Drosha-mediated excision of a pre-miRNA from the genome could induce the cleavage of the replicated viral RNA.^{328,329} Furthermore, it has been suggested that any HIV-1 miRNA would need to play an important evolutionary role in viral replication to compensate for the degradation of viral genomic RNAs by Drosha cleavage.¹⁹ On the other hand, HIV-1 might encode functional miRNAs because the viral genomic RNA is reverse transcribed in the cytoplasm into a double-stranded DNA molecule that is then imported into the nucleus for integration into the host genome.³³⁰ As mentioned above, RNA viruses have been successfully engineered to express functional miRNAs without reducing viral genome replication.²¹ Furthermore, bovine leukemia virus, a retrovirus, avoids Drosha-mediated cleavage of its genome by encoding miRNAs that are produced by RNA polymerase III. This creates miRNAs that are too short to be processed by Drosha/DGCR8 complexes and are instead processed directly by Dicer in the cytoplasm.³³¹

In 2004, five putative HIV miRNAs were computationally identified,³³² and it was later reported that an HIV miRNA derived from the *nef* gene, termed miR-N367, targeted the long



terminal repeat (LTR) U3 region.^{333,334} However, two reports subsequently reported that HIV miRNAs – including miR-N367 – were not detectable by conventional sequencing of small RNAs in HIV-infected HeLa cells¹⁸ or persistently-infected ACH-2 T cells.¹⁹ Using *in vitro* studies, Klase et al reported that the HIV-1 TAR element, a hairpin structure ~50 nucleotides in length at the 5' end of the viral genome, was processed by Dicer to create viral miRNA that was capable of inhibiting LTR-driven gene expression. This suggested a post transcriptional silencing method that could contribute to viral latency.¹⁵ Primer extension and RNase protection assays derived a TAR miRNA duplex that encoded miR-TAR-5p and miR-TAR-3p,¹⁷ and in another study by Klase et al, HIV-1 miR-TAR-3p/5p was found to protect infected cells from apoptosis.¹⁶ Another HIV-encoded miRNA, miR-H3, was recently identified through computational prediction and deep sequencing. Deletion assays suggested that it targeted the HIV 5' LTR TATA box to activate viral transcription.³³⁵

Deep sequencing studies of HIV-infected T cells identified several HIV small RNAs, including an 18-nucleotide RNA antisense to the HIV tRNA primer binding site.³³⁶ Similarly, Schopman et al used deep sequencing to identify small RNAs from HIV-infected T cells. One set of small RNAs originated from secondary hairpin structures formed by the viral genomic RNA and were attributed as a class of miRNAs. Although they were found to be in very low proportion in the total small RNAs, three of them were able to prevent luciferase activity when cloned into a reporter construct, including the previously reported TAR miRNA. Antagomirs also increased HIV replication of infected 293T cells.³³⁷

A recent study by Whisnant et al pointed out that miRNAs <22 nucleotides are more consistent with RNA breakdown products than other miRNAs and that a decisive seed region on the 5' end of the miRNA must be considered.²⁰ They also highlighted that low-frequency miRNAs, representing <0.1% of the total miRNA pool, are not likely to be biologically relevant.³³⁸ They examined small RNAs in two HIV-infected cell lines, human primary CD4+ peripheral blood mononuclear cells (PBMCs), and human macrophages, and did not identify any HIV miRNAs.²⁰ By using PAR-CLIP, they found a small subset of expressed cellular miRNAs that exhibited complementarity to HIV genome sequences. However, they determined that these miRNAs are largely unable to interact with the HIV RNA genome due to extensive viral secondary structures. Since HIV-1 has been implicated in the induction of several cellular miRNAs from infected cells (described below), this finding generates another layer of controversy in the matter. Together, these studies emphasize that additional confirmatory evidence is needed to establish a body of evidence to support or refute whether HIV-1 indeed encodes its own miRNA.

Cellular miRNAs induced by HIV-1 infection. Studies have begun to shed light on the involvement of cellular miRNAs during HIV-1 infection. As mentioned above, additional

studies will be needed to verify the biological relevance of these antisense oligonucleotides at the concentrations found in infected cells. For biomarker discovery, the relative presence or absence of miRNAs (or breakdown products) during infection is a more important consideration than their absolute concentration, as long as they are consistently detectable in a validated manner.

During HIV infection, several cellular miRNAs have been reported to be modulated that indirectly impact the replication of the virus. In 2007, Triboulet et al used microarray studies of HIV-infected Jurkat or PBMC to identify 11 cellular miRNAs that were upregulated upon infection and were dependent upon Drosha and Dicer machinery. Viral replication occurred faster in the absence of these miRNAs. Notably, miR-122, miR-297, miR-370, and miR-373* were only expressed in HIV+ cells, while the miR-17/92 cluster (that includes miR-17-5p/3p, miR-18, miR-19a, miR-19b-1, miR-20a, and miR-92-1) was downregulated.³³⁹

A challenge of using PBMCs to examine HIV-associated cellular miRNAs is that miRNAs are expressed differently in the various peripheral blood cells in the context of different environments. In an attempt to address this concern, Chang et al infected the CD4+ SUP-T1 T cell line with HIV-1 or UV-inactivated HIV-1 and used deep sequencing to identify miRNAs. At 24 hours post infection, 65 and 39 cellular miRNAs were upregulated or downregulated, respectively, in the HIV-1-infected cells.³⁴⁰ Similarly, HIV-1 infection of the CEMx174 hybrid B/T cell line resulted in the increased or decreased expression of 72 and 106 miRNAs, respectively, as assessed by the miRNA microarray.³⁴¹ The results varied with different cell types or HIV strains, making comparison of the studies difficult.

In addition to affecting viral replication, cellular miRNAs have also been reported to directly target HIV-1 regulatory and accessory genes. A cluster of cellular miRNAs have been shown to target the 3' end of the HIV-1 RNA, common to nearly all HIV-1 mRNA 3'-UTRs, to inhibit HIV-1 infection of primary CD4 T cells. Termed the “anti-HIV miRNAs,” this cluster includes miR-28, miR-125b, miR-150, miR-223, and miR-382.³⁴² Combined inhibition of these five miRNAs increased virus production in CD4T cells isolated from HIV-1+ individuals on highly active antiretroviral therapy (HAART), indicating the miRNAs may be involved in maintaining latent infection in resting CD4 T cells. Furthermore, another group has shown that miR-150 and miR-223 are expressed at high levels in CD4+ T cells isolated from healthy controls compared with the HIV-1-infected individuals.³⁴³ A study using microarray to examine miRNA levels in the PBMC of HIV-1+ individuals with high viral load found that miR-223 was downregulated in this patient population, but miR-150 expression was unchanged.³⁴⁴

It has been speculated that the anti-HIV miRNAs may be reduced in the differentiation of monocytes to macrophages, thereby creating an HIV-promoting intracellular environment.



However, microarray studies showed that only miR-223 exhibited downregulation upon monocyte differentiation into macrophages. Meanwhile, miR-28-3p expression only slightly declined, miR-125b and miR-382 did not differ from background levels, and miR-150 was strongly upregulated.³⁴⁵

Several studies have examined the role of the miR-29 family during HIV infection, and target sites for miR-29a and miR-29b have been predicted within the HIV-1 *nef* 3'-UTR.^{346,347} Inhibiting miR-29a in 293T cells enhanced virus production and increased infectivity, while an miR-29a mimic suppressed HIV-1 production.³⁴⁶ In support of this, Patel et al noted that miR-29a levels were higher in PBMCs from control patients than from HIV+ patients, suggesting a possible role for miR-29a in HIV latency. Interestingly, plasma levels of miR-29a showed an opposite trend.³⁴⁸

miRNAs as potential biomarkers of HIV infection. Several groups have examined the regulation of cellular miRNAs during HIV infection to validate the potential of miRNAs as biomarkers (Table 11). Moreover, recent studies have specifically examined the potential of miRNAs to distinguish between different groups of HIV-infected individuals. Munshi et al recently analyzed miR-16, miR-146b-5p, miR-150, miR-191, and miR-223 levels in the PBMCs and serum of HIV/AIDS patients in different stages of disease. miR-150 and miR-146b-5p were found to distinguish HIV+ individuals as well as those on HAART or developing drug resistance. The levels of miR-150 were found to be decreased in the PBMCs of HIV/AIDS patients before treatment, and HAART was able to restore levels of miR-150. Individuals who developed drug resistance reduced miR-150 expression to the levels observed in the symptomatic HIV/AIDS patients.³⁴⁹ In contrast, plasma levels of miR-150 were increased during infection and reduced upon treatment, prompting the authors to propose that miR-150 in the plasma may be derived from damaged tissues or released exosomes. A positive trend was also generally observed between PBMC miR-150 and CD4 T cell counts.

Some individuals infected with HIV, known as elite suppressors (ES), maintain an undetectable viral load and high CD4 T cell counts in the absence of any treatment. A study that characterized the miRNAs present in the PBMCs of ES

versus untreated HIV/AIDS patients determined that the two groups actually share expression patterns of several miRNAs, although a few miRNAs were differentially expressed. miR-31, miR-31*, and miR-150 were significantly downregulated, while miR-155 and miR-22 were moderately upregulated in active HIV/AIDS versus ES patients.³⁵⁰ This study also found a correlation of particular miRNAs with CD4 T cells counts in infected individuals compared to uninfected controls: miR-181b (negative) and miR-31, miR-31*, miR-29a, and miR-150 (positive) all significantly correlated with CD4 T cell count ($P < 0.05$). Recently, Reynoso et al performed a similar study examining miRNAs in the plasma of ES versus chronically infected individuals and normal donors. No significant differences were noted between normal and ES donors, although miR-29b-3p, miR-33a-5p, and miR-146a-5p were upregulated in ES versus chronically infected patients. Several miRNAs were also identified that correlated with CD4 T cell counts.³⁵¹

While viral titers and CD4 T cell counts are important for predicting disease progression, it has been suggested that CD4 T cell counts do not necessarily correlate well with virologic failure in patients being treated with ART,³⁵² emphasizing the importance of the characterization and validation of potential HIV biomarkers.

Potential miRNA therapeutics for HIV. At first thought, the cluster of anti-HIV miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) is of interest as potential antiretroviral drugs due to their reported ability to target HIV-1 mRNAs. However, several of these miRNAs are expressed in normal CD4 T cells,³⁵³ which are still capable of being infected by HIV. Recently, Whisnant et al found that HIV-1 transcripts are refractory to miRNA binding, likely due to secondary structure of viral mRNAs.²⁰ This implied that these anti-HIV miRNAs may not be able to target the virus directly. This does not preclude their possible role, however, in modulating expression of genes that could create a cellular environment that is not susceptible or permissive to infection. Considering that a single miRNA is capable of affecting the transcription of many genes and therefore possibly inducing off-target effects, additional research is warranted concerning the utility of miRNAs as antiretroviral drugs.

Table 11. Biomarker potential of host miRNAs associated with HIV-1 infection (>2-fold change in miRNA): selected studies.

SAMPLE TYPE	miRNA UPREGULATED	miRNA DOWNREGULATED	REFERENCES
HIV-infected CD4+CD8- PBMCs	miR-223	miR-29a/b, miR-155, miR-21	446
HIV-infected Jurkat cells	miR-122, miR-297, miR-370, miR-373*	miR17/92 cluster	339
PBMCs from HIV-infected patients		miR-15b, miR-23a, miR-23b, miR-26a, miR27a, miR-92, miR-144, miR-210, miR-320, miR-337, miR-342, miR-451	447
PBMCs from HIV-infected patients	miR-9, miR-181b	miR-31, miR-31*, let-7 g, miR- 125b, miR-150	350
Plasma and PBMCs from HIV-infected patients	miR-150, miR-146b-5p		349



Concluding Remarks

Over 200 biomarker-related clinical trials involving miRNAs are listed on the Clinicaltrials.gov website (<https://clinicaltrials.gov/ct2/results?term=miRNA>), demonstrating that miRNAs continue to have great potential as biomarkers of diagnosis, disease progression, and treatment efficacy. As such, the use of miRNA biomarkers during diseases of viral origin also warrants further investigation. Several DNA viruses of clinical importance, namely, herpesviruses and polyomaviruses, encode unique viral miRNAs that could prove promising to differentiate viruses that have similar clinical symptoms. The use of miRNAs specifically expressed at different stages of infection could also help in accurately determining disease status. For the RNA viruses or retroviruses described above, profiling panels of cellular miRNAs could provide a means of identifying viruses or classifying disease stage. miRNAs that can be detected in PBMCs, plasma, or exosomes and mirror expression at the local site of infection or pathology are particularly attractive, providing a means to avoid invasive biopsies and better monitor disease using less-invasive methods. However, future miRNA assays based upon next-generation sequencing will require additional technical expertise and standardization. In addition, the presence of specific miRNAs in individuals of different backgrounds has not been thoroughly studied, and the conservation of miRNAs between clinical strains of the same virus also needs to be further explored.

Elucidating the biological functions of specific miRNAs has allowed us to contemplate the role of specific miRNAs as therapeutics or as therapeutic targets. However, drugs based upon RNAi have not been without multiple technical difficulties, including vehicle design, antisense oligonucleotide biostability, targeted delivery to cells of interest (and to necessary intracellular compartments), and off-target effects. Nonetheless, new strategies have been and continue to be developed to combat these initial roadblocks and build better therapeutics.^{354,355} Preliminary results from Phase 2a studies of miravirsin, an LNA that targets miR-122 during HCV infection, are cautiously promising.²⁵⁵ The results of additional clinical trials of miravirsin and other antagomirs will be of great interest in ascertaining the therapeutic potential of miRNAs and their inhibitors.

Author Contributions

Made critical revisions and approved the final version: JL, MB. All listed authors wrote sections of the manuscript and approved the final manuscript.

REFERENCES

- Kola I. The state of innovation in drug development. *Clin Pharmacol Ther.* 2008;83(2):227–30.
- Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol.* 2009;10(2):126–39.
- Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet.* 2010;11(9):597–610.
- Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J.* 2002;21(17):4663–70.
- 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(1):15–20.
- Grimson A, Farh KKH, Johnston WK, Garrett-Engle P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell.* 2007;27(1):91–105.
- Kim YK, Heo I, Kim VN. Modifications of small RNAs and their associated proteins. *Cell.* 2010;143(5):703–9.
- Chi SW, Zang JB, Mele A, Darnell RB. Ago HITS-CLIP decodes miRNA-mRNA interaction maps. *Nature.* 2009;460(7254):479–86.
- Haecker I, Gay LA, Yang Y, et al. Ago HITS-CLIP expands understanding of Kaposi's sarcoma-associated herpesvirus miRNA function in primary effusion lymphomas. *PLoS Pathog.* 2012;8(8):e1002884.
- Hafner M, Landthaler M, Burger L, et al. Transcriptome-wide Identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell.* 2010;141(1):129–41.
- Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods.* 2007;4(9):721–6.
- Pfeffer S, Zavolan M, Grässer FA, et al. Identification of virus-encoded microRNAs. *Science.* 2004;304(5671):734–6.
- Cox JE, Sullivan CS. Balance and stealth: the role of noncoding RNAs in the regulation of virus gene expression. *Annu Rev Virol.* 2014;1(1):89–109.
- Grundhoff A, Sullivan CS. Virus-encoded microRNAs. *Virology.* 2011;411(2):325–43.
- Klase Z, Kale P, Winograd R, et al. HIV-1 TAR element is processed by Dicer to yield a viral micro-RNA involved in chromatin remodeling of the viral LTR. *BMC Mol Biol.* 2007;8:63.
- Klase Z, Winograd R, Davis J, et al. HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. *Retrovirology.* 2009;6:18.
- Ouellet DL, Plante I, Landry P, et al. Identification of functional microRNAs released through asymmetrical processing of HIV-1 TAR element. *Nucleic Acids Res.* 2008;36(7):2353–65.
- Pfeffer S, Sewer A, Lagos-Quintana M, et al. Identification of microRNAs of the herpesvirus family. *Nat Methods.* 2005;2(4):269–76.
- Lin J, Cullen BR. Analysis of the interaction of primate retroviruses with the human RNA interference machinery. *J Virol.* 2007;81(22):12218–26.
- Whisnant AW, Bogerd HP, Flores O, et al. In-depth analysis of the interaction of HIV-1 with cellular microRNA biogenesis and effector mechanisms. *MBio.* 2013;4(2):1–13.
- Rouha H, Thurner C, Mandl CW. Functional microRNA generated from a cytoplasmic RNA virus. *Nucleic Acids Res.* 2010;38(22):8328–37.
- Dobson AT, Sederati F, Devi-Rao G, et al. Identification of the latency-associated transcript promoter by expression of rabbit beta-globin mRNA in mouse sensory nerve ganglia latently infected with a recombinant herpes simplex virus. *J Virol.* 1989;63(9):3844–51.
- Farrell MJ, Dobson AT, Feldman LT. Herpes simplex virus latency-associated transcript is a stable intron. *Proc Natl Acad Sci U S A.* 1991;88(3):790–4.
- Krause PR, Ostrove JM, Straus SE. The nucleotide sequence, 5' end, promoter domain, and kinetics of expression of the gene encoding the herpes simplex virus type 2 latency-associated transcript. *J Virol.* 1991;65(10):5619–23.
- Spivack JG, Fraser NW. Expression of herpes simplex virus type 1 latency-associated transcripts in the trigeminal ganglia of mice during acute infection and reactivation of latent infection. *J Virol.* 1988;62(5):1479–85.
- Mador N, Goldenberg D, Cohen O, Panet A, Steiner I. Herpes simplex virus type 1 latency-associated transcripts suppress viral replication and reduce immediately gene mRNA levels in a neuronal cell line. *J Virol.* 1998;72(6):5067–75.
- Chen SH, Kramer MF, Schaffer PA, Coen DM. A viral function represses accumulation of transcripts from productive-cycle genes in mouse ganglia latently infected with herpes simplex virus. *J Virol.* 1997;71(8):5878–84.
- Garber DA, Schaffer PA, Knipe DM. A LAT-associated function reduces productive-cycle gene expression during acute infection of murine sensory neurons with herpes simplex virus type 1. *J Virol.* 1997;71(8):5885–93.
- Kozomara A, Griffiths-Jones S. MiRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.* 2014;42(D1):D68–73.
- Jurak I, Kramer MF, Mellor JC, et al. Numerous conserved and divergent microRNAs expressed by herpes simplex viruses 1 and 2. *J Virol.* 2010;84(9):4659–72.
- Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature.* 2008;454(7205):780–3.
- 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(3):1433–42.
- Flores O, Nakayama S, Whisnant AW, Javanbakht H, Cullen BR, Bloom DC. Mutational inactivation of herpes simplex virus 1 microRNAs identifies viral mRNA targets and reveals phenotypic effects in culture. *J Virol.* 2013;87(12):6589–603.



34. Du T, Han Z, Zhou G, Roizman B. Patterns of accumulation of miRNAs encoded by herpes simplex virus during productive infection, latency, and on reactivation. *Proc Natl Acad Sci U S A*. 2015;112(1):E49–55.
35. Kramer MF, Jurak I, Pesola JM, Boissel S, Knipe DM, Coen DM. Herpes simplex virus 1 microRNAs expressed abundantly during latent infection are not essential for latency in mouse trigeminal ganglia. *Virology*. 2011;417(2):239–47.
36. Belter A, Gudanis D, Rolle K, et al. Mature miRNAs form secondary structure, which suggests their function beyond RISC. *PLoS One*. 2014;9(11):e113848.
37. Pan D, Flores O, Umbach JL, et al. A neuron-specific host microRNA targets herpes simplex virus-1 ICP0 expression and promotes latency. *Cell Host Microbe*. 2014;15(4):446–56.
38. Tang S, Bertke AS, Patel A, Wang K, Cohen JJ, Krause PR. An acutely and latently expressed herpes simplex virus 2 viral microRNA inhibits expression of ICP34.5, a viral neurovirulence factor. *Proc Natl Acad Sci U S A*. 2008;105(31):10931–6.
39. Tang S, Bertke AS, Patel A, Margolis TP, Krause PR. Herpes simplex virus 2 microRNA miR-H6 is a novel latency-associated transcript-associated microRNA, but reduction of its expression does not influence the establishment of viral latency or the recurrence phenotype. *J Virol*. 2011;85(9):4501–9.
40. Grinfeld E, Kennedy PGE. Translation of varicella-zoster virus genes during human ganglionic latency. *Virus Genes*. 2004;29(3):317–9.
41. Umbach JL, Nagel MA, Cohrs RJ, Golden DH, Cullen BR. Analysis of human alphaherpesvirus microRNA expression in latently infected human trigeminal ganglia. *J Virol*. 2009;83(20):10677–83.
42. Glazov EA, Horwood PF, Assavalapsakul W, et al. Characterization of microRNAs encoded by the bovine herpesvirus 1 genome. *J Gen Virol*. 2010;91(1):32–41.
43. Anselmo A, Flori L, Jaffrezic F, et al. Co-expression of host and viral microRNAs in porcine dendritic cells infected by the pseudorabies virus. *PLoS One*. 2011;6(3):e17374.
44. Timoneda O, Núñez-Hernández F, Balcells I, et al. The role of viral and host microRNAs in the Aujeszky's disease virus during the infection process. *PLoS One*. 2014;9(1):e86965.
45. Pomeranz LE, Reynolds AE, Hengartner CJ. Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. *Microbiol Mol Biol Rev*. 2005;69(3):462–500.
46. Cullen BR. MicroRNAs as mediators of viral immune evasion. *Nat Immunol*. 2013;14(3):205–10.
47. Klinke O, Feederle R, Delecluse HJ. Genetics of Epstein-Barr virus microRNAs. *Semin Cancer Biol*. 2014;26:52–9.
48. Thorley-Lawson DA, Allday MJ. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat Rev Microbiol*. 2008;6(12):913–24.
49. Cai X, Schäfer A, Lu S, et al. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog*. 2006;2(3):0236–47.
50. Seto E, Moosmann A, Grömminger S, Walz N, Grundhoff A, Hammerschmidt W. Micro RNAs of Epstein-Barr virus promote cell cycle progression and prevent apoptosis of primary human B cells. *PLoS Pathog*. 2010;6(8):69–70.
51. Feederle R, Linnstaedt SD, Bannert H, et al. A viral microRNA cluster strongly potentiates the transforming properties of a human herpesvirus. *PLoS Pathog*. 2011;7(2):e1001294.
52. Qiu J, Cosmopoulos K, Pegtel M, et al. A novel persistence associated EBV miRNA expression profile is disrupted in neoplasia. *PLoS Pathog*. 2011;7(8):e1002484.
53. Robertson ES, Tomkinson B, Kieff E. An Epstein-Barr virus with a 58-kilobase-pair deletion that includes BARF0 transforms B lymphocytes in vitro. *J Virol*. 1994;68(3):1449–58.
54. Barth S, Pfuhl T, Mamiani A, et al. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. *Nucleic Acids Res*. 2008;36(2):666–75.
55. Riley KJ, Rabinowitz GS, Yario TA, Luna JM, Darnell RB, Steitz JA. EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. *EMBO J*. 2012;31(9):2207–21.
56. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer*. 2004;4(10):757–68.
57. Lo AK, To KF, Lo KW, et al. Modulation of LMP1 protein expression by EBV-encoded microRNAs. *Proc Natl Acad Sci U S A*. 2007;104(41):16164–9.
58. Lu JJ-Y, Chen J-Y, Hsu T-Y, Yu WCY, Su I-J, Yang C-S. Induction of apoptosis in epithelial cells by Epstein-Barr virus latent membrane protein 1. *J Gen Virol*. 1996;77(8):1883–92.
59. Choy EY, Siu KL, Kok KH, et al. An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. *J Exp Med*. 2008;205(11):2551–60.
60. Skalsky RL, Corcoran DL, Gottwein E, et al. The viral and cellular microRNA targetome in lymphoblastoid cell lines. *PLoS Pathog*. 2012;8(1):e1002484.
61. Marquitz AR, Mathur A, Nam CS, Raab-Traub N. The Epstein-Barr Virus BART microRNAs target the pro-apoptotic protein Bim. *Virology*. 2011;412(2):392–400.
62. Shinozaki-Ushiku A, Kunita A, Isogai M, et al. Profiling of virus-encoded microRNAs in Epstein-Barr virus-associated gastric carcinoma and their roles in gastric carcinogenesis. *J Virol*. 2015;89(10):5581–91.
63. 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(4):376–85.
64. Xia T, O'Hara A, Araujo I, et al. EBV microRNAs in primary lymphomas and targeting of CXCL-11 by ebv-mir-BHRF1-3. *Cancer Res*. 2008;68(5):1436–42.
65. Cox JE, McClure LV, Goga A, Sullivan CS. Pan-viral-microRNA screening identifies interferon inhibition as a common function of diverse viruses. *Proc Natl Acad Sci*. 2015;112(6):1856–61.
66. Wen KW, Damania B. Kaposi sarcoma-associated herpesvirus (KSHV): molecular biology and oncogenesis. *Cancer Lett*. 2010;289(2):140–50.
67. Cai X, Cullen BR. Transcriptional origin of Kaposi's sarcoma-associated herpesvirus microRNAs. *J Virol*. 2006;80(5):2234–42.
68. Samols MA, Hu J, Skalsky RL, Renne R. Cloning and identification of a microRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus. *J Virol*. 2005;79(14):9301–5.
69. Marshall V, Parks T, Bagni R, et al. Conservation of virally encoded microRNAs in Kaposi sarcoma-associated herpesvirus in primary effusion lymphoma cell lines and in patients with Kaposi sarcoma or multicentric Castlemann disease. *J Infect Dis*. 2007;195(5):645–59.
70. Gottwein E, Corcoran DL, Mukherjee N, et al. Viral microRNA targetome of KSHV-infected primary effusion lymphoma cell lines. *Cell Host Microbe*. 2011;10(5):515–26.
71. Lin Y-T, Sullivan CS. Expanding the role of Drosha to the regulation of viral gene expression. *Proc Natl Acad Sci U S A*. 2011;108(27):11229–34.
72. Bellare P, Ganem D. Regulation of KSHV lytic switch protein expression by a virus-encoded microRNA: an evolutionary adaptation that fine-tunes lytic reactivation. *Cell Host Microbe*. 2009;6(6):570–5.
73. Lin X, Liang D, He Z, Deng Q, Robertson ES, Lan K. miR-K12-7-5p encoded by Kaposi's sarcoma-associated herpesvirus stabilizes the latent state by targeting viral ORF50/RTA. *PLoS One*. 2011;6(1):e16224.
74. Plaisance-Bonstaff K, Choi HS, Beals T, et al. KSHV miRNAs decrease expression of lytic genes in latently infected PEL and endothelial cells by targeting host transcription factors. *Viruses*. 2014;6(10):4005–23.
75. Lu F, Stedman W, Yousef M, Renne R, Lieberman PM. Epigenetic regulation of Kaposi's sarcoma-associated herpesvirus latency by virus-encoded microRNAs that target Rta and the cellular Rbl2-DNMT pathway. *J Virol*. 2010;84(6):2697–706.
76. Yan Q, Li W, Tang Q, et al. Cellular microRNAs 498 and 320d regulate herpes simplex virus 1 induction of Kaposi's sarcoma-associated herpesvirus lytic replication by targeting RTA. *PLoS One*. 2013;8(2):e55832.
77. Yan Q, Ma X, Shen C, et al. Inhibition of Kaposi's sarcoma-associated herpesvirus lytic replication by HIV-1 Nef and cellular microRNA hsa-miR-1258. *J Virol*. 2014;88(9):4987–5000.
78. McClure LV, Kincaid RP, Burke JM, Grundhoff A, Sullivan CS. Comprehensive mapping and analysis of Kaposi's sarcoma-associated herpesvirus 3' UTRs identify differential posttranscriptional control of gene expression in lytic versus latent infection. *J Virol*. 2013;87(23):12838–49.
79. Bai Z, Huang Y, Li W, et al. Genomewide mapping and screening of Kaposi's sarcoma-associated herpesvirus (KSHV) 3' untranslated regions identify bicistronic and polycistronic viral transcripts as frequent targets of kshv microRNAs. *J Virol*. 2013;88(1):377–92.
80. Yang Y, Boss IW, McIntyre LM, Renne R. A systems biology approach identified different regulatory networks targeted by KSHV miR-K12-11 in B cells and endothelial cells. *BMC Genomics*. 2014;15(1):668.
81. Samols MA, Skalsky RL, Maldonado AM, et al. Identification of cellular genes targeted by KSHV-encoded microRNAs. *PLoS Pathog*. 2007;3(5):0611–8.
82. Gottwein E, Cullen BR. A human herpesvirus microRNA inhibits p21 expression and attenuates p21-mediated cell cycle arrest. *J Virol*. 2010;84(10):5229–37.
83. Gottwein E, Mukherjee N, Sachse C, et al. A viral microRNA functions as an orthologue of cellular miR-155. *Nature*. 2007;450(7172):1096–9.
84. Thai TH, Calado DP, Casola S, et al. Regulation of the germinal center response by microRNA-155. *Science*. 2007;316:604–8.
85. Costinean S, Zanesi N, Pekarsky Y, et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci U S A*. 2006;103(18):7024–9.
86. 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(22):11670–8.
87. Manzano M, Shamulailatpam P, Raja AN, Gottwein E. Kaposi's sarcoma-associated herpesvirus encodes a mimic of cellular miR-23. *J Virol*. 2013;87(21):11821–30.
88. Skalsky RL, Samols MA, Plaisance KB, et al. Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. *J Virol*. 2007;81(23):12836–5.
89. Tsai Y-H, Wu M-F, Wu Y-H, et al. The M type K15 protein of Kaposi's sarcoma-associated herpesvirus regulates microRNA expression via its SH2-binding motif to induce cell migration and invasion. *J Virol*. 2009;83(2):622–32.



90. Lagos D, Pollara G, Henderson S, et al. miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. *Nat Cell Biol.* 2010;12(5):513–9.
91. Hook L, Hancock M, Landais I, Grabski R, Britt W, Nelson JA. Cytomegalovirus microRNAs. *Curr Opin Virol.* 2014;7(1):40–6.
92. Grey F, Antoniewicz A, Allen E, et al. Identification and characterization of human cytomegalovirus-encoded microRNAs. *J Virol.* 2005;79(18):12095–9.
93. Shen ZZ, Pan X, Miao LF, et al. Comprehensive analysis of human cytomegalovirus microRNA expression during lytic and quiescent infection. *PLoS One.* 2014;9(2):1–11.
94. Stern-Ginossar N, Saleh N, Goldberg MD, Prichard M, Wolf DG, Mandelboim O. Analysis of human cytomegalovirus-encoded microRNA activity during infection. *J Virol.* 2009;83(20):10684–93.
95. Grey F, Meyers H, White EA, Spector DH, Nelson J. A human cytomegalovirus-encoded microRNA regulates expression of multiple viral genes involved in replication. *PLoS Pathog.* 2007;3(11):1593–602.
96. Murphy E, Vanicek J, Robins H, Shenk T, Levine AJ. Suppression of immediate-early viral gene expression by herpesvirus-coded microRNAs: implications for latency. *Proc Natl Acad Sci U S A.* 2008;105(14):5453–8.
97. Petrucelli A, Rak M, Grainger L, Goodrum F. Characterization of a novel Golgi apparatus-localized latency determinant encoded by human cytomegalovirus. *J Virol.* 2009;83(11):5615–29.
98. Huang Y, Qi Y, Ma Y, et al. Down-regulation of human cytomegalovirus UL138, a novel latency-associated determinant, by hcmv-miR-UL36. *J Biosci.* 2013;38(3):479–85.
99. Paulus C, Nevels M. The human cytomegalovirus major immediate-early proteins as antagonists of intrinsic and innate antiviral host responses. *Viruses.* 2009;1(3):760–79.
100. Grey F, Tirabassi R, Meyers H, et al. A viral microRNA down-regulates multiple cell cycle genes through mRNA 5'UTRs. *PLoS Pathog.* 2010;6(6):e1000967.
101. Stern-Ginossar N, Elefant N, Zimmermann A, et al. Host immune system gene targeting by a viral miRNA. *Science.* 2007;317(5836):376–81.
102. Kim Y, Lee S, Kim S, Kim D, Ahn JH, Ahn K. Human cytomegalovirus clinical strain-specific microRNA miR-UL148D targets the human chemokine RANTES during infection. *PLoS Pathog.* 2012;8(3):e1002577.
103. Kim S, Lee S, Shin J, et al. Human cytomegalovirus microRNA miR-US4-1 inhibits CD8+ T cell responses by targeting the aminopeptidase ERAP1. *Nat Immunol.* 2011;12(10):984–91.
104. 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(3):1638–49.
105. Tsao EH, Kellam P, Sin CSY, Rasaiyaah J, Griffiths PD, Clark DA. Microarray-based determination of the lytic cascade of human herpesvirus 6B. *J Gen Virol.* 2009;90(11):2581–91.
106. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol.* 1999;17:189–220.
107. Wilhelmus KR. Antiviral treatment and other therapeutic interventions for herpes simplex virus epithelial keratitis. *Cochrane Database Syst Rev.* 2010;2010(12):CD002898.
108. Bancheau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity.* 2006;25(3):383–92.
109. García-Sastre A, Biron CA. Type 1 interferons and the virus-host relationship: a lesson in détente. *Science.* 2006;312(5775):879–82.
110. Welsh RM, Bahl K, Marshall HD, Urban SL. Type 1 interferons and antiviral CD8 T-cell responses. *PLoS Pathog.* 2012;8(1):e1002352.
111. Kotton CN. CMV: prevention, diagnosis and therapy. *Am J Transplant.* 2013;13(3):24–40.
112. Kawano Y, Iwata S, Kawada J, et al. Plasma viral microRNA profiles reveal potential biomarkers for chronic active Epstein–Barr virus infection. *J Infect Dis.* 2013;208(5):771–9.
113. Zhang G, Zong J, Lin S, et al. Circulating Epstein–Barr virus microRNAs miR-BART7 and miR-BART13 as biomarkers for nasopharyngeal carcinoma diagnosis and treatment. *Int J Cancer.* 2015;136(5):E301–12.
114. Chugh PE, Sin SH, Ozgur S, et al. Systemically circulating viral and tumor-derived microRNAs in KSHV-associated malignancies. *PLoS Pathog.* 2013;9(7):e1003484.
115. Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, et al. Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci U S A.* 2010;107(14):6328–33.
116. Meckes DG, Shair KHY, Marquitz AR, Kung C-P, Edwards RH, Raab-Traub N. Human tumor virus utilizes exosomes for intercellular communication. *Proc Natl Acad Sci U S A.* 2010;107(47):20370–5.
117. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science.* 2008;319(5866):1096–100.
118. Mackenzie EF, Poulding JM, Harrison PR, Amer B. Human polyoma virus (HPV) – a significant pathogen in renal transplantation. *Proc Eur Dial Transplant Assoc.* 1978;15:352–60.
119. Hirsch HH, Randhawa P. BK virus in solid organ transplant recipients. *Am J Transplant.* 2009;9(suppl 4):S136–46.
120. Azzi A, Cesaro S, Laszlo D, et al. Human polyomavirus BK (BKV) load and haemorrhagic cystitis in bone marrow transplantation patients. *J Clin Virol.* 1999;14(2):79–86.
121. Weinreb DB, Desman GT, Amolat-Apiado MJM, Burstein DE, Godbold JH, Johnson EM. Polyoma virus infection is a prominent risk factor for bladder carcinoma in immunocompetent individuals. *Diagn Cytopathol.* 2006;34(3):201–3.
122. Roberts ISD, Besarani D, Mason P, Turner G, Friend PJ, Newton R. Polyoma virus infection and urothelial carcinoma of the bladder following renal transplantation. *Br J Cancer.* 2008;99(9):1383–6.
123. van Aalderen MC, Yapici Ü, van der Pol JA, et al. Polyomavirus BK in the pathogenesis of bladder cancer. *Neth J Med.* 2013;71(1):26–8.
124. Robles C, Viscidi R, Malats N, et al. Bladder cancer and seroreactivity to BK, JC and Merkel cell polyomaviruses: the Spanish bladder cancer study. *Int J Cancer.* 2013;133(3):597–603.
125. Hassan S, Alirhayim Z, Ahmed S, Amer S. Polyoma BK virus: an oncogenic virus? *Case Rep Nephrol.* 2013;2013:1–4.
126. Maginnis MS, Ströh LJ, Gee GV, et al. Progressive multifocal leukoencephalopathy-associated mutations in the JC polyomavirus capsid disrupt lactoseries tetrasaccharide c binding. *MBio.* 2013;4(3):1–11.
127. Rollison DE. JC virus infection: a cause of colorectal cancer? *J Clin Gastroenterol.* 2010;44(7):466–8.
128. van der Meijden E, Janssens RWA, Lauber C, Bavincq JNB, Gorbalenya AE, Feltkamp MCW. Discovery of a new human polyomavirus associated with Trichodysplasia Spinulosa in an immunocompromized patient. *PLoS Pathog.* 2010;6(7):1–10.
129. Bauman Y, Mandelboim O. MicroRNA based immunoevasion mechanism of human polyomaviruses. *RNA Biol.* 2011;8(4):591–4.
130. Bauman Y, Nachmani D, Vitenstein A, et al. An identical miRNA of the human JC and BK polyoma viruses targets the stress-induced ligand ULBP3 to escape immune elimination. *Cell Host Microbe.* 2011;9(2):93–102.
131. 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(7042):682–6.
132. You X, Zhang Z, Fan J, Cui Z, Zhang XE. Functionally orthologous viral and cellular microRNAs studied by a novel dual-fluorescent reporter system. *PLoS One.* 2012;7(4):e36157.
133. Lee S, Paulson KG, Murchison EP, et al. Identification and validation of a novel mature microRNA encoded by the Merkel cell polyomavirus in human Merkel cell carcinomas. *J Clin Virol.* 2011;52(3):272–5.
134. Seo GJ, Fink LHL, O'Hara B, Atwood WJ, Sullivan CS. Evolutionarily conserved function of a viral microRNA. *J Virol.* 2008;82(20):9823–8.
135. Link A, Balaguer F, Nagasaka T, Boland CR, Goel A. MicroRNA miR-J1-5p as a potential biomarker for JC virus infection in the gastrointestinal tract. *PLoS One.* 2014;9(6):1–8.
136. Lagatie O, Van Loy T, Tritsmans L, Stuyver LJ. Viral miRNAs in plasma and urine divulge JC polyomavirus infection. *Virol J.* 2014;11(158):1–9.
137. Lagatie O, Van Loy T, Tritsmans L, Stuyver LJ. Circulating human microRNAs are not linked to JC polyomavirus serology or urinary viral load in healthy subjects. *Virol J.* 2014;11(1):41.
138. Seo GJ, Chen CJ, Sullivan CS. Merkel cell polyomavirus encodes a microRNA with the ability to autoregulate viral gene expression. *Virology.* 2009;383(2):183–7.
139. Pipas JM, Levine AJ. Role of T antigen interactions with p53 in tumorigenesis. *Semin Cancer Biol.* 2001;11(1):23–30.
140. Ganem D, Prince A. Hepatitis B virus infection—natural history and clinical consequences. *N Engl J Med.* 2004;350(11):1118–29.
141. Dienstag J. Hepatitis B virus infection. *N Engl J Med.* 2008;359(14):1486–500.
142. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87–108.
143. Jin WB, Wu FL, Kong D, Guo AG. HBV-encoded microRNA candidate and its target. *Comput Biol Chem.* 2007;31(2):124–6.
144. Wei Y-F, Cui G-Y, Ye P, Chen J-N, Diao H-Y. MicroRNAs may solve the mystery of chronic hepatitis B virus infection. *World J Gastroenterol.* 2013;19(30):4867–76.
145. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol.* 2002;12(9):735–9.
146. Chang J, Nicolas E, Marks D, et al. miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. *RNA Biol.* 2004;1(2):106–13.
147. Hou J, Lin L, Zhou W, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell.* 2011;19(2):232–43.
148. Tsai WC, Hsu SD, Hsu CS, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest.* 2012;122(8):2884–97.



149. Jopling C. Liver-specific microRNA-122: biogenesis and function. *RNA Biol.* 2012;9(2):137–42.
150. Wang S, Qiu L, Yan X, et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1) -modulated P53 activity. *Hepatology.* 2012;55(3):730–41.
151. Qiu L, Fan H, Jin W, et al. MiR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. *Biochem Biophys Res Commun.* 2010;398(4):771–7.
152. Protzer U, Seyfried S, Quasdorff M, et al. Antiviral activity and hepatoprotection by heme oxygenase-1 in hepatitis B virus infection. *Gastroenterology.* 2007;133(4):1156–65.
153. Chen Y, Shen A, Rider PJ, et al. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J.* 2011;25(12):4511–21.
154. Song K, Han C, Zhang J, et al. Epigenetic regulation of miR-122 by PPAR γ and hepatitis B virus X protein in hepatocellular carcinoma cells. *Hepatology.* 2013;58(5):1681–92.
155. Li Y, Masaki T, Yamane D, McGivern DR, Lemon SM. Competing and noncompeting activities of miR-122 and the 5' exonuclease Xrn1 in regulation of hepatitis C virus replication. *Proc Natl Acad Sci U S A.* 2013;110(5):1881–6.
156. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene.* 2009;28(40):3526–36.
157. Fan CG, Wang CM, Tian C, et al. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol Rep.* 2011;26(5):1281–6.
158. Li C, Wang Y, Wang S, et al. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol.* 2013;87(4):2193–205.
159. Wang Y, Jiang L, Ji X, Yang B, Zhang Y, Fu XD. Hepatitis B viral RNA directly mediates down-regulation of the tumor suppressor microRNA miR-15a/miR-16-1 in hepatocytes. *J Biol Chem.* 2013;288(25):18484–93.
160. Liu Y, Zhao JJ, Wang CM, et al. Altered expression profiles of microRNAs in a stable hepatitis B virus-expressing cell line. *Chin Med J (Engl).* 2009;122(1):10–4.
161. Wu G, Yu F, Xiao Z, et al. Hepatitis B virus X protein downregulates expression of the miR-16 family in malignant hepatocytes in vitro. *Br J Cancer.* 2011;105(1):146–53.
162. Liu N, Zhang J, Jiao T, et al. Hepatitis B virus inhibits apoptosis of hepatoma cells by sponging the microRNA 15a/16 cluster. *J Virol.* 2013;87(24):13370–8.
163. Zhang GL, Li YX, Zheng SQ, Liu M, Li X, Tang H. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. *Antiviral Res.* 2010;88(2):169–75.
164. Potenza N, Papa U, Mosca N, Zerbini F, Nobile V, Russo A. Human microRNA hsa-miR-125a-5p interferes with expression of hepatitis B virus surface antigen. *Nucleic Acids Res.* 2011;39(12):5157–63.
165. Zhang X, Zhang E, Ma Z, et al. Modulation of hepatitis B virus replication and hepatocyte differentiation by microRNA-1. *Hepatology.* 2011;53(5):1476–85.
166. Datta J, Kutay H, Nasser MW, et al. Methylation mediated silencing of microRNA-1 gene and its role in hepatocellular carcinogenesis. *Cancer Res.* 2008;68(13):5049–58.
167. Yuan K, Lian Z, Sun B, Clayton MM, Ng IOL, Feitelson MA. Role of mir-148a in hepatitis B associated hepatocellular carcinoma. *PLoS One.* 2012;7(4):1–12.
168. Xu X, Fan Z, Kang L, et al. Hepatitis B virus X protein represses miRNA-148a to enhance tumorigenesis. *J Clin Invest.* 2013;123(2):630–45.
169. Zhang Y, Jia Y, Zheng R, et al. Plasma microRNA-122 as a biomarker for viral, alcohol, and chemical-related hepatic diseases. *Clin Chem.* 2010;56(12):1830–8.
170. Nathwani RA, Pais S, Reynolds TB, Kaplowitz N. Serum alanine aminotransferase in skeletal muscle diseases. *Hepatology.* 2005;41(2):380–2.
171. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology.* 2008;47(4):1363–70.
172. Adams LA. Biomarkers of liver fibrosis. *J Gastroenterol Hepatol.* 2011;26(5):802–9.
173. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology.* 2009;49(4):1335–74.
174. Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol.* 1986;2(2):165–73.
175. Saludes V, González V, Planas R, Matas L, Ausina V, Martró E. Tools for the diagnosis of hepatitis C virus infection and hepatic fibrosis staging. *World J Gastroenterol.* 2014;20(13):3431–42.
176. CDC. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR Recomm Rep.* 2008;57(RR-8):1–10.
177. Li LM, Hu ZB, Zhou ZX, et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res.* 2010;70(23):9798–807.
178. Qi P, Cheng S, Wang H, Li N, Chen Y, Gao C. Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. *PLoS One.* 2011;6(12):e28486.
179. Waidmann O, Bihrer V, Pleli T, et al. Serum microRNA-122 levels in different groups of patients with chronic hepatitis B virus infection. *J Viral Hepatol.* 2012;19(2):e58–65.
180. Li L, Guo Z, Wang J, Mao Y, Gao Q. Serum miR-18a: a potential marker for hepatitis B virus-related hepatocellular carcinoma screening. *Dig Dis Sci.* 2012;57(11):2910–6.
181. Akamatsu S, Hayes CN, Tsuge M, et al. Differences in serum microRNA profiles in hepatitis B and C virus infection. *J Infect.* 2015;70(3):273–87.
182. Zhang H, Li QY, Guo ZZ, et al. Serum levels of microRNAs can specifically predict liver injury of chronic hepatitis B. *World J Gastroenterol.* 2012;18(37):5188–96.
183. Winther TN, Bang-Berthelsen CH, Heiberg IL, Pociot F, Høgh B. Differential plasma microRNA profiles in HBeAg positive and HBeAg negative children with chronic hepatitis B. *PLoS One.* 2013;8(3):e58236.
184. Brunetto MR, Cavallone D, Oliveri F, et al. A serum microRNA signature is associated with the immune control of chronic hepatitis B virus infection. *PLoS One.* 2014;9(10):e110782.
185. Zhou J, Yu L, Gao X, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol.* 2011;29(36):4781–8.
186. Tan Y, Ge G, Pan T, et al. A serum microRNA panel as potential biomarkers for hepatocellular carcinoma related with hepatitis B virus. *PLoS One.* 2014;9(9):e107986.
187. Ura S, Honda M, Yamashita T, et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology.* 2009;49(4):1098–112.
188. Jiang J, Gusev Y, Aderca I, et al. Association of microRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res.* 2008;14(2):419–27.
189. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene.* 2006;25(17):2537–45.
190. Arbuthnot P, Thompson LJ. Harnessing the RNA interference pathway to advance treatment and prevention of hepatocellular carcinoma. *World J Gastroenterol.* 2008;14(11):1670–81.
191. Chen Y, Cheng G, Mahato RI. RNAi for treating hepatitis B viral infection. *Pharm Res.* 2008;25(1):72–86.
192. Arbuthnot P, Longshaw V, Naidoo T, Weinberg MS. Opportunities for treating chronic hepatitis B and C virus infection using RNA interference. *J Viral Hepatol.* 2007;14(7):447–59.
193. Romano PR, McCallus DE, Pachuk CJ. RNA interference-mediated prevention and therapy for hepatocellular carcinoma. *Oncogene.* 2006;25(27):3857–65.
194. McCaffrey AP, Nakai H, Pandey K, et al. Inhibition of hepatitis B virus in mice by RNA interference. *Nat Biotechnol.* 2003;21(6):639–44.
195. Weinberg MS, Ely A, Barichev S, et al. Specific inhibition of HBV replication in vitro and in vivo with expressed long hairpin RNA. *Mol Ther.* 2007;15(3):534–41.
196. Giladi H, Ketzinel-Gilad M, Rivkin L, Felig Y, Nussbaum O, Galun E. Small interfering RNA inhibits hepatitis B virus replication in mice. *Mol Ther.* 2003;8(5):769–76.
197. Hamasaki K, Nakao K, Matsumoto K, Ichikawa T, Ishikawa H, Eguchi K. Short interfering RNA-directed inhibition of hepatitis B virus replication. *FEBS Lett.* 2003;543(1–3):51–4.
198. Klein C, Bock CT, Wedemeyer H, et al. Inhibition of hepatitis B virus replication in vivo by nucleoside analogues and siRNA. *Gastroenterology.* 2003;125(1):9–18.
199. Arbuthnot P. MicroRNA-like antivirals. *Biochim Biophys Acta.* 2011;1809(11–12):746–55.
200. Carmona S, Jørgensen MR, Kolli S, et al. Controlling HBV replication in vivo by intravenous administration of triggered PEGylated siRNA-nanoparticles. *Mol Pharm.* 2009;6(3):706–17.
201. Ely A, Naidoo T, Mufamadi S, Crowther C, Arbuthnot P. Expressed anti-HBV primary microRNA shuttles inhibit viral replication efficiently in vitro and in vivo. *Mol Ther.* 2008;16(6):1105–12.
202. Morrissey DV, Blanchard K, Shaw L, et al. Activity of stabilized short interfering RNA in a mouse model of hepatitis B virus replication. *Hepatology.* 2005;41(6):1349–56.
203. Morrissey DV, Lockridge JA, Shaw L, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. *Nat Biotechnol.* 2005;23(8):1002–7.
204. Carmona S, Ely A, Crowther C, et al. Effective inhibition of HBV replication in vivo by anti-HBx short hairpin RNAs. *Mol Ther.* 2006;13(2):411–21.
205. Kim YH, Lee JH, Paik NW, Rho HM. RNAi-based knockdown of HBx mRNA in HBx-transformed and HBV-producing human liver cells. *DNA Cell Biol.* 2006;25(7):412–7.



