REVIEW

RNAi and miRNA in Viral Infections and Cancers

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Abstract

   Since the first report of RNA interference (RNAi) less than a decade ago, this type of molecular intervention has been introduced to repress gene expression *in vitro* and also for *in vivo* studies in mammals. Understanding the mechanisms of action of synthetic small interfering RNAs (siRNAs) underlies use as therapeutic agents in the areas of cancer and viral infection. Recent studies have also promoted different theories about cell-specific targeting of siRNAs. Design and delivery strategies for successful treatment of human diseases are becomingmore established and relationships between miRNA and RNAi pathways have been revealed as virus-host cell interactions. Although both are well conserved in plants, invertebrates and mammals, there is also variabilityand a more complete understanding of differences will be needed for optimal application. RNA interference (RNAi) is rapid, cheap and selective in complex biological systems and has created new insight sin fields of cancer research, genetic disorders, virology and drug design. Our knowledge about the role of miRNAs and siRNAs pathways in virus-host cell interactions in virus infected cells is incomplete. There are different viral diseases but few antiviral drugs are available. For example, acyclovir for herpes viruses, alpha-interferon for hepatitis C and B viruses and anti-retroviral for HIV are accessible. Also cancer is obviously an important target for siRNA-based therapies, but the main problem in cancer therapy is targeting metastatic cells which spread from the original tumor. There are also other possible reservations and problems that might delay or even hinder siRNA-based therapies for the treatment of certain conditions; however, this remains the most promising approach for a wide range of diseases. Clearly, more studies must be done to allow efficient delivery and better understanding of unwanted side effects of siRNA-based therapies. In this review miRNA and RNAi biology, experimental design, anti-viral and anti-cancer effects are discussed.

Keywords: RNA interference - micro RNA - antiviral agent- anticancer agent

Interference of RNA (RNAi-SiRNA)

   Small ribonucleic acid (RNA) can act as a specific regulator of gene expression. This discovery has been an exciting breakthrough in Biological Sciences of the past decade, climax in last year's Nobel Prize in Physiology or Medicine awarded to Andrew Fire and Craig Mello in 2006. Building on previous work mainly in plants found that exogenous double-stranded RNA can be used to specifically interfere with gene function. This event was called RNA interference (RNAi) (Kozielski et al., 2013; Nishimura et al., 2013). They also consider that organisms might use double-stranded RNA naturally as a way of silencing genes. It was then shown that RNA interference was mediated by 22 nucleotide single-stranded RNAs termed small interfering RNAs (siRNAs) derived from the longer double-stranded RNA precursors (Schott et al., 2012). The small interfering RNAs were discovered to suppress genes by removing the corresponding messenger RNA transcripts, and thus, blocking protein synthesis. SiRNAs regulate the degradation of mRNA molecules identical in sequence to that of the corresponding siRNA, resulting in the silencing of the corresponding gene and the shutting down of protein synthesis (Luo et al., 2004; Overhoff et al., 2005). The main mechanism of action of siRNA is the mRNA cleavage function. There are no genes that encode for siRNAs. siRNAs can also make gene expression silence by triggering promoter gene methylation and chromatin condensation (de Almeida et al., 2008; Dua et al., 2011; Chen et al., 2012).

Micro RNAs (miRNAs)

   The first miRNA was discovered in 1993 by Victor Ambros and colleagues Rosalind Lee and Rhonda Feinbaum. A genetic screen in the roundworm

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Caenorhabditis elegans, a millimeter-long animal used as a model organism in biological research, recognized genes involved in developmental timing (Cevec et al., 2010). Surprisingly, one of the genes, termed *lin-4*, did not encode a protein but instead a new 22-nucleotide small RNA. Seven years later, Reinhart et al. discovered a second 22-nucleotide small RNA of this type, *let-7*, a gene also involved in *C elegans* developmental timing (Cevec et al., 2008). The *lin-4* and *let-7* small regulatory RNAs soon became very exciting for two reasons. Firstly, similar *let-7* gene were identified in other animals including humans. The conservation of *let-7* across species suggested an important and essential biological role for this small RNA (Zhu et al., 2011). Secondly, the mechanism of RNA interference (RNAi) was discovered at that time, and it became clear that miRNA and RNAi pathways were intricately linked and shared common constituents. Within the following year, more than 100 additional small regulatory RNAs similar to *lin-4* and *let-7* were identified in worms, the fruit fly Drosophila, and in humans These small non-coding RNAs were named microRNAs (miRNAs) (Schott et al., 2012).

Compare between SiRNA and miRNA

Comparing with siRNAs, miRNAs are encoded by specific miRNA genes as short hairpin pri-miRNAs in the nucleus miRNAS are also small noncoding RNAs, but they seem to need only a 7-8base-pair "seed" match between the 5' region of the miRNA and the 3'UTR of the target (Auyeung et al., 2013). It would seem that the majority of miRNA targets are translationally repressed; however, reduction of the target mRNA can also happen. The main mechanism of action of miRNA may be the inhibition of mRNA translation, although the cleavage of mRNA is also an important role (Figure 1) (Branscheid, et al., 2011). siRNAs are synthesized from double-stranded segments of matched mRNA via RNA-dependent RNA polymerase. miRNAs are synthesized from an unmatched segment of RNA precursor featuring a hairpin turn.
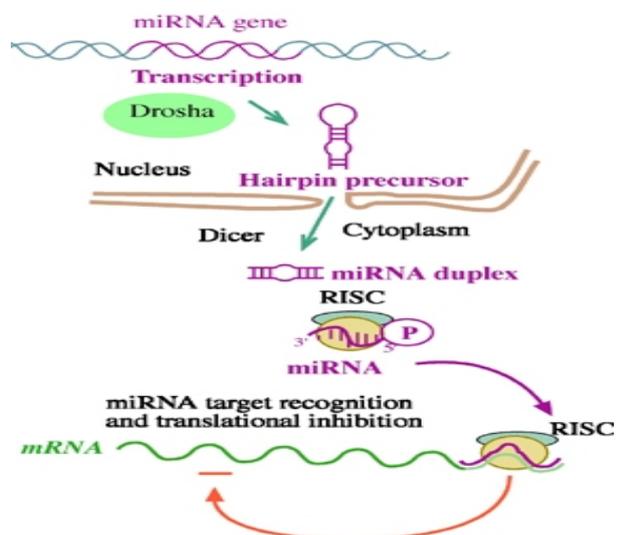
siRNAs are often extracted from repetitive DNA sequences and associated transposons and centromeres, forming heterochromatin structures (Tsutsumi, et al., 2011). Both siRNAs and miRNAs are produced by Dicer-mediated cleavage of longer double-stranded RNA precursors. However, miRNAs are completely endogenous, whereas siRNAs may be endogenous or xogenously derived from viruses (Figure 2). Also, miRNAs cluster in "families" closely related as far as the sequence is concerned or as individual units. siRNAs can exist as repeated elements. Although miRNAs and siRNAs are inhibitors of specific mRNAs and both are under extreme study in anticancer drug development and there are significant differences between them which are highlighted in Table 1 (Monteys et al., 2010). Transcriptional profiling data have shown that the expression of miRNAs is highly normal in tissue and tumor-specific. miRNAs have been localized in chromosomal loci in the human genome associated with cancer, including fragile sites and areas of gene amplification (Lee, et al., 2011). Gains and losses of miRNA expression have been associated with cancer development and progression. However, the evidence that miRNAs directly participate in cancer development is limited (Chatterjee et al., 2013).

Mechanisms of RNA Interference in Mammalian Cells

As shown in the Figure 2 pathway at the bottom left, cytoplasmic double-stranded RNAs (dsRNAs) are processed by a complex consisting of Dicer, TAR RNA-binding protein (TRBP) and protein activator of protein kinase PKR (PACT) into small interfering RNAs (siRNAs),which are loaded into Argonaute 2 (AGO2) and the RNA-induced silencing complex (RISC). The siRNA guide strand recognizes target sites to direct mRNA cleavage, which is carried out by the catalytic domain of AGO2. siRNAs complementary to promoter regions direct transcriptional gene silencing in the nucleus through chromatin changes involving histone methylation
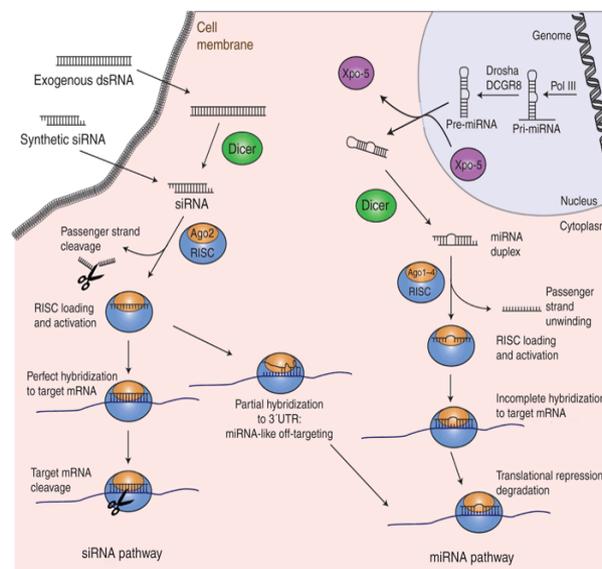

Figure 1. The Main Mechanism of Action of miRNA may be the Inhibition of mRNA Translation (Izquierdo, 2005)

Figure 2. Comparison between siRNA and miRNA Pathways (Kanasty et al., 2012)

Table 1. Comparison Properties in SiRNA and miRNA

| Properties	SiRNA	MiRNA
Primary mode of action	Inhibition of translation	mRNA cleavage
Secondary mode of action	mRNA cleavage	Chromatin silencing
Encoded by genes in nucleus	Yes	No
Matched RNA source	Yes	No
Synthesis	Endogenous	Endogenous or exogenous dsRNA
Phylogenitically conserved	Yes	No
Synthesis enzymes	RNA depended -RNA polymerase , Dicer	RNA polymerase II , Drosha,Dicer
Precursor stage	Nucleus	Nucleus
Length	19-23 bp	19-24 bp
Potential use in cancer diagnosis	Unknown	Yes
Cancer prognostic significance	Unknown	Yes
Use in research and drug discovery	Yes	Yes
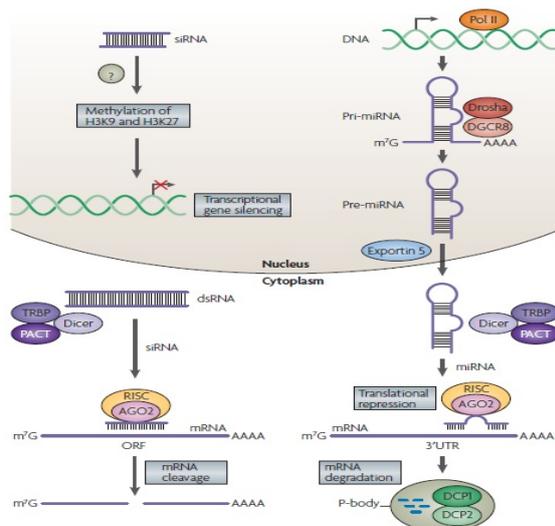
Figure 3. Mechanisms of RNA Interference in Mammalian Cells (Daniel et al., 2007). H3K9, histone 3 lysine 9; H3K27, histone 3 lysine 27;m7G, 7-methylguanylate; ORF, open reading frame

(top left); the precise molecular details of this pathway in mammalian cells are currently unclear. As shown in the pathway on the right, endogenously encoded primary microRNA transcripts (pri-miRNAs) are transcribed by RNA polymerase II (Pol II) and initially processed by Drosha–DGCR8 (DiGeorge syndrome critical region gene 8) to generate precursor miRNAs (pre-miRNAs). These precursors are exported to the cytoplasm by exportin 5 and subsequently bind to the Dicer–TRBP–PACT complex, which processes the pre-miRNA for loading into AGO2 and RISC. The mature miRNA recognizes target sites in the 3' untranslated region (3' UTR) of mRNAs to direct translational inhibition and mRNA degradation in processing (P)-bodies that contain the decapping enzymes DCP1 and DCP2 (Geoghegan, et al., 2012; Kim et al., 2007) (Figure 3).

RISC Loading and Activation

Double-stranded RNAs (dsRNAs) and precursor microRNAs (pre-miRNAs) are processed by a complex comprising Dicer, TAR RNA-binding protein (TRBP) and protein activator of protein kinase PKR (PACT), facilitating loading of the small interfering RNA (siRNA) or microRNA (miRNA) duplex into Argonaute 2 (AGO2)
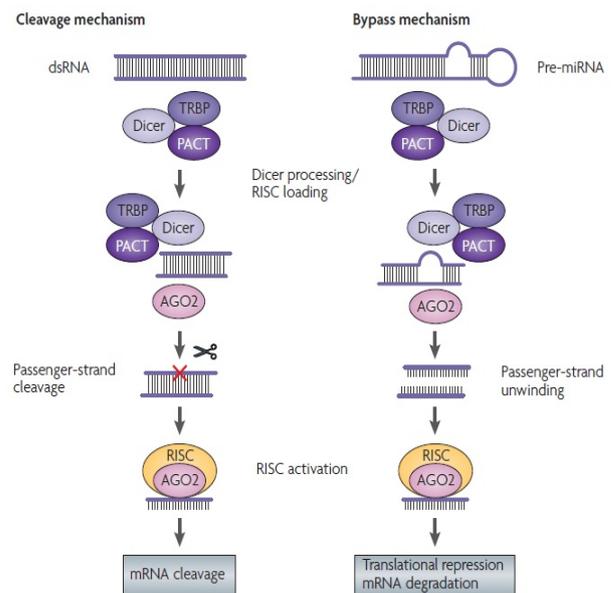

Figure 4. RISC Loading and Activation (Daniel et al., 2007)

and RNA-induced silencing complex (RISC) (Gu et al., 2012). When the RNA duplex loaded into RISC has perfect sequence complementarity, AGO2 cleaves the passenger strand so that active RISC is produced that contains the guide strand,which is complementary to the target sequence (Rand et al., 2005; Ye et al., 2011). When the RNA duplex loaded into RISC has imperfect sequence complementarity a bypass mechanism is used, in which a helicase activity is required to unwind the passenger strand from the guide strand and to generate the mature miRNA strand, producing active RISC (Kim, 2007) (Figure 4).

Immunostimulatory Effects of RNA Interference

Double-stranded RNAs (dsRNAs) longer than 30 base pairs activate protein kinase R (PKR) and induce an interferon (IFN) response, as do blunt-ended, small interfering RNAs (siRNAs),which are detected by RIG-I (Lu et al., 2011). siRNAs that are incorporated into endosomes can activate Toll-like receptors (TLRs), depending on the presence of immunostimulatory motifs, inducing a type I interferon response by activating the transcription factors nuclear factor-κB (NFκB), activating transcription factor 2 (ATF2) and interferon regulatory

factor (IRF) (Xu et al., 2009; Sioud et al., 2010) (Figure 5).

miRNA and Cancer Diagnosis

The miRNA signature seems to be specific for a given normal tissue or malignancy. Investigators have reported that the miRNA signatures for poorly differentiated human neoplasms are more tumor-specific than the corresponding mRNA expression patterns (Cao et al., 2011). Moreover, profiling for miRNA expression in human solid tumors has confirmed a number of miRNAs as potential targets of anticancer therapy (Castaneda et al., 2011). In 2005, three reports provided the first mechanistic insight into how miRNAs might contribute to carcinogenesis. Two independent studies described the relationship between a miRNA cluster, mir-17-92, and the Myc oncogenic pathway (Jay et al., 2007). A third report demonstrated an interaction between let-7 miRNA and the RAS proto-oncogene . mir-17–92, is shown to be a potential oncogene by its action in an in vivo model of human B-cell lymphoma (Mitani et al., 2013). A cluster of microRNAs on human chromosome 13 has been found to be regulated by c-Myc, an important transcription factor that is overexpressed in many human cancers. And analysis of microRNA expression in over 300 individuals shows that microRNA profiles could be of value in cancer diