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REVIEW

MicroRNAs and their role in viral infection

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Abstract Recently, a class of about 22 nucleotides (nt) small RNA has been discovered in many eukaryotes, termed microRNAs (miRNAs), which have a variety of functions. Many recent findings have demonstrated that viruses can also encode their own miRNAs. Meanwhile, other findings reveal a relationship between host miRNA and viral infection. These findings suggest a tight relationship between host and viral infection via miRNA pathway. This article introduces the miRNAs encoded by viruses and reviews the advances of the interaction of the mammalian host miRNAs and viral infection.

Keywords microRNA (miRNA), viral infection, small RNA

Viruses have threatened people's life and health throughout history. They have caused enormous damages to human society, such as the smallpox which had devastated mankind for centuries, the 1918 Spanish Flu pandemic, and AIDS, the modern plague whose first case was reported in 1981. The hosts of viruses include not only human, but also other animals, plants, fungi, bacteria, etc. During the long history of co-evolution, viruses and their hosts have been battling against one another. Recently, it has been discovered that microRNAs play an important role in the interaction of virus and host.

MicroRNAs (miRNAs) are small RNAs common in eukaryotes. They do not have open reading frames (ORF) or encode any proteins. They are 20–24 nucleotides (nt) in length and may have a length variance of 1–2 nt at the 3′ end. Mature miRNAs have a phosphate group in its 5′ end and a hydroxide at 3′ end. The miRNA genes exist in clusters in genome, and the miRNAs in the same gene cluster have high homologous feature, whereas those in different clusters are less homologous. Many miRNAs are highly conserved across species. On the other hand, miRNAs are expressed in a tissue-specific and developmental stage-specific manner, that is to say, different miRNAs are expressed at different periods of development, or in different tissues, and this specificity implies that they are involved in the complex regulation process of gene expression in organism (Zhang et al., 2005).

The precursor of miRNA, a PolII transcript of hundreds or thousands nucleotides in length, named pri-miRNA, is cleaved by RNase III protein Drosha in nucleus into a short fragment of 60–80 nt incomplete hairpin termed as pre-miRNA. Subsequently, it is transported out of nucleus by nucleus transportation protein: Exportin-5 and Ran, and again undergoes another cleavage process by Dicer in cytoplasm, then degrades messenger RNA (mRNA) in a similar mechanism as small interfering RNA (siRNA) does. On the other hand, miRNAs may also bind to the untranslational region (UTR) of 3′ end of a particular mRNA, and suppress subsequent translation (Sullivan et al., 2005). By modulating gene expression, these non-coding small RNAs play important roles in the process of development, lipid metabolism, differentiation, proliferation, apoptosis, tumerigenesis, etc. (Schutz and Sarnow, 2006).

Small in length, and not inducing antibody reaction as protein does, miRNA obviously is a highly efficient and effective way of gene regulation for viruses, life with the smallest genome in the whole living world. Recent studies reveal more and more virus genome in mammalian encode miRNAs. These miRNAs may play a pivotal role in viral infection, and the whole viral life circle. Meanwhile, miRNAs in mammalian genome which interact with virus have also been found, implying the importance of miRNAs in the process of viral infection.

1 miRNAs encoded by DNA virus

1.1 miRNAs encoded by herpesvirus

Herpesvirus is a kind of DNA virus which can cause persistent infection. It has been discovered in recent years that miRNAs are encoded in many herpesvirus genome (Nair and Zavolan, 2006) (Fig. 1).