206. Ren X, Luo G, Xie Z, Zhou L, Kong X, Xu A. Inhibition of multiple gene expression and virus replication of HBV by stable RNA interference in 2.2.15 cells. *J Hepatol*. 2006;44(4):663–70.
207. Ren XR, Zhou LJ, Luo GB, Lin B, Xu A. Inhibition of hepatitis B virus replication in 2.2.15 cells by expressed shRNA. *J Viral Hepat*. 2005;12(3):236–42.
208. Shlomai A, Shaul Y. Inhibition of hepatitis B virus expression and replication by RNA interference. *Hepatology*. 2003;37(4):764–70.
209. Uprichard SL, Boyd B, Althage A, Chisari FV. Clearance of hepatitis B virus from the liver of transgenic mice by short hairpin RNAs. *Proc Natl Acad Sci U S A*. 2005;102(3):773–8.
210. Grimm D, Streetz KL, Jopling CL, et al. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature*. 2006;441(7092):537–41.
211. McBride JL, Boudreau RL, Harper SQ, et al. Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: implications for the therapeutic development of RNAi. *Proc Natl Acad Sci U S A*. 2008;105(15):5868–73.
212. Boudreau RL, Martins I, Davidson BL. Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in vitro and in vivo. *Mol Ther*. 2009;17(1):169–75.
213. Keck K, Volper EM, Spengler RM, et al. Rational design leads to more potent RNA interference against hepatitis B virus: factors effecting silencing efficiency. *Mol Ther*. 2009;17(3):538–47.
214. Ely A, Naidoo T, Arbutnot P. Efficient silencing of gene expression with modular trimeric Pol II expression cassettes comprising microRNA shuttles. *Nucleic Acids Res*. 2009;37(13):e91.
215. Arbutnot P, Ely A, Weinberg MS. Hepatic delivery of RNA interference activators for therapeutic application. *Curr Gene Ther*. 2009;9(2):91–103.
216. Mowa MB, Crowther C, Arbutnot P. Therapeutic potential of adenoviral vectors for delivery of expressed RNAi activators. *Expert Opin Drug Deliv*. 2010;7(12):1373–85.
217. Kota J, Chivukula RR, O'Donnell KA, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell*. 2009;137(6):1005–17.
218. Xiangji L, Feng X, Qingbao C, et al. Knockdown of HBV surface antigen gene expression by a lentiviral microRNA-based system inhibits HBV replication and HCC growth. *J Viral Hepat*. 2011;18(9):653–60.
219. Wang Y, Kato N, Jazag A, et al. Hepatitis C virus core protein is a potent inhibitor of RNA silencing-based antiviral response. *Gastroenterology*. 2006;130(3):883–92.
220. Ji J, Glaser A, Wernli M, Berke JM, Moradpour D, Erb P. Suppression of short interfering RNA-mediated gene silencing by the structural proteins of hepatitis C virus. *J Gen Virol*. 2008;89(11):2761–6.
221. Chang C, Lin n, Hsieh W-L, Lai H-W, Tsai C, Cheng Y. MicroRNA expression profiling in PBMCs: a potential diagnostic biomarker of chronic hepatitis C. *Dis Markers*. 2014;2014:1–9.
222. Liu X, Wang T, Wakita T, Yang W. Systematic identification of microRNA and messenger RNA profiles in hepatitis C virus-infected human hepatoma cells. *Virology*. 2010;398(1):57–67.
223. Varnholt H, Drebber U, Schulze F, et al. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. *Hepatology*. 2008;47(4):1223–32.
224. 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(5740):1577–81.
225. Jangra RK, Yi M, Lemon SM. Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122. *J Virol*. 2010;84(13):6615–25.
226. 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(8):3193–8.
227. Roberts APE, Lewis AP, Jopling CL. MiR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. *Nucleic Acids Res*. 2011;39(17):7716–29.
228. Shimakami T, Yamane D, Jangra RK, et al. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. *Proc Natl Acad Sci U S A*. 2012;109(3):941–6.
229. Wilson JA, Zhang C, Huys A, Richardson CD. Human Ago2 is required for efficient microRNA 122 regulation of hepatitis C virus RNA accumulation and translation. *J Virol*. 2011;85(5):2342–50.
230. Mukherjee R, Burns A, Rodden D, et al. Diagnosis and management of hepatitis C virus infection. *J Lab Autom*. 2015;20(5):519–38.
231. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. *Toxicology*. 2008;245(3):194–205.
232. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet*. 2001;357(9262):1069–75.
233. Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem*. 2005;51(10):1867–73.
234. Laterza OF, Lim L, Garrett-Engle PW, et al. Plasma microRNAs as sensitive and specific biomarkers of tissue injury. *Clin Chem*. 2009;55(11):1977–83.
235. Diaz G, Melis M, Tice A, et al. Identification of microRNAs specifically expressed in hepatitis C virus-associated hepatocellular carcinoma. *Int J Cancer*. 2013;133(4):816–24.
236. Murakami Y, Aly HH, Tajima A, Inoue I, Shimotohno K. Regulation of the hepatitis C virus genome replication by miR-199a*. *J Hepatol*. 2009;50(3):453–60.
237. Hou W, Tian Q, Zheng J, Bonkovsky HL. MicroRNA-196 represses Bach1 protein and hepatitis C virus gene expression in human hepatoma cells expressing hepatitis C viral proteins. *Hepatology*. 2010;51(5):1494–504.
238. Pedersen IM, Cheng G, Wieland S, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. *Nature*. 2007;449(7164):919–22.
239. Scagnolari C, Zingariello P, Vecchiet J, et al. Differential expression of interferon-induced microRNAs in patients with chronic hepatitis C virus infection treated with pegylated interferon alpha. *Virology*. 2010;7:311.
240. Braconi C, Huang N, Patel T. MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology*. 2010;51(3):881–90.
241. Bruni R, Marcantonio C, Tritarelli E, et al. An integrated approach identifies IFN-regulated microRNAs and targeted mRNAs modulated by different HCV replication clones. *BMC Genomics*. 2011;12(1):485.
242. De Jong YP, Jacobson IM. Antisense therapy for hepatitis C virus infection. *J Hepatol*. 2014;60(1):227–8.
243. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*. 2001;358(9286):958–65.
244. Poynard T, Yuen M-F, Ratziu V, Lung Lai C. Viral hepatitis C. *Lancet*. 2003;362(9401):2095–100.
245. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med*. 2013;368(20):1878–87.
246. Afdhal N, Zeuzem S, Kwo P, et al; ION-3 Investigators. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med*. 2014;370:1889–98.
247. Kowdley KV, Gordon SC, Reddy KR, et al; ION-3 Investigators. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med*. 2014;370:1879–88.
248. Poordad F, McCone J, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med*. 2011;364:1195–206.
249. Zeuzem S, Buti M, Ferenci P, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *J Hepatol*. 2006;44(1):97–103.
250. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology*. 2003;38(3):645–52.
251. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med*. 2002;347:975–82.
252. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology*. 2002;36(5 1):237–44.
253. Young DD, Connelly CM, Grohmann C, Deiters A. Small molecule modifiers of microRNA miR-122 function for the treatment of hepatitis C virus infection and hepatocellular carcinoma. *J Am Chem Soc*. 2010;132(23):7976–81.
254. Lanford RE, Hildebrandt-eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C infection. *Science*. 2010;327(5962):198–201.
255. Janssen HL, Reesink HW, Lawitz EJ, et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med*. 2013;368(18):1685–94.
256. Banaudha K, Kaliszewski M, Korolnek T, et al. MicroRNA silencing of tumor suppressor DLC-1 promotes efficient hepatitis C virus replication in primary human hepatocytes. *Hepatology*. 2011;53(1):53–61.
257. Hoffmann TW, Duverlie G, Bengrine A. MicroRNAs and hepatitis C virus: toward the end of miR-122 supremacy. *Virology*. 2012;9(1):109.
258. Yang X, Haurigot V, Zhou S, Luo G, Couto LB. Inhibition of hepatitis C virus replication using adeno-associated virus vector delivery of an exogenous anti-hepatitis C virus microRNA cluster. *Hepatology*. 2010;52(6):1877–87.
259. United States Centers for Disease Control and Prevention. Chapter 11: Human Papillomavirus. In: Hamborsky J, Kroger A, Wolfe S, eds. *Epidemiology and Prevention of Vaccine-Preventable Diseases*. 13th ed. Washington, DC: Public Health Foundation; 2015:175–86.
260. Nominé Y, Masson M, Charbonnier S, et al. Structural and functional analysis of E6 oncoprotein: Insights in the molecular pathways of human papillomavirus-mediated pathogenesis. *Mol Cell*. 2006;21(5):665–78.
261. Ristriani T, Fournane S, Orfanoudakis G, Travé G, Masson M. A single-codon mutation converts HPV16 E6 oncoprotein into a potential tumor suppressor, which induces p53-dependent senescence of HPV-positive HeLa cervical cancer cells. *Oncogene*. 2009;28(5):762–72.
262. Green DR, Kroemer G. Cytoplasmic functions of the tumour suppressor p53. *Nature*. 2009;458(7242):1127–30.



263. Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53 regulated genes. *Nat Rev Mol Cell Biol.* 2008;9:402–12.
264. Au Yeung CL, Tsang TY, Yau PL, Kwok TT. Human papillomavirus type 16 E6 induces cervical cancer cell migration through the p53/microRNA-23b/urokinase-type plasminogen activator pathway. *Oncogene.* 2011;30(21):2401–10.
265. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. *Nature.* 2007;447(7148):1130–4.
266. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell.* 2007;26(5):745–52.
267. Raver-Shapira N, Marciano E, Meiri E, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell.* 2007;26(5):731–43.
268. McKenna DJ, McDade SS, Patel D, McCance DJ. MicroRNA 203 expression in keratinocytes is dependent on regulation of p53 levels by E6. *J Virol.* 2010;84(20):10644–52.
269. Martinez I, Gardiner AS, Board KF, Monzon FA, Edwards RP, Khan SA. Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. *Oncogene.* 2008;27(18):2575–82.
270. Goto Y, Kojima S, Nishikawa R, et al. The microRNA-23b/27b/24-1 cluster is a disease progression marker and tumor suppressor in prostate cancer. *Oncotarget.* 2014;5(17):7748–59.
271. Majid S, Dar AA, Saini S, et al. MicroRNA-23b functions as a tumor suppressor by regulating Zeb1 in bladder cancer. *PLoS One.* 2013;8(7):e67686.
272. Pellegrino L, Stebbing J, Braga VM, et al. miR-23b regulates cytoskeletal remodeling, motility and metastasis by directly targeting multiple transcripts. *Nucleic Acids Res.* 2013;41(10):5400–12.
273. Sun H, Tian J, Xian W, Xie T, Yang X. miR-34a inhibits proliferation and invasion of bladder cancer cells by targeting orphan nuclear receptor HNF4G. *Dis Markers.* 2015;2015:1–8.
274. Xu X, Chen W, Miao R, et al. miR-34a induces cellular senescence via modulation of telomerase activity in human hepatocellular carcinoma by targeting FoxM1/c-Myc pathway. *Oncotarget.* 2015;6(6):3988–4004.
275. Li N, Fu H, Tie Y, et al. miR-34a inhibits migration and invasion by down-regulation of c-Met expression in human hepatocellular carcinoma cells. *Cancer Lett.* 2009;275(1):44–53.
276. Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene.* 2007;26(34):5017–22.
277. Hailer A, Grunewald TGP, Orth M, Reiss C, Spahn M, Butt E. Loss of tumor suppressor mir-203 mediates overexpression of LIM and SH3 Protein 1 (LASP1) in high-risk prostate cancer thereby increasing cell proliferation and migration. *Oncotarget.* 2014;5(12):4144–53.
278. Wang N, Liang H, Zhou Y, et al. miR-203 suppresses the proliferation and migration and promotes the apoptosis of lung cancer cells by targeting SRC. *PLoS One.* 2014;9(8):1–10.
279. Michel C, Malumbres M. microRNA-203: tumor suppression and beyond. *MicroRNA.* 2013;2(2):118–26.
280. Petrocca F, Visone R, Onelli MR, et al. E2F1-regulated microRNAs impair TGFβ-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer Cell.* 2008;13(3):272–86.
281. Lu Y, Zhang L, Miu M, Waye Y, Fu W, Zhang J. MiR-218 Mediates tumorigenesis and metastasis: perspectives and implications. *Exp Cell Res.* 2015;334(1):173–82.
282. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A.* 2006;103(7):2257–61.
283. Gonzalez SL, Strelau M, He X, Basile JR, Münger K. Degradation of the retinoblastoma tumor suppressor by the human papillomavirus type 16 E7 oncoprotein is important for functional inactivation and is separable from proteasomal degradation of E7. *J Virol.* 2001;75(16):7583–91.
284. Woods K, Thomson JM, Hammond SM. Direct regulation of an oncogenic microRNA cluster by E2F transcription factors. *J Biol Chem.* 2007;282(4):2130–4.
285. Bueno MJ, Gómez de Cedrón M, Laresgoiti U, Fernández-Piqueras J, Zubiaga AM, Malumbres M. Multiple E2F-induced microRNAs prevent replicative stress in response to mitogenic signaling. *Mol Cell Biol.* 2010;30(12):2983–95.
286. Wang X, Tang S, Le SY, et al. Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. *PLoS One.* 2008;3(7):e2557.
287. Zheng Z-M, Wang X. Regulation of cellular miRNA expression by human papillomaviruses. *Biochim Biophys Acta.* 2011;1809(11–12):668–677.
288. Myklebust MP, Bruland O, Fluge Ø, Skarstein A, Balteskard L, Dahl O. MicroRNA-15b is induced with E2F-controlled genes in HPV-related cancer. *Br J Cancer.* 2011;105(11):1719–25.
289. McKenna DJ, Patel D, McCance DJ. MiR-24 and miR-205 expression is dependent on HPV onco-protein expression in keratinocytes. *Virology.* 2014;448:210–6.
290. Melar-New M, Laimins LA. Human papillomaviruses modulate expression of microRNA 203 upon epithelial differentiation to control levels of p63 proteins. *J Virol.* 2010;84(10):5212–21.
291. Greco D, Kivi N, Leivonen SK, Auvinen P, Auvinen E. Human papillomavirus 16 E5 modulates the expression of host microRNAs. *PLoS One.* 2011;6(7):1–13.
292. Cervigne NK, Reis PP, Machado J, et al. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. *Hum Mol Genet.* 2009;18(24):4818–29.
293. Xiao W, Bao ZX, Zhang CY, et al. Upregulation of miR-31* is negatively associated with recurrent/newly formed oral leukoplakia. *PLoS One.* 2012;7(6):e38648.
294. Kozaki KI, Imoto I, Mogi S, Omura K, Inazawa J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. *Cancer Res.* 2008;68(7):2094–105.
295. Ma YJ, Yang J, Fan XL, et al. Cellular microRNA let-7c inhibits M1 protein expression of the H1 N1 influenza A virus in infected human lung epithelial cells. *J Cell Mol Med.* 2012;16(10):2539–46.
296. Hu X, Schwarz JK, Lewis JS Jr, et al. A microRNA expression signature for cervical cancer prognosis. *Cancer Res.* 2010;70(4):1441–8.
297. Lee JW, Choi CH, Choi JJ, et al. Altered microRNA expression in cervical carcinomas. *Clin Cancer Res.* 2008;14(9):2535–42.
298. Pereira PM, Marques JP, Soares AR, Carreto L, Santos MA. MicroRNA expression variability in human cervical tissues. *PLoS One.* 2010;5(7):e11780.
299. Ayaz L, Görür A, Yaroğlu HY, Özcan C, Tamer L. Differential expression of microRNAs in plasma of patients with laryngeal squamous cell carcinoma: potential early-detection markers for laryngeal squamous cell carcinoma. *J Cancer Res Clin Oncol.* 2013;139(9):1499–506.
300. Kikkawa N, Hanazawa T, Fujimura L, et al. miR-489 is a tumour-suppressive miRNA target PTPN11 in hypopharyngeal squamous cell carcinoma. *Br J Cancer.* 2010;103(6):877–84.
301. Sethi N, Wright A, Wood H, Rabbitts P. MicroRNAs and head and neck cancer: reviewing the first decade of research. *Eur J Cancer.* 2014;50(15):2619–35.
302. Avissar M, Christensen BC, Kelsey KT, Marsit CJ. MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma. *Clin Cancer Res.* 2009;15(8):2850–5.
303. Childs G, Fazzari M, Kung G, et al. Low-level expression of microRNAs let-7d and miR-205 are prognostic markers of head and neck squamous cell carcinoma. *Am J Pathol.* 2009;174(3):736–45.
304. Cao P, Zhou L, Zhang J, et al. Comprehensive expression profiling of microRNAs in laryngeal squamous cell carcinoma. *Head Neck.* 2013;35(5):720–8.
305. Scapoli L, Palmieri A, Lo Muzio L, et al. MicroRNA expression profiling of oral carcinoma identifies new markers of tumor progression. *Int J Immunopathol Pharmacol.* 2010;23(4):1229–34.
306. Ma D, Zhang YY, Guo YL, Li ZJ, Geng L. Profiling of microRNA-mRNA reveals roles of microRNAs in cervical cancer. *Chin Med J (Engl).* 2012;125(23):4270–6.
307. Lui W-O, Pourmand N, Patterson BK, Fire A. Patterns of known and novel small RNAs in human cervical cancer. *Cancer Res.* 2007;67(13):6031–43.
308. Hsu C, Lin P-M, Wang Y, Chen Z-J, Lin S-F, Yang M-Y. Circulating miRNA is a novel marker for head and neck squamous cell carcinoma. *Tumor Biol.* 2012;33:1933–42.
309. Liu CJ, Tsai MM, Hung PS, et al. miR-31 ablates expression of the HIF regulatory factor FIH to activate the HIF pathway in head and neck carcinoma. *Cancer Res.* 2010;70(4):1635–44.
310. Wong TS, Liu XB, Wong BYH, Ng RWM, Yuen APW, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. *Clin Cancer Res.* 2008;14(9):2588–92.
311. Hui AB, Lenarduzzi M, Krushel T, et al. Comprehensive microRNA profiling for head and neck squamous cell carcinomas. *Clin Cancer Res.* 2010;16(4):1129–39.
312. Chang SS, Jiang WW, Smith I, et al. MicroRNA alterations in head and neck squamous cell carcinoma. *Int J Cancer.* 2008;123(12):2791–7.
313. Rentoft M, Fahlén J, Coates PJ, et al. miRNA analysis of formalin-fixed squamous cell carcinomas of the tongue is affected by age of the samples. *Int J Oncol.* 2011;38(1):61–9.
314. Yang Y, Li Y, Yang X, Jiang L, Zhou Z, Zhu Y. Progress risk assessment of oral premalignant lesions with saliva miRNA analysis. *BMC Cancer.* 2013;13(1):129.
315. Lajer CB, Garnæs E, Friis-Hansen L, et al. The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. *Br J Cancer.* 2012;106(9):1526–34.
316. Liu CJ, Lin SC, Yang CC, Cheng HW, Chang KW. Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma. *Head Neck.* 2012;34(2):219–24.
317. Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. *Oral Dis.* 2010;16(4):360–4.



318. Lajer CB, Nielsen FC, Friis-Hansen L, et al. Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study. *Br J Cancer*. 2011;104(5):830–40.
319. Wald A, Hoskins E, Wells S, Ferris RL, Khan SA. Alteration of microRNA profiles in squamous cell carcinoma of the head and neck cell lines by human papillomavirus. *Head Neck*. 2011;33:504–12.
320. Wiklund ED, Gao S, Hulf T, et al. MicroRNA alterations and associated aberrant DNA methylation patterns across multiple sample types in oral squamous cell carcinoma. *PLoS One*. 2011;6(11):e27840.
321. Ramdas L, Giri U, Ashorn CL, et al. miRNA expression profiles in head and neck squamous cell carcinoma and adjacent normal tissue. *Head Neck*. 2009;31(5):642–54.
322. Fletcher AM, Heaford AC, Trask DK. Detection of metastatic head and neck squamous cell carcinoma using the relative expression of tissue-specific mir-205. *Transl Oncol*. 2008;1(4):202–8.
323. Jiang J, Lee EJ, Gusev Y, Schmittgen TD. Real-time expression profiling of microRNA precursors in human cancer cell lines. *Nucleic Acids Res*. 2005;33(17):5394–403.
324. Barker EV, Cervigne NK, Reis PP, et al. microRNA evaluation of unknown primary lesions in the head and neck. *Mol Cancer*. 2009;8:127.
325. Huang L, Lin J, Yu Y, Zhang M, Wang H, Zheng M. Downregulation of six microRNAs is associated with advanced stage, lymph node metastasis and poor prognosis in small cell carcinoma of the cervix. *PLoS One*. 2012;7(3):e33762.
326. Wang X, Meyers C, Guo M, Zheng ZM. Upregulation of p18Ink4c expression by oncogenic HPV E6 via p53-miR-34a pathway. *Int J Cancer*. 2011;129(6):1362–72.
327. Wang X, Wang HK, McCoy JP, et al. Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6. *RNA*. 2009;15(4):637–47.
328. Cullen BR. Five questions about viruses and microRNAs. *PLoS Pathog*. 2010;6(2):2–4.
329. Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. *Annu Rev Microbiol*. 2010;64:123–41.
330. Swaminathan G, Navas-Martín S, Martín-García J. MicroRNAs and HIV-1 infection: antiviral activities and beyond. *J Mol Biol*. 2014;426(6):1178–97.
331. Kincaid RP, Burke JM, Sullivan CS. From the Cover: RNA virus microRNA that mimics a B-cell oncomiR. *Proc Natl Acad Sci U S A*. 2012;109(8):3077–82.
332. Bennasser Y, Le S, Yeung ML, Jeang K. HIV-1 encoded candidate micro-RNAs and their cellular targets. *Retrovirology*. 2004;1:43.
333. Omoto S, Ito M, Tsutsumi Y, et al. HIV-1 nef suppression by virally encoded microRNA. *Retrovirology*. 2004;1:44.
334. Omoto S, Fujii YR. Regulation of human immunodeficiency virus 1 transcription by nef microRNA. *J Gen Virol*. 2005;86(3):751–5.
335. Zhang Y, Fan M, Geng G, et al. A novel HIV-1-encoded microRNA enhances its viral replication by targeting the TATA box region. *Retrovirology*. 2014;11(1):23.
336. Yeung ML, Bennasser Y, Watashi K, Le SY, Houzet L, Jeang KT. Pyrosequencing of small non-coding RNAs in HIV-1 infected cells: evidence for the processing of a viral-cellular double-stranded RNA hybrid. *Nucleic Acids Res*. 2009;37(19):6575–86.
337. Schopman NC, Willemsen M, Liu YP, et al. Deep sequencing of virus-infected cells reveals HIV-encoded small RNAs. *Nucleic Acids Res*. 2012;40(1):414–27.
338. Mullokandov G, Baccarini A, Ruzo A, et al. High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. *Nat Methods*. 2013;9(8):840–6.
339. Triboulet R, Mari B, Lin YL, et al. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science*. 2007;315(5818):1579–82.
340. Chang ST, Thomas MJ, Sova P, Green RR, Palermo RE, Katze MG. Next-generation sequencing of small RNAs from HIV-infected cells identifies phased microRNA expression patterns and candidate novel microRNAs differentially expressed upon infection. *MBio*. 2013;4(1):1–10.
341. Hayes AM, Qian S, Yu L, Boris-Lawrie K. Tat RNA silencing suppressor activity contributes to perturbation of lymphocyte miRNA by HIV-1. *Retrovirology*. 2011;8(1):36.
342. Huang J, Wang F, Argyris E, et al. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. *Nat Med*. 2007;13(10):1241–7.
343. Swaminathan S, Murray DD, Kelleher AD. Mirnas and HIV: unforeseen determinants of host-pathogen interaction. *Immunol Rev*. 2013;254(1):265–80.
344. Duskova K, Nagilla P, Le HS, et al. MicroRNA regulation and its effects on cellular transcriptome in human immunodeficiency virus-1 (HIV-1) infected individuals with distinct viral load and CD4 cell counts. *BMC Infect Dis*. 2013;13(1):250.
345. Sisk JM, Clements JE, Witwer KW. miRNA profiles of monocyte-lineage cells are consistent with complicated roles in HIV-1 restriction. *Viruses*. 2012;4(10):1844–64.
346. Nathans R, Chu CY, Serquina AK, Lu CC, Cao H, Rana TM. Cellular microRNA and P bodies modulate host-HIV-1 interactions. *Mol Cell*. 2009;34(6):696–709.
347. Sun G, Rossi JJ. MicroRNAs and their potential involvement in HIV infection. *Trends Pharmacol Sci*. 2011;32(11):675–81.
348. Patel P, Ansari MY, Bapat S, Thakar M, Gangakhedkar R, Jameel S. The microRNA miR-29a is associated with human immunodeficiency virus latency. *Retrovirology*. 2014;11(1):1–5.
349. Munshi SU, Panda H, Holla P, Rewari BB, Jameel S. MicroRNA-150 is a potential biomarker of HIV/AIDS disease progression and therapy. *PLoS One*. 2014;9(5):e95920.
350. Witwer KW, Watson AK, Blankson JN, Clements JE. Relationships of PBMC microRNA expression, plasma viral load, and CD4+ T-cell count in HIV-1-infected elite suppressors and viremic patients. *Retrovirology*. 2012;9(1):5.
351. Reynoso R, Laufer N, Hackl M, et al. MicroRNAs differentially present in the plasma of HIV elite controllers reduce HIV infection in vitro. *Sci Rep*. 2014;4:5915.
352. Moore DM, Awor A, Downing R, et al. CD4+ T-cell count monitoring does not accurately identify HIV-infected adults with virologic failure receiving antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2008;49(5):477–84.
353. Wang X, Ye L, Hou W, et al. Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. *Blood*. 2009;113(3):671–4.
354. Videira M, Arranja A, Rafael D, Gaspar R. Preclinical development of siRNA therapeutics: towards the match between fundamental science and engineered systems. *Nanomedicine*. 2014;10(4):689–702.
355. Deng Y, Wang CC, Choy KW, et al. Therapeutic potentials of gene silencing by RNA interference: principles, challenges, and new strategies. *Gene*. 2014;538(2):217–27.
356. Vejnár CE, Zdobnov EM. MiRmap: comprehensive prediction of microRNA target repression strength. *Nucleic Acids Res*. 2012;40(22):11673–83.
357. Kertesz M, Iovino N, Unnerstall U, Gaul U, Segal E. The role of site accessibility in microRNA target recognition. *Nat Genet*. 2007;39(10):1278–84.
358. Krek A, Grün D, Poy MN, et al. Combinatorial microRNA target predictions. *Nat Genet*. 2005;37(5):495–500.
359. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human microRNA targets. *PLoS Biol*. 2004;2(11):e363.
360. Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R. Fast and effective prediction of microRNA/target duplexes. *RNA*. 2004;10(2003):1507–17.
361. Kiriakidou M, Nelson PT, Kouranov A, et al. A combined computational-experimental approach predicts human microRNA targets. *Genes Dev*. 2004;18(10):1165–78.
362. Gaidatzis D, van Nimwegen E, Hausser J, Zavolan M. Inference of miRNA targets using evolutionary conservation and pathway analysis. *BMC Bioinformatics*. 2007;8:69.
363. Marin RM, Vaníek J. Efficient use of accessibility in microRNA target prediction. *Nucleic Acids Res*. 2011;39(1):19–29.
364. Zeng L, Yu J, Huang T, et al. Differential combinatorial regulatory network analysis related to venous metastasis of hepatocellular carcinoma. *BMC Genomics*. 2012;13(suppl 8):S14.
365. Jung YJ, Kim JW, Park SJ, et al. c-Myc-mediated overexpression of miR-17-92 suppresses replication of hepatitis B virus in human hepatoma cells. *J Med Virol*. 2013;85(6):969–78.
366. Connolly E, Melegari M, Landgraf P, et al. Elevated expression of the miR-17-92 polycistron and miR-21 in hepatitis B virus-associated hepatocellular carcinoma contributes to the malignant phenotype. *Am J Pathol*. 2008;173(3):856–64.
367. Liu WH, Yeh SH, Lu CC, et al. MicroRNA-18a prevents estrogen receptor- α expression, promoting proliferation of hepatocellular carcinoma cells. *Gastroenterology*. 2009;136(2):683–93.
368. Qiu X, Dong S, Qiao F, et al. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene*. 2013;32(27):3296–305.
369. Liu C, Yu J, Yu S, et al. MicroRNA-21 acts as an oncomir through multiple targets in human hepatocellular carcinoma. *J Hepatol*. 2010;53(1):98–107.
370. Jiang R, Deng L, Zhao L, et al. miR-22 promotes HBV-related hepatocellular carcinoma development in males. *Clin Cancer Res*. 2011;17(17):5593–603.
371. Zhang J, Yang Y, Yang T, et al. microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumorigenicity. *Br J Cancer*. 2010;103(8):1215–20.
372. Shi C, Xu X. MicroRNA-22 is down-regulated in hepatitis B virus-related hepatocellular carcinoma. *Biomed Pharmacother*. 2013;67(5):375–80.
373. Salvi A, Sabelli C, Moncini S, et al. MicroRNA-23b mediates urokinase and c-met downmodulation and a decreased migration of human hepatocellular carcinoma cells. *FEBS J*. 2009;276(11):2966–82.
374. Chen L, Zheng J, Zhang Y, et al. Tumor-specific expression of microRNA-26a suppresses human hepatocellular carcinoma growth via cyclin-dependent and -independent pathways. *Mol Ther*. 2011;19(8):1521–8.



375. Yang X, Liang L, Zhang XF, et al. MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma by targeting interleukin-6-Stat3 pathway. *Hepatology*. 2013;58(1):158–70.
376. Kong G, Zhang J, Zhang S, Shan C, Ye L, Zhang X. Upregulated microRNA-29a by hepatitis B virus X protein enhances hepatoma cell migration by targeting PTEN in cell culture model. *PLoS One*. 2011;6(5):1–10.
377. Wang CM, Wang Y, Fan CG, et al. MiR-29c targets TNFAIP3, inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma. *Biochem Biophys Res Commun*. 2011;411(3):586–92.
378. Yang P, Li QJ, Feng Y, et al. TGF- β -miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-Positive hepatocellular carcinoma. *Cancer Cell*. 2012;22(3):291–303.
379. Li D, Liu X, Lin L, et al. MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma. *J Biol Chem*. 2011;286(42):36677–85.
380. Wei X, Xiang T, Ren G, et al. MiR-101 is down-regulated by the hepatitis B virus x protein and induces aberrant DNA methylation by targeting DNA methyltransferase 3A. *Cell Signal*. 2013;25(2):439–46.
381. Au SL, Wong CC, Lee JM, et al. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. *Hepatology*. 2012;56(2):622–31.
382. Wang L, Zhang X, Jia LT, et al. C-Myc-mediated epigenetic silencing of microRNA-101 contributes to dysregulation of multiple pathways in hepatocellular carcinoma. *Hepatology*. 2014;59(5):1850–63.
383. Su H, Yang JR, Xu T, et al. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer Res*. 2009;69(3):1135–42.
384. Li S, Fu H, Wang Y, et al. MicroRNA-101 regulates expression of the v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS) oncogene in human hepatocellular carcinoma. *Hepatology*. 2009;49(4):1194–202.
385. Nishida N, Mimori K, Fabbri M, et al. MicroRNA-125a-5p is an independent prognostic factor in gastric cancer and inhibits the proliferation of human gastric cancer cells in combination with trastuzumab. *Clin Cancer Res*. 2011;17(9):2725–33.
386. Coppola N, Potenza N, Pisaturo M, et al. Liver microRNA hsa-miR-125a-5p in HBV chronic infection: correlation with HBV replication and disease progression. *PLoS One*. 2013;8(7):e65336.
387. Park SO, Kumar M, Gupta S. TGF- β and iron differently alter HBV replication in human hepatocytes through TGF- β /BMP signaling and cellular microRNA expression. *PLoS One*. 2012;7(6):1–11.
388. Hu W, Wang X, Ding X, et al. MicroRNA-141 represses HBV replication by targeting PPARA. *PLoS One*. 2012;7(3):e34165.
389. Zhang X, Liu S, Hu T, Liu S, He Y, Sun S. Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. *Hepatology*. 2009;50(2):490–9.
390. Noh JH, Chang YG, Kim MG, et al. MiR-145 functions as a tumor suppressor by directly targeting histone deacetylase 2 in liver cancer. *Cancer Lett*. 2013;335(2):455–62.
391. Yang XW, Zhang LJ, Huang XH, et al. miR-145 suppresses cell invasion in hepatocellular carcinoma cells: miR-145 targets ADAM17. *Hepatol Res*. 2013;44(5):551–9.
392. Wang S, Zhang X, Ju Y, et al. MicroRNA-146a feedback suppresses T cell immune function by targeting Stat1 in patients with chronic hepatitis B. *J Immunol*. 2013;191(1):293–301.
393. Zhang J-P, Zeng C, Xu L, Gong J, Fang J-H, Zhuang S-M. MicroRNA-148a suppresses the epithelial-mesenchymal transition and metastasis of hepatoma cells by targeting Met/Snail signaling. *Oncogene*. 2014;33(31):4069–76.
394. Huang J, Wang Y, Guo Y, Sun S. Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology*. 2010;52(1):60–70.
395. Su C, Hou Z, Zhang C, Tian Z, Zhang J. Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. *Viral J*. 2011;8(1):354.
396. Xie Q, Chen X, Lu F, et al. Aberrant expression of microRNA 155 may accelerate cell proliferation by targeting sex-determining region y box 6 in hepatocellular carcinoma. *Cancer*. 2012;118(9):2431–42.
397. Fornari F, Milazzo M, Chieco P, et al. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res*. 2010;70(12):5184–93.
398. Henry JC, Park JK, Jiang J, et al. miR-199a-3p targets CD44 and reduces proliferation of CD44 positive hepatocellular carcinoma cell lines. *Biochem Biophys Res Commun*. 2010;403(1):120–5.
399. Murakami Y, Toyoda H, Tanaka M, et al. The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. *PLoS One*. 2011;6(1):2–9.
400. Zhang T, Zhang J, Cui M, et al. Hepatitis B virus X protein inhibits tumor suppressor miR-205 through inducing hypermethylation of miR-205 promoter to enhance carcinogenesis. *Neoplasia*. 2013;15(11):1282–IN26.
401. Wong QW, Ching AK, Chan AW, et al. MiR-222 overexpression confers cell migratory advantages in hepatocellular carcinoma through enhancing AKT signaling. *Clin Cancer Res*. 2010;16(3):867–75.
402. Scisciani C, Vossio S, Guerrieri F, et al. Transcriptional regulation of miR-224 upregulated in human HCCs by NF κ B inflammatory pathways. *J Hepatol*. 2012;56(4):855–61.
403. Lan SH, Wu SY, Zuchini R, et al. Autophagy suppresses tumorigenesis of hepatitis B virus-associated hepatocellular carcinoma through degradation of microRNA-224. *Hepatology*. 2014;59(2):505–17.
404. Wang Y, Lee AT, Ma JZ, et al. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. *J Biol Chem*. 2008;283(19):13205–15.
405. Wang J, Liu X, Wu H, et al. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res*. 2010;38(16):5366–83.
406. Guo H, Liu H, Mitchelson K, et al. MicroRNAs-372/373 promote the expression of hepatitis B virus through the targeting of nuclear factor I/B. *Hepatology*. 2011;54(3):808–19.
407. Jin J, Tang S, Xia L, et al. MicroRNA-501 promotes HBV replication by targeting HBXIP. *Biochem Biophys Res Commun*. 2013;430(4):1228–33.
408. Li Y, Xie J, Xu X, et al. MicroRNA-548 down-regulates host antiviral response via direct targeting of IFN-. *Protein Cell*. 2013;4(2):130–41.
409. Yang L, Ma Z, Wang D, Zhao W, Chen L, Wang G. MicroRNA-602 regulating tumor suppressive gene RASSF1 A is overexpressed in hepatitis B virus-infected liver and hepatocellular carcinoma. *Cancer Biol Ther*. 2010;9(10):803–8.
410. Ji J, Zhao L, Budhu A, et al. Let-7g targets collagen type I alpha 2 and inhibits cell migration in hepatocellular carcinoma. *J Hepatol*. 2010;52(5):690–7.
411. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell*. 2005;120(5):635–47.
412. Xu J, Wu C, Che X, et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog*. 2011;50(2):136–42.
413. Hayes CN, Akamatsu S, Tsuge M, et al. Hepatitis B virus-specific miRNAs and argonaute2 play a role in the viral life cycle. *PLoS One*. 2012;7(10):e47490.
414. Yu C-H, Xu C-F, Li Y-M. Association of microRNA-223 expression with hepatic ischemia/reperfusion injury in mice. *Dig Dis Sci*. 2009;54(11):2362–6.
415. Marquez RT, Bandyopadhyay S, Wendlandt EB, et al. Correlation between microRNA expression levels and clinical parameters associated with chronic hepatitis C viral infection in humans. *Lab Invest*. 2010;90(12):1727–36.
416. Shirasaki T, Honda M, Shimakami T, et al. MicroRNA-27a regulates lipid metabolism and inhibits hepatitis C virus replication in human hepatoma cells. *J Virol*. 2013;87(9):5270–86.
417. Bandyopadhyay S, Friedman RC, Marquez RT, et al. Hepatitis C virus infection and hepatic stellate cell activation downregulate miR-29: miR-29 overexpression reduces hepatitis C viral abundance in culture. *J Infect Dis*. 2011;203(12):1753–62.
418. Yao J, Liang L, Huang S, et al. MicroRNA-30d promotes tumor invasion and metastasis by targeting galphai2 in hepatocellular carcinoma. *Hepatology*. 2010;51(3):846–56.
419. Randall G, Panis M, Cooper JD, et al. Cellular cofactors affecting hepatitis C virus infection and replication. *Proc Natl Acad Sci U S A*. 2007;104(31):12884–9.
420. Hou W, Bukong TN, Kodys K, Szabo G. Alcohol facilitates HCV RNA replication via up-regulation of miR-122 expression and inhibition of cyclin G1 in human hepatoma cells. *Alcohol Clin Exp Res*. 2013;37(4):599–608.
421. Yoshikawa T, Takata A, Otsuka M, et al. Silencing of microRNA-122 enhances interferon- α signaling in the liver through regulating SOCS3 promoter methylation. *Sci Rep*. 2012;2:1–10.
422. Zeng B, Li Z, Chen R, et al. Epigenetic regulation of miR-124 by Hepatitis C Virus core protein promotes migration and invasion of intrahepatic cholangiocarcinoma cells by targeting SMYD3. *FEBS Lett*. 2012;586(19):3271–8.
423. Zheng F, Liao YJ, Cai MY, et al. The putative tumour suppressor microRNA-124 modulates hepatocellular carcinoma cell aggressiveness by repressing ROCK2 and EZH2. *Gut*. 2012;61(2):278–89.
424. Bhanja Chowdhury J, Shrivastava S, Steele R, Di Bisceglie AM, Ray R, Ray RB. Hepatitis C virus infection modulates expression of interferon stimulatory gene IFITM1 by upregulating miR-130A. *J Virol*. 2012;86(18):10221–5.
425. Wong CC, Wong CM, Tung EK, et al. The microRNA miR-139 suppresses metastasis and progression of hepatocellular carcinoma by down-regulating rho-kinase 2. *Gastroenterology*. 2011;140(1):322–31.
426. Zhang Y, Wei W, Cheng N, et al. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology*. 2012;56(5):1631–40.
427. Mekky RY, El-Ekiaby NM, Hamza MT, et al. Mir-194 is a hepatocyte gate keeper hindering HCV entry through targeting CD81 receptor. *J Infect*. 2015;70(1):78–87.



428. Fornari F, Gramantieri L, Ferracin M, et al. MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene*. 2008;27(43):5651–61.
429. Gramantieri L, Fornari F, Ferracin M, et al. MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. *Clin Cancer Res*. 2009;15(16):5073–81.
430. Sarma NJ, Tiriveedhi V, Subramanian V, et al. Hepatitis C virus mediated changes in miRNA-449a modulates inflammatory biomarker YKL40 through components of the NOTCH signaling pathway. *PLoS One*. 2012;7(11):e50826.
431. Cheng JC, Yeh YJ, Tseng CP, et al. Let-7b is a novel regulator of hepatitis C virus replication. *Cell Mol Life Sci*. 2012;69(15):2621–33.
432. Lan FF, Wang H, Chen YC, et al. Hsa-let-7 g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-Myc and upregulation of p16INK4 A. *Int J Cancer*. 2011;128(2):319–31.
433. Shrivastava S, Mukherjee A, Ray RB. Hepatitis c virus infection, microRNA and liver disease progression. *World J Hepatol*. 2013;5(9):479–86.
434. Shwetha S, Gouthamchandra K, Chandra M, Ravishankar B, Khaja MN, Das S. Circulating miRNA profile in HCV infected serum: novel insight into pathogenesis. *Sci Rep*. 2013;3:1555.
435. Ishida H, Tatsumi T, Hosui A, et al. Alterations in microRNA expression profile in HCV-infected hepatoma cells: involvement of miR-491 in regulation of HCV replication via the PI3 kinase/Akt pathway. *Biochem Biophys Res Commun*. 2011;412(1):92–7.
436. Steuerwald NM, Parsons JC, Bennett K, Bates TC, Bonkovsky HL. Parallel microRNA and mRNA expression profiling of (genotype 1b) human hepatoma cells expressing hepatitis C virus. *Liver Int*. 2010;30(10):1490–504.
437. Xiong Y, Fang JH, Yun JP, et al. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology*. 2010;51(3):836–45.
438. Ji J, Yamashita T, Budhu A, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM – positive hepatic cancer stem cells. *Hepatology*. 2009;50(2):472–80.
439. Lena AM, Shalom-Feuerstein R, Rivetti di Val Cervo P, et al. miR-203 represses “stemness” by repressing DeltaNp63. *Cell Death Differ*. 2008;15(7):1187–95.
440. Yao T, Lin Z. MiR-21 is involved in cervical squamous cell tumorigenesis and regulates CCL20. *Biochim Biophys Acta*. 2012;1822(2):248–60.
441. Dreher A, Rossing M, Kaczkowski B, et al. Differential expression of cellular microRNAs in HPV 11, -16, and -45 transfected cells. *Biochem Biophys Res Commun*. 2011;412(1):20–5.
442. Rao Q, Shen Q, Zhou H, Peng Y, Li J, Lin Z. Aberrant microRNA expression in human cervical carcinomas. *Med Oncol*. 2012;29(2):1242–8.
443. Li Y, Liu J, Yuan C, Cui B, Zou X, Qiao Y. High-risk human papillomavirus reduces the expression of microRNA-218 in women with cervical intraepithelial neoplasia. *J Int Med Res*. 2010;38(5):1730–6.
444. Sun G, Li H, Wu X, et al. Interplay between HIV-1 infection and host microRNAs. *Nucleic Acids Res*. 2012;40(5):2181–96.
445. Houzet L, Yeung ML, de Lame V, Desai D, Smith SM, Jeang K-T. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. *Retrovirology*. 2008;5(class 1):118.
446. Nicoll MP, Proença JT, Efstathiou S. The molecular basis of herpes simplex virus latency. *FEMS Microbiol Rev*. 2012;36:684–706.