SURVEY AND SUMMARY

Cellular versus viral microRNAs in host–virus interaction

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ABSTRACT

MicroRNAs (miRNAs) mark a new paradigm of RNA-directed gene expression regulation in a wide spectrum of biological systems. These small non-coding RNAs can contribute to the repertoire of host-pathogen interactions during viral infection. This interplay has important consequences, both for the virus and the host. There have been reported evidences of host-cellular miRNAs modulating the expression of various viral genes, thereby playing a pivotal role in the host–pathogen interaction network. In the hide-and-seek game between the pathogens and the infected host, viruses have evolved highly sophisticated gene-silencing mechanisms to evade host-immune response. Recent reports indicate that virus too encode miRNAs that protect them against cellular antiviral response. Furthermore, they may exploit the cellular miRNA pathway to their own advantage. Nevertheless, our increasing knowledge of the host–virus interaction at the molecular level should lead us toward possible explanations to viral tropism, latency and oncogenesis along with the development of an effective, durable and nontoxic antiviral therapy. Here, we summarize the recent updates on miRNA-induced gene-silencing mechanism, modulating host–virus interactions with a glimpse of the miRNA-based antiviral therapy for near future.

INTRODUCTION

MicroRNAs (miRNAs) are small $\sim$22 nucleotide (nt) non-coding RNAs (ncRNAs) that play an important role in the post-transcriptional regulation of gene expression in a wide range of organisms from unicellular eukaryotes to multicellular eukaryotes by a variety of mechanisms. Initially, these were discovered in *Caenorhabditis elegans* (1,2), but now they are known to be widespread in nature (3–8). It therefore comes as no surprise that viruses, which typically employ many components of the host gene expression machinery, also encode miRNAs (9–16). Thus far, 8619 miRNA genes have been annotated from 87 organisms of which *Homo sapiens* top the list with 695 miRNA genes identified till date. These data are available at microRNA Registry database managed by researchers at University of Manchester and the Wellcome Trust Sanger Institute (http://microrna.sanger.ac.uk/sequences) (17). Over the course of evolution, viruses have developed highly sophisticated mechanisms to exploit the biosynthetic machinery of host cells and elude the cellular defense mechanisms (18,19). Present research advances reveal that the complex interaction between viruses and host cells also involves miRNA-mediated RNA-silencing pathways (20).

Viruses have a more intricate interaction with the host cell, which creates problem in inactivating a virus without doing any harm to the host cell. Combating viral infection by targeting viral proteins and pathways unique to the viral life cycle has become possible for a few viruses without unacceptable host cell toxicity (21). Hence, only a few effective antiviral drugs exist. Viral resistance, sequence diversity and drug toxicity are significant problems for all antiviral therapies. This has lead toward harnessing the potential of RNA interference (RNAi) as an innate antiviral defense mechanism (22,23).

RNAi represents a vital component of the innate antiviral immune response in plants and invertebrate animals. Furthermore, it serves as a host gene-regulation mechanism that is triggered by the expression of highly structured miRNA molecules. However, role of cellular miRNAs in the defense against viral infection in mammalian organisms has thus far remained elusive. Hence, it is important to understand the intricate details regarding the influence of viral replication on the abundance and

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distribution of miRNAs within the host cell. It has been proposed that cellular miRNAs may have a substantial effect on viral evolution and have the potential to regulate the tissue tropism of viruses *in vivo* (20). Viruses too exploit miRNA-induced gene-silencing pathway by encoding their own miRNAs (9,16,24–27). Thus, studying the changes in miRNA landscape during viral replication may help us understand the molecular regulation of host defenses and the attempt by viruses to overcome host defense during infection. A wide range of complex interactions is possible through miRNA–mRNA coupling during host–virus interaction (21). In this game of pathogen-host interaction, viruses strive to succeed by effective usage of host machinery and expressing viral proteins, whereas efficient hosts limit viral invasion by putting up innate and adaptive antiviral defenses.

The present review discusses the existing intricate details about the role of miRNAs in virus–host interaction. Furthermore, it discusses its therapeutic implications along with the existing resources needed to study such interaction.

VIRAL miRNAs

Thomas Tuschl and his group at Rockefeller University reported the existence of viral miRNAs for the first time in Epstein-Barr virus (EBV) (9). Till now, 141 miRNA genes have been identified in 15 viruses from three viral families, herpesvirus, polyomavirus and retrovirus. Herpesvirus family with three subfamilies, *viz*., α-, β- and γ-herpesvirinae express a large number of distinct miRNAs. Among these, γ-herpesvirus encodes maximum number of miRNAs (9,28,29). EBV of γ-herpesvirus subfamily has the highest number of miRNAs (17). Kaposi's sarcoma-associated viruses (KSHV), a member of the γ-herpesvirus subfamily encodes an array of 13 distinct miRNAs, all of which are expressed at readily detectable levels in latently KSHV-infected cells. The remaining three members encoding miRNAs are murine γ-herpesvirus 68 (MHV68) (10,30), Rhesus monkey rhadino virus (RRV) (31) and Rhesus lymphocryptovirus (rLCV) (28).

Furthermore, reports have been published on identification of miRNAs encoded by polyomaviruses, *viz*., BK polyomavirus (BKV), JC polyomavirus (JCV) and simian virus 40 (SV40) (11,32), Human cytomegalovirus (HCMV) (10,25,33); Herpes Simplex virus-1(HSV-1) (16,26), HSV-2 (34), Murine cytomegalovirus (MCMV) (13,35) and Marek's disease virus type 1 and 2 (MDV-1 and MDV-2) (36–38). The details about the viruses, their pathogenicity in host and miRNAs encoded by them are provided in Table 1.

miRNA-biogenesis pathway (Figure 1) poses some serious problem for RNA viruses and a group of DNA viruses (poxviruses) to encode miRNAs (4). However, it is possible to overcome these problems by adopting nonconventional ways. Omoto *et al.* (46) have reported the presence of miRNAs in HIV-1 (which is an RNA virus) infected cells, although extensive studies by Pfeffer *et al.* (10) as well as Lin and Cullen (47) have failed to confirm the existence of viral miRNAs in HIV. Recently, Ouellet

et al. (15) have also identified miRNAs within HIV-1 trans-activation responsive (TAR) element. It is definitely essential and still needs further investigation to find whether the expression of TAR miRNAs influences viral replication or the efficiency of host-antiviral defenses.

BIOGENESIS OF miRNAs

Any understanding of the potential role of miRNAs in viral pathogenesis and studies into the entire spectrum of host–virus interactions at the miRNA level requires an appreciation of the genomic location, transcription and processing of miRNAs (48,49).

Organization of miRNA genes

Viral miRNA genes are found as single or clustered transcription units (50–52). The genomic location of the virus-encoded miRNAs is very important and to some extent linked with their function. miRNAs of α-herpesviruses, namely HSV-1, MDV-1 and 2 and those of the γ-herpesviruses, namely KSHV, are located closed to and within the latency-associated transcript. These miRNAs are associated with latent transcription (16,36,38,41). The organization of viral miRNA genes within their genomes is provided in Table 1.

Maturation and processing of miRNAs

miRNA biogenesis initiates with the transcription of a pri-miRNA precursor, typically of length ranging from ~200 nt to several thousand nts by RNA polymerase II (Pol II) (53). On the contrary, work of Borchert *et al.* (54) showed a miRNA cluster (C19MC) in the human chromosome 19, interspersed among Alu repeats requires RNA polymerase III (Pol III) for transcription (54).Viral miRNAs too undergoes similar processing by Pol II except in a few cases, namely MHV68 miRNAs, which are transcribed by Pol III (10).

Next step in miRNA processing involves the recognition and nuclear cleavage of pri-miRNAs by RNase III enzyme Drosha acting in concert with the double-stranded RNA-binding protein DGCR8 (DiGeorge-syndrome critical-region protein 8) in vertebrates (55,56). This generates ~60-nt pre-miRNA hairpin, which is transported into the cytoplasm by exportin-5 complexed with Ran-GTP (Figure 1). Here, GTP hydrolysis results in the release of the pre-miRNA.

Drosha-mediated processing of miRNA genes located within the open-reading frames and translation of these protein-coding transcripts must be mutually exclusive. As in the case of KSHV miRNAs, processing of miR-K10 and miR-12 within the nucleus must be modulated in such a way, so that a substantial percentage of KSHV mRNA is able to exit the nucleus before Drosha cleavage (40). Hence, it is possible that the regulation of viral mRNA expression by modulation of Drosha cleavage efficiency has a role in several different virus replication cycles.

Drosha independent miRNA processing has been previously observed in the case of mirtrons (57). The TAR element in HIV-1 having structural similarities with human pre-miRNA let-7a-3 (15) is too short for Drosha

Table 1. List of viruses encoding microRNAs, their genomic location, hosts and diseases caused by these viruses

DNA virus families	Species	Diseases	Host	Nos.	miRNAs	Genomic location	Functions	References
α-Herpesviruses	Herpes Simplex Virus-1	Cold sores	Human	6	hsv1-miR-H1	450-bp upstream of TSS of LAT region	Maintenance of viral latency	16,26
					hsv1-miR-H(2-6)	Four are encoded in LAT region and fifth one in unknown transcript		
	Herpes Simplex Virus-2	Genital herpes	Human	1	hsv2-miR-I	Encoded in LAT exon-2	Controls viral replication in neurons	34
	Marek's disease virus	Marek's disease (MD); Neurolymphomatosis; Ocular lymphomatosis	Chicken	14	mdv1-miR-M (1–13)	Clustered in MEQ and LAT regions	Neoplastic transformation, antiapoptotic	36,38
	Marek's disease virus type 2	Nononcogenic; Neurolymphomatosis; Ocular lymphomatosis	Chicken	17	mdv2-miR-M (14–30)	Sixteen are clustered in a long repeat region, encoding R-LORF2 to R-LORF5. The single miRNA is within the C-terminal region of the ICP4 homolog.		37
β-Herpesviruses	Human Cytomegalovirus	Congenital CMV infection; CMV mononucleosis; CMV pneumonitis; CMV retinitis	Human	11	hcmv-miR-UL22A, hcmv-miR-UL36, hcmv-miR-UL70, hcmv-miR-UL112, hcmv-miR-UL148D, hcmv-miR-US4, hcmv-miR-US5-1, hcmv-miR-US5-2, hcmv-miR-US25-1, hcmv-miR-US25-2, hcmv-miR-US33	Intergenic and intronic	Act as immune-response inhibitor, help in viral replication	10,25,30,33,39
	Mouse Cytomegalovirus		Murine	18	mcmv-miR-M23-1, mcmv-miR-M23-2, mcmv-miR-M44-1, mcmv-miR-M55-1, mcmv-miR-M87-1, mcmv-miR-M95-1, mcmv-miR-m01-1, mcmv-miR-m01-2, mcmv-miR-m01-3, mcmv-miR-m01-4, mcmv-miR-m21-1, mcmv-miR-m22-1, mcmv-miR-m59-1, mcmv-miR-m59-2, mcmv-miR-m88-1, mcmv-miR-m107-1,	Organized into five genomic regions and three clusters	Could influence viral infection and latency	13,35

(continued)

Table 1. Continued

DNA virus families	Species	Diseases	Host	Nos.	miRNAs	Genomic location	Functions	References
γ-Herpesviruses	Kaposi's sarcoma-associated Herpesvirus	Kaposi's sarcoma, primary effusion lymphoma (PEL), multicentric Castleman's disease	Human	13	mcmv-miR-m108-1, mcmv-miR-m108-2 kshv-miR-K12-(1-9), kshv-miR-K12-10(a,b), kshv-miR-K12-(11-12)	Located within 3.6-kb intergenic region and Kaposin gene	May regulate kaposin gene; down-regulates thrombospondin 1, which has anti-proliferative, anti-angiogenic activity	10,27,40–42
	Epstein-Barr Virus	Pfeiffer's disease; Burkitt's lymphoma; gastric carcinoma; Nasopharyngeal carcinoma	Human	23	ebv-miR-BART(1-20), ebv-miR-BHRF1-(1-3)	Twenty genes are within the introns of BARTs and remaining in BHRF1	Tumorigenesis of EBVaGCs; regulates LMP1 expression (Nasopharyngeal Carcinoma); down-regulate the viral DNA polymerase BALF5;	9,28,29,43–45
	Rhesus Lymphocryptovirus	Lymphadenopathy	Simian	16	rlcv-miR-rL1-(1-16)	Clustered in BART and BHRF1		28
	Rhesus Monkey Rhadinovirus	Multicentric lymphoproliferative disorder	Simian	7	rrv-miR-rR1-(1-7)	Located within a single cluster at the same genomic location as of KSHV		31
	Mouse Gamma Herpesvirus 68	Infectious mononucleosis		9	mghv-miR-M1-(1-9)	Intergenic and clustered		10,30
Polyomaviruses	Simian Virus 40	Tumors	Simian	1	sv40-miR-S1		Down-regulate the expression of viral early genes	11
	BK Polyomavirus	Polyomavirus-associated nephropathy	Human	1	bkv-miR-B1		-do-	32
	JC Polyomavirus	Progressive Multifocal Leuko-encephalopathy	Human	1	jcv-miR-J1		-do-	32
RNA virus families								
HIV	Human immunodeficiency virus 1	AIDS	Human	3	hiv1-miR-H1, hiv1-miR-N367, hiv1-miR-TAR	Found in nef gene and TAR element	Supress Nef function; supress HIV-1 virulence	15,46,47

TSS: transcription start site; LAT: latency-associated transcript; BART: BamA rightward transcript; BHRF1: BamH1 fragment H rightward open reading frame 1; TAR: trans-acting responsive element; nef: negative factor.
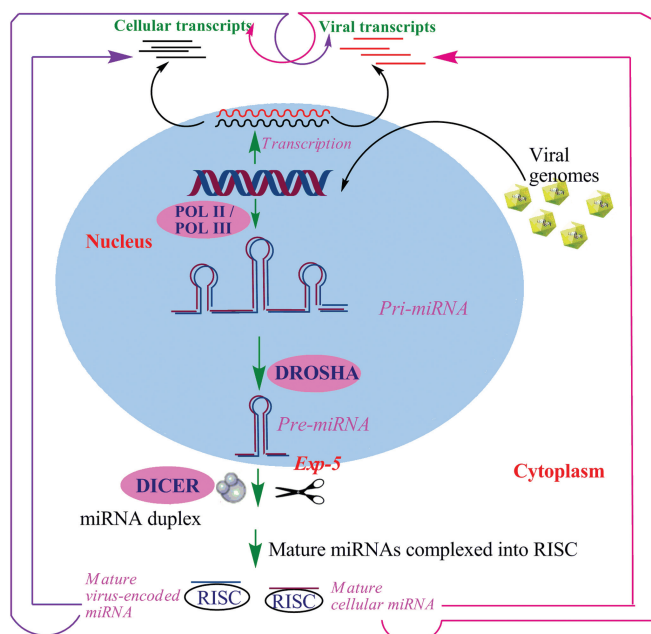
Figure 1. Biogenesis of microRNAs. Processing of both host miRNAs (violet) and virus-encoded miRNAs (blue) is assumed to occur through the same pathway. The arrows (pink) indicate the effects of cellular miRNAs on their own cellular transcripts as well as on viral transcripts. The violet arrow shows the effects of virus-encoded miRNAs on both cellular and viral transcripts. Both these arrows suggest possible miRNA-mediated interactions between viruses and their hosts.

processing whose pri-miRNA substrates contain a stem of approx. three helical turns (~33 bp). Therefore, experimental evidences suggest Drosha independent processing of HIV-1 TAR miRNAs.

The pre-miRNA resulting from Drosha processing is cleaved thereafter by cytoplasmic RNase III enzyme Dicer acting in concert with its cofactor TRBP (transactivating region RNA-binding protein) (58). The terminal loop is removed, generating the miRNA duplex intermediate. Dicer facilitates assembly of the miRNA strand (having weaker 5′ bp) of the duplex into the miRNA effector complexes, called RNA-induced silencing complexes (RISCs) (58). The unincorporated strand termed as 'passenger strand' is released and degraded. Although the composition of RISC is not completely defined, the key constituents of it are miRNA and one of the four Argonaute (Ago) proteins (59). The miRNA then directs RISC to complementary mRNAs (60), which is either cleaved or undergone translational repression depending on the degree of complementarity between the RISC-bound miRNA and the target mRNA. A seed sequence within the miRNA (nts 2–8) is known to be critical for binding and target recognition. Perfect complementarity results in mRNA degradation/cleavage, which is rare in animals but not in plants. Such an example is exhibited by the polyoma virus SV40 miRNAs, which are perfectly complementary to early mRNAs transcribed antisense to the pre-miRNA and direct the RISC-mediated cleavage of these early transcripts, responsible for generating strong cytotoxic T-cell (20). In major instances, imperfect/partial complementarity with the target is observed, leading to

translational repression of the mRNA transcripts by miRNA-RISC (61,62). In addition to repressing translation, miRNA interactions can lead to deadenylation or target decapping, leading to rapid mRNA decay (63–66).

miRNA editing influences processing pathway

The levels of mature miRNAs expressed within a cell are not simply determined by the transcription of miRNA genes; rather it depends on one or more steps in the processing pathway (67) like RNA editing of pri-/pre-miRNAs. Edited pri-/pre-miRNAs do not undergo Drosha or Dicer cleavage, which eventually reduces the production of mature miRNAs. In certain cases, pri-miRNAs are transported out of the nucleus into the cytoplasm where Drosha fails to process them and they are destroyed (68). Adensosine deaminase editing of specific pri-miRNAs has been reported. This A-I editing event leads to decreased processing of the miRNA by Drosha and increases turnover by the Tudor-SN nuclease, a component of RISC and also a ribonuclease specific to inosine-containing dsRNAs (69). The human and mouse pre-miRNAs of miR-22 are edited at several positions, including sites in the mature miRNA, which are predicted to influence its biogenesis and function (70). Notably, the viral miRNA, kshv-miR-K12-10, with a single adenosine residue substituted by guanosine (miR-K12-10b) is frequently detected among cDNA isolates identified by the small RNA-cloning method. The editing of this particular site does not inhibit pri-miR-K12-10 RNA processing, but leads to expression of mature miRNA with the edited sequence (10). Evidence of RNA editing has also been observed in miR-M7 of MDV, although its effect is unknown (38). However, RNA editing in the seed sequence of a miRNA could re-direct it to a new set of targets (71). All these indicate that the miRNAs originated from the same pre-miRNA may target more corresponding complementary mRNA, making the fine-tuning of the virus-host interaction network more complicated.

EVOLUTIONARY ASPECTS OF CELLULAR VERSUS VIRAL miRNA GENES

Cellular miRNAs and their target sequences are frequently conserved (72), which facilitate computational biologists toward *in silico* prediction of cellular miRNAs and their targets. Interestingly, viral miRNAs, unlike their vertebrate counterparts do not share a high level of homology, even within members of the same family or with that of the host. However, miRNAs of closely related viruses such as RRV and KSHV are encoded in the same genomic region but do not exhibit sequence homology (31). The miRNAs encoded by chicken α-herpesviruses MDV-1 and MDV-2 are clustered in homologous regions of the viral genomes, which are transcribed during viral latency, but are not homologous in sequence (36–38). In contrary to these, Cai *et al.* (28) have shown that eight of EBV miRNAs are conserved with rLCV miRNAs, thus arguing for their importance in viral life cycle.

The lack of conservation in viral miRNA sequences attributes to the higher rate of mutations and faster

evolution in viruses when compared to eukaryotes. This would mean an evolutionary advantage for rapid adaptation to the host and environmental conditions. However, it offers a challenge to computational biologists as most of the algorithms for miRNA prediction rely heavily on conservation and would prove inadequate in case of viruses. Even a single-point mutation in the seed region can lead to a dramatic shift in miRNA function due to the loss or acquisition of a large number of cellular or viral mRNA targets.

VIRUS-ENCODED miRNAs—ORTHOLOGS OF CELLULAR miRNAs

In general, viral miRNAs and cellular miRNAs do not bear seed homology. But presumably, due to the presence of highly evolved gene-regulatory networks, some viral miRNAs have seed homology with cellular miRNAs. Recent report suggests that miR-K12-11 encoded by KSHV shares the first eight nts with hsa-miR-155 (27,73) (refer Figure 2). MiR-155 is processed from a primary transcript, termed as BIC gene (B-cell Integration Cluster), whose upstream region was identified as a common site of integration of the avian leucosis virus (ALV) (74) in lymphomas. miRNA-profiling studies

(a)

```
UUAAUGCUAAUCGUGAUAGGGGU  hsa-miR-155 (MIMAT0000646)
|||||||| |  | ||       |
UUAAUGCUUAGCCUGUGUCCGA   kshv-miR-K12-11 (MIMAT0002181)

UUAAUGCUAAUCGUGAUAGGGGU  hsa-miR-155 (MIMAT0000646)
||||||||          ||
UUAAUGCUGUAUCGGAACCCUUC  mdv1-miR-M4 (MIMAT0003924)
||||||||          ||
UUAAUGCUAAUCGUGAUAGGGG   gga-miR-155 (MIMAT0001106)
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(b)

```
UCGAGGAGCUCACAGUCUAGU    hsa-miR-151-5p(MIMAT0004697)
||||||||| |    |  |
UCGAGGAGCACGUGUUAUUCUA   mghv-miR-M1-4 (MIMAT0001567)
||||||||| |    |  |  |
UCGAGGAGCUCACAGUCUAGU    mmu-miR-151-5p (MIMAT0004536)
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(c)

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UAAGGUGCAUCUAGUGCAGAUAG  hsa-miR-18a (MIMAT0000072)
 ||||||| ||||||
CAAGGUGAAUAUAGCUGCCCAUCG ebv-miR-BART5 (MIMAT0003413)
 ||||||| ||||||
UAAGGUGCAUCUAGUGCAGUUAG  hsa-miR-18b (MIMAT0001412)

UAAGGUGCAUCUAGUGCAGAUAG  mmu-miR-18a (MIMAT0000528)
||||||| |    |  |
AAAGGUGGAGGUGCGGUAACCU   mghv-miR-M1-7-5p (MIMAT0001570)
||||||| |    |  |
UAAGGUGCAUCUAGUGCUGUUAG  mmu-miR-18b (MIMAT0004858)

UAAGGUGCAUCUAGUGCAGAUAG  hsa-miR-18a(MIMAT0000072)
|||||||  ||  | |||
UAAGGUGAAUAUAGCUGCCCAUUG rlcv-miR-rL1-8 (MIMAT0003437)
|||||||  ||  | |||
UAAGGUGCAUCUAGUGCAGUUAG  hsa-miR-18b (MIMAT0001412)
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Figure 2. Virus-encoded microRNAs as orthologs of cellular microRNAs. Encircled boxes show sequence homology of viral miRNAs to human/mouse miRNAs. The homologous bases are shown in blue. (a) Orthologs of cellular miR-155; (b) orthologs of cellular miR-151; (c) orthologs of cellular miR-18a and miR-18b.

have shown increased expression of miR-155 in a wide range of cancers including lymphomas (75). Gottwein *et al.* (73) reported that miR-155 and miR-K12-11 regulate similar set of targets including genes with known roles in cell-growth regulation. It has been shown that BACH-1 is one of the predicted mRNAs, targeted by both miR-155 and miR-K12-11 (27). Transient expression of miR-155 occurs in macrophages, T and B lymphocytes and miR-155 knockout mice revealed defects in adaptive immune responses. Furthermore, overexpression of miR-155 in B-cells is associated with the development of B-cell lymphomas in humans, mice and chickens (74) although the mechanism is unknown. Given the apparent role of miR-155 in tumorigenesis and miR-K12-11 being an ortholog of miR-155, it is tempting to speculate that miR-K12-11 may contribute to the development of B-cell tumors seen in KSHV-infected individuals. Inspite of being a distantly related γ-herpesvirus, EBV miRNAs do not bear homology to miR-155 (76). However, previous reports have shown the expression of BIC during EBV infection expressing the full repertoire of EBV latency genes, which implies the role of EBV latency genes in inducing BIC gene (77).

Analyzing the entire set of viral miRNAs known till today, such seed homology is observed in a few more cases. One of the interesting cases is the MDV-1 miRNA miR-M4, which bears the same $5^/$terminal 8 nts as miR-K12-11 and hence might function as an ortholog of miR-155. Since MDV-1 encodes meq oncogene apart from other proteins, miR-M4 might contribute to tumorigenesis in chickens. Furthermore, miR-M1-4 of MHV68 shares $5^/$terminal 9 nts with murine miR-151. The function of this cellular miRNA is still unknown. Potential cellular orthologs of other viral miRNAs having limited seed homology (nts 2–7) (refer Figure 2), corresponding to the minimal miRNA seed region, include ebv-miR-BART5, rlcv-miR-rL1-8 and mghv-miR-M1-7-5p, which have miR-18a and miR-18b as their cellular counterpart. These two cellular miRNAs are encoded in the miR-17-92 cluster, which has oncogenic function (78).

VIRAL miRNAs-REGULATING GENE EXPRESSION

Regulatory impact on viral transcripts

Viral miRNAs have a regulatory effect on their protein-coding genes. The level of regulation depends on the degree of complementarity of the viral miRNAs with the $3^/$UTR (untranslated region) of the regulated mRNAs (79). These regulations are beneficiary to the virus toward maintaining its replication, latency and evading the host-immune system (Figure 3).

MiR-BART2 of EBV exhibits perfect complementarity to the $3^/$UTR of BALF5, which encodes the viral DNA polymerase (9). Recently, Barth *et al.* (14) have shown that miR-BART2 down-regulates BALF5. Induction of the lytic viral replication cycle results in a reduction of the level of miR-BART2. Hence, there is a decrease in cleavage of the BALF5 $3^/$UTR. Forced expression of miR-BART2 during lytic replication resulted in a 40–50% reduction of the level of BALF5 protein and

a 20% reduction of the amount of virus released from EBV-infected cells. It might be the situation that latently expressed miR-BART2 specifically has evolved to target BALF5, and such an interaction may be essential for maintaining EBV latency. The other EBV miRNAs miR-BART-1p, miR-BART16 and miR-BART17-5p (having imperfect match with the targets) target 3′UTR of the mRNA coding for the latency-associated membrane protein LMP-1 and repress its expression. This regulation decreases LMP-1-mediated activation of nuclear factor-kappa B (NF-κB) as well as apoptosis resistance (44). Hence, these miR-BART miRNA-mediated regulations on LMP1 may explain the discrepancy between LMP1 transcript and protein detection in nasopharyngeal carcinoma. This further highlights the role of the EBV miRNAs in regulating LMP1 downstream signaling to promote cancer development (44).

Grey *et al.* (79) predicted that miR-UL112-1 of HCMV targets the viral immediate-early protein 1(IE1) mRNA, a transcription factor required for the expression of many viral genes. To test this prediction, mutant viruses were generated that were unable to express miRNA or encoded an IE1 mRNA lacking its target site. Analysis of RNA and protein within infected cells demonstrated that miR-UL112-1 inhibits expression of the major IE1 protein. Such miRNA-mediated suppression of IE genes might be a part of the strategy of these viruses to enter the host and maintain latency.

SV40 miRNAs (miR-S1-5p and miR-S1-3p) are perfectly complementary to early viral mRNAs and target the mRNAs for a protein known as T antigen, leading to its cleavage. On entering into the cell, viral replication is triggered by the production of this T antigen. Furthermore, T antigen serves as a target for host immune (T) cells, which destroys infected cells and prevents the virus from spreading. Thus, the corresponding miRNAs (targeting T antigenic mRNA) accumulate at late times in infection when enough viral replication has been done. Furthermore, it has been shown that cells infected with a mutant virus (that does not produce SVmiRNAs) are more likely to get killed by cytotoxic immune cells rather than the wild-type ones (11). Thus, it is shown that viral evolution has taken advantage of the miRNA pathway to generate effectors that enhance the probability of successful infection.

MDV latency-associated transcripts include miR-M6 to miR-M8 and miR-M10 and miR-M13, which maps to a large intron at the 5′ end (38,80). This is presumably derived from a large 10-kb transcript that maps antisense to the ICP4 gene, which implies a probable role of these miRNAs toward modulating ICP4 transcript to inhibit entry of the virus into the lytic cycle.

In HIV-1-infected and nef-transduced cells, nef-derived miRNA miR-N367 inhibits HIV-1 transcription in human T cells (46), thus facilitating both viral replication and disease progression. Recently, annotated HSV-1 miRNAs, miR-H2-3p and miR-H6 are reported to down-regulate the expression of ICP0 and ICP4 proteins, respectively. Such miRNA-induced down-regulation helps HSV-1 to maintain latency (16). Furthermore, miR-I of HSV-2 regulates expression of a key viral neurovirulence factor, thereby affecting the establishment of latency (34).