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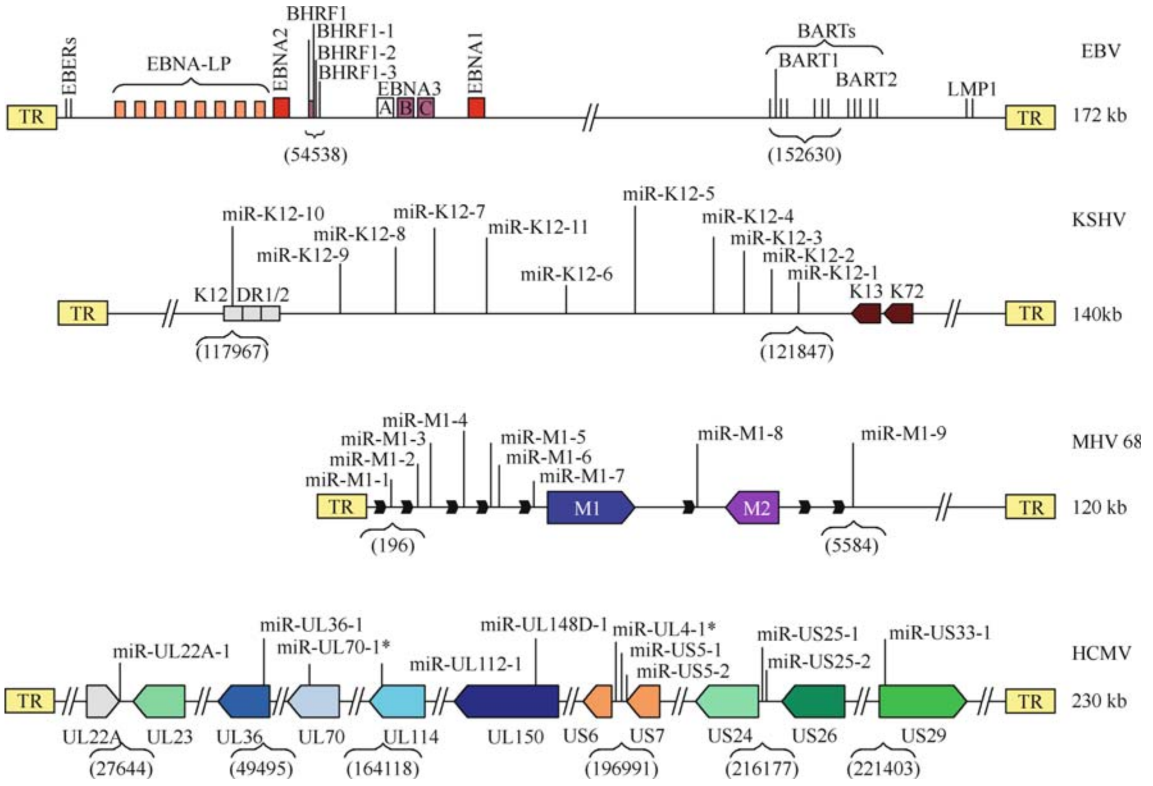


Approximate genomic locations of miRNAs encoded by each of the viruses are indicated by the nucleotide positions shown in brackets

Fig. 1 Schematic diagram showing the structure of EBV, KSHV, MHV 68, and HCMV genomes

1.1.1 miRNAs encoded by Epstein–Barr virus (EBV)

EBV, a member of herpesvirus *γ* subfamily, is the first virus demonstrated to encode miRNAs. Pfeffer et al. (2004) identified five miRNAs from a B cell line latently infected with EBV. However, none of these miRNAs has homologous features in any known host encoded miRNAs. In the latent infected cells, EBV encoded miRNAs are found in two major regions: three miRNAs are beside BHRF1, and the others lie within the virus gene BART. The expression of BART miRNA is tissue-specific whereas the expression of BHRF1 miRNA is developmental stage-specific. The induction of viral lytic replication may cause many, but not all the expression of virus miRNAs.

Computational predictions suggest that some of these miRNAs may target chemokines, cytokines, and apoptotic and cell growth control genes and many elements in the signal transduction pathway. Among them, the target of miRNA-BART2 may be the DNA polymerase BALF5 encoded by virus. The sequence of miRNA-BART2 is completely complementary with that of the 3′ UTR of BALF5, and it leads to the degradation of BALF5 mRNA via RNA interfering pathway. These phenomena illustrate that EBV may regulate both itself and its host via miRNA, and attain persistent latent infection with the mechanism which does not incur immune reaction.

1.1.2 miRNAs encoded by Kaposi's sarcoma-associated herpesvirus (KSHV)

Kaposi's sarcoma-associated herpesvirus, another member of herpesvirus *γ* subfamily, is the causative agent of Kaposi's sarcoma, and is also linked to several rare lympohoproliferative syndromes. Some groups of researchers (Cai et al., 2005; Pfeffer et al., 2005; Samols et al., 2005), using traditional RACE-like cloning, have identified miRNAs in KSHV and there is broad agreement among these studies that a cluster of 11 KSHV miRNAs are encoded in a single 4.5 kb region of the genome. Among them, miR-K12-10 lies in ORF which encodes a family of proteins (kaposins A, B and C). Kaposin A affects cell growth control, and kaposin B enhances cytokine production by infected cells. Both proteins are tightly regulated in latency, as overexpression of either is deleterious to cells. The other 10 miRNAs reside in the intron of kaposin primary transcript. Computational predictions suggest that the KSHV miRNAs target at viral genes such as ORF 23, 27, 31, 52, 49, 61, 68, K7, K13 and K14, as well as cellular targets involved in such activities as apoptosis, signaling and B cell regulation (McCormick and Ganem, 2005; Kliche et al., 2001). Although not validated yet, these predictions indicate KSHV might modulate both their own gene expression and that of their host's, and play a pivotal role in viral replication and pathogenesis.

1.1.3 miRNAs encoded by murine herpesvirus 68 (MHV 68)

Murine herpesvirus 68 is a causative agent of small rodent animal, and is classified into herpesvirus *γ* subfamily. It is highly homologous with human *γ* herpesvirus and is therefore often used in small animal model studying *γ* herpesvirus. Presently, it has been detected that MHV 68 encodes nine miRNAs, and all cluster within a 6-kb region near the M1 terminus of the linear genome, right downstream of the predicted viral tRNA gene. Further research reveals that MHV pre-miRNA may start tRNA promoter through PolIII transcription, whereas all previously studied mammalian pre-miRNA was processed by PolII transcript (Pfeffer et al., 2005). The target of miRNA of MHV 68 in viral and host still needs validation. However, experiment shows that a mutant deleted of this miRNA cluster leads to changes in tissue specificity as well as the latency depending on infection pathway (Clambey et al., 2002).

1.1.4 miRNAs encoded by human cytomegalovirus (HCMV)

Human cytomegalovirus is classified into the *β* herpesvirus subfamily. It can cause extensive primary or periodic infection, with the symptoms varying from subclinical to severe acute disease. In contrast to MHV 68, HCMV encodes nine miRNAs spread throughout the viral genome. Among them, three are transcribed from the complimentary strand of known ORFs, which may associate with autoregulation of virus, five are located in intergenic regions and one is within an intron (Pfeffer et al., 2005). Since all nine miRNAs were cloned from lytically infected cells, it is likely that miRNAs are not solely associated with the latency program. Hence, many more miRNAs await discovery in the latently infected cells.

1.1.5 miRNAs encoded by herpes simplex virus (HSV)

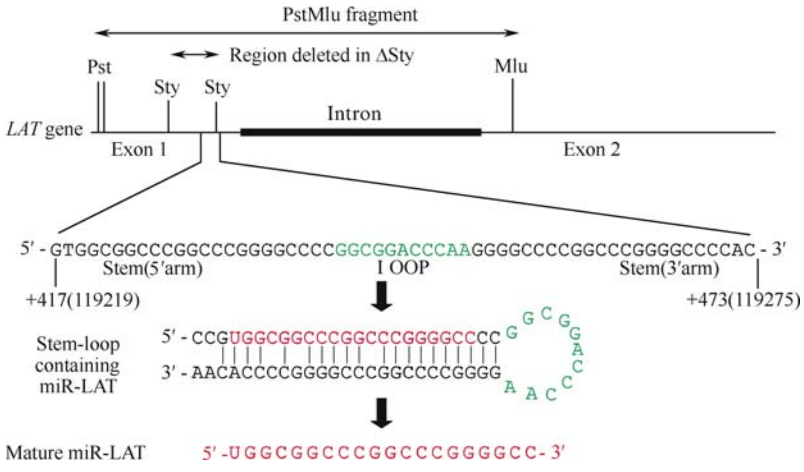
Herpes simplex virus belongs to *α* herpesvirus subfamily, and is the first virus discovered as human herpesvirus. Recent studies reveal HSV also encodes miRNAs. The latency-associated transcript (LAT) gene of HSV-1 is the sole viral gene expressed in latent infection in nerve cell. Latency-associated transcript inhibits apoptosis and maintains latency by increasing the survival of nerve cell. However, the mechanism of apoptosis avoidance and other proteins involved in this process is still unclear. Gupta et al. (2006) found and validated a LAT-associated miRNA, termed miR-LAT, located in the first extron of LAT gene. Latency-associated transcript miRNA is demonstrated to accumulate in cells transfected with LAT gene or infected with wide type HSV-1. An experiment was conducted with a mutant virus with all the 372-nt fragment of the miRNA region deleted, and resulted in apoptosis in cells and no miRNA production (Fig. 2). Further studies show that miR-LAT antagonize apoptosis by lowering the expression of TGF-β and SMAD3 in TGF-β pathway. Hence, HSV-1 escapes the host immune system, avoids the host cell apoptosis and realizes persistent latent infection.

1.2 miRNAs encoded by other DNA viruses

Besides herpesvirus, many other DNA viruses are reported to encode miRNAs.

1.2.1 miRNAs encoded by simian virus40 (SV40) and other polyomaviruses

Polyomaviruses are small DNA viruses which are widespread in mammalians. The best-known polyomavirus is SV40, which is often used as model virus studying oncogenesis. It was recently discovered to encode some miRNAs (Sullivan et al., 2005) These miRNAs are all produced from a single pre-miRNA, and the targets of them have been identified. This pre-miRNA resides in the late strain of viral genome (the strain that transcript after the replication of viral DNA) and overlaps with the early viral mRNA sequence. These miRNAs are perfectly complementary with early mRNA, and consequently the transcripts are excised. In fact, the 3′



Bottom panel: sequence conservation of miR-LAT-encoding region in 17＋, F and McKrae strains of HSV

Fig. 2 The LAT gene of HSV-1 codes for a miRNA polyadenylated product predicted from such excision has already been detected in cells, and the mutant experiment on pre-miRNA resulted in the disappearance of this product, demonstrating that they are derived from the miRNA action. In the late period of viral replication, these miRNAs remarkably down-regulate the expression of early viral genes, including T antigen, product of a major early gene. When SV40 enters host cell, it produces T antigen and initiates the onset of viral DNA replication. However, viral T antigen is also a target of T cell of the host immune system. T cell can therefore destroy the infected cell and thus stop the spread of virus. The miRNAs which target T antigen gene will be expressed in a large amount during the late period of infection when T antigen is no longer important to viral replication. T antigen expression level will be lowered owing to miRNAs, and virus is therefore able to escape the host immune system. These pre-miRNAs have been found in other polyomaviruses such as BKV, JCV and SA12, indicating these miRNAs play an important biological role in viral infection.

Recently, it has been discovered that miRNA in mouse polyomavirus also plays a regulatory role in the expression of viral middle and big antigen (Sullivan et al., 2005). However, the miRNAs of mouse polyomavirus bear no homology with that of SV40, and emanate from a different region of the late pre-miRNA. The two completely unrelated miRNAs from different regions have the same function: down-regulating the expression of viral T antigen and consequently evading host immune response.

1.2.2 miRNAs encoded by adenovirus (ADV)

Adenovirus is a dsDNA virus which is prevalent in nature. It was lately discovered that ADV encodes a non-coding RNA of 160 nt, termed VA1, which exists in the late period of lytic replication. The expression level of VA1 is as high as 108 copies per cell. Synthesized by RNA PolIII in nucleus, VA1 RNA is then transported to cytoplasm by EXP5 and RAN, and then inhibits antivirus enzyme: protein Kinese R (PKR). Since VA1 is carried out of nucleus as pre-miRNA, and bares the motif that can be recognized by EXP5, and the same motif can also be recognized by heterogenous dimmer Dicer-TRBP, it is highly probable that VA1 be processed by Dicer. But this process might be low efficient and only 1% of these RNAs could be processed into ADV miRNA. Cullen (2006) found that VA1 could inhibit the expression of RNAi in vivo. VA1 can inhibit the RNAi induced by shRNA or the precursor of miRNA, whereas it has no effect on the artificial small fragment of RNAi. Then, it is hypothesized that VA1 inhibits RNAi by inhibiting the nuclear transportation of shRNA or the pre-miRNA by means of competing the biding of EXP5 or binding straightly with Dicer (Cullen, 2006).

2 miRNAs encoded by RNA viruses

Although the RNA viruses are far more than DNA viruses, to date, RNA virus encoding miRNAs is not a prevalent phenomenon as DNA virus has been reported. Pfeffer et al. (2005) conducted computational analysis and experimental confirmation on hepatitis C virus (HCV) and yellow-fever virus (YFV) genome but found no miRNA encoded. No RNA virus except retroviruses has been found to encode miRNA.

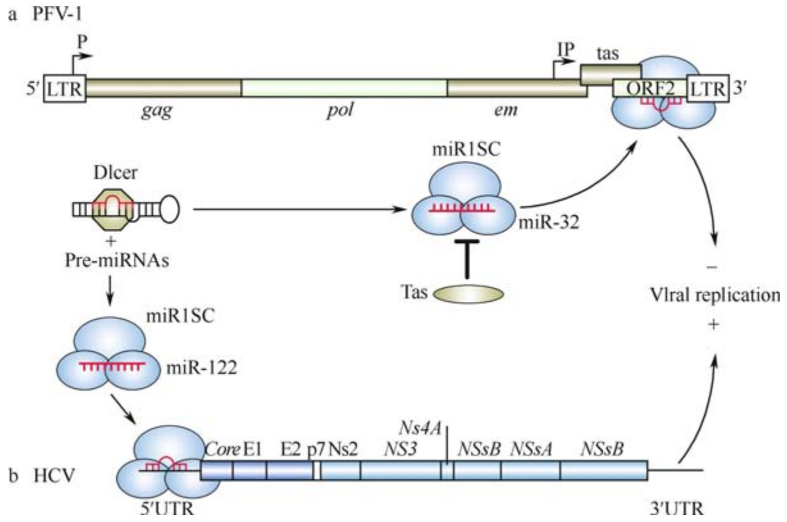
Retroviruses are very important RNA viruses, and among them, human immunodeficiency virus (HIV) is the one studied most. Computational analysis on HIV genome reveals that some pre-miRNAs are encoded, and the subsequent miRNAs may modulate HIV gene and the host gene associated with HIV infection, such as CD28, CD4, IL (Hariharan et al., 2005; Couturier and Root-Bernstein, 2005). In a research on some HIV infected people who do not have clinical symptom, Omoto and Fujii found that the miRNA from HIV Nef gene, named miR-N367, can inhibit the function of Nef and decrease virulence via RNAi pathway. Further studies reveal miR-N367 reduces the HIV LTR promoter activity by acting on the replication element on 5′-LTR U3 region, and thus it can inhibit HIV-1 transcription via two pathways (Omoto et al., 2004; Omoto and Fujii, 2005). The down regulation of its own transcription by miR-N367 acting on LTR U3 negative element indicates that HIV-1 may achieve its goal of persistent latent infection through miRNA autoregulation of both transcription and replication.

3 Host miRNA and its interaction with viral infection

After infected, mamalian cells can initiate many defending measures to prevent or inhibit viral replication. Take interferon for example, it can activate the production of many defending proteins, and among them are the PKR depending on dsRNAs, which may phosphorylate eIF-2*α*and therefore inhibit translation; Mx protein GTPase, which can arrest viral gene expression; and oligoadenylate synthetase (OAS) which activates RNase L and then degradates mRNAs and ribosome RNAs (Gao et al., 2002). The miRNAs are another choice of defense for host cells. Almost all the high eukaryotes encode miRNAs. And the expression map of miRNA may vary considerably in different cells and tissues, and therefore may have different functions. Some play a positive regulatory role in viral infection, enhancing infection and engendering a series of clinical symptoms; whereas others may play a negative role and inhibit viral infection (Sarnow et al., 2006).

3.1 miRNAs playing a positive regulation role in viral infection

MicroRNA 122 (miR-122) is a miRNA which is specifically expressed and highly abundant in hepatocyte. Sarnow et al. (2006) studied this small RNA which counts for 70% of the total hepatocyte miRNAs, tried silencing miR-122 by transfecting the complementary sequence of miR-22 into Huh7 hepatocyte cell line (expressing HCV RNA replicon) and



(a): Binding of the host-cell miRNA miR-32 to a site in primate foamy virus type 1 (PFV-1) RNA restricts viral RNA accumulation. The viral protein Tas functions as a silencing suppressor to relieve this repression; (b): Interaction of the liver-specific miRNA miR-122 with a target site close to the 5′ end of HCV RNA results in an increase in the amount of viral RNA, probably owing to stimulation of viral replication or to localization of viral RNA

Fig. 3 Interaction of cellular miRNAs with viral genomes (Sarnow et al., 2006)

learned that when miR-122 was deactivated, the copies of HCV RNA replicons would be reduced 80%. Mutations were introduced into the two predicted miR-122 binding site (within the non-coding region of RNA genome), and the results show that the mutant of 3′ non-coding region has no significant effect on viral replication whereas mutant of 5′ non-coding leads to the arresting of viral replication. However, ectopic expressing miR-122 enables HCV copies mount a come back. These phenomena indicate that miRNA plays a pivotal role in HCV replication. Likewise, in the EBV transformed lymphocyte, the expression of miR-155 has been remarkably upregulated, indicating that miR-155 may involve in the oncogenesis of EBV related malignant tumor (Kluiver et al., 2006).

3.2 miRNAs playing a negative regulation role in viral infection

During the infection of prototype foamy virus type-1, host cell miRNAs regulate viral infection in another way. Lecellier et al. (2005) found that miR-32, an endogenous miRNA, inhibits the replication of PFV in human cells. By transfecting the antisense competing RNA of miR-32, viral replication level was found to be doubled. On the other hand, virus does not wait for its doom facing the menace of RNAi by host miRNAs. Yeung et al. analysed miRNA expression map of the HIV infected human cells and found that the expression of four miRNAs, miR-93, miR-148b, miR-221 and miR-16 have been reduced. Meanwhile they discovered HIV itself encodes the precursor of siRNA and inhibits RNAi by Tat interference of Dicer processing dsRNA (Bennasser et al., 2005; Yeung et al., 2005). The Tas of PFV can also inhibit RNAi in mammalian cells. Since Dicer is needed in both siRNA and miRNA pathways, it is possible that virus may act on Dicer by protein encoded by itself and thus inhibit host siRNA and miRNA pathways and attain persistent latency.

The preliminary studies of our lab reveal that Borf-1, transcription activator of bovine foamy virus (BFV) can inhibit let-7, a miRNA that is common in human cells (data not published). This discovery suggests that different members of the foamy virus subfamily may antagonize host siRNA and miRNA interaction via a similar mechanism.

4 Summary and outlook

As a small regulatory factor on RNA level, miRNAs are prevalent in organism and are an ancient biology phenomenon, though discovered just recently. During the long period of coevolution of virus and host, in order to escape the immune system of the host, viruses encode miRNAs, which are economic and do not activate the host immune response and manage to regulate the gene expressions of both theirs and their host's. Or they utilize the host's miRNAs to achieve infection and completeness of life circle. On the other hand, the hosts do not wait for their doom, as well as antagonizing viruses via immune response, the hosts utilize another strategy: encoding miRNAs to inhibit the infection and replication of virus. And conversely, viruses also encode proteins to counteract this inhibition. In sum, the interaction between virus and host exists in various levels such as protein–protein, RNA–protein, RNA–RNA, RNA–DNA level and forms a complex and multidimensional relationship. In the millions of years of evolution, the viruses and their host have been battling against each other, and evolved together. Further research on the interaction between virus and host on miRNA level will surely lead to deeper understanding of this interaction and may probably influence the treatment of many human diseases.

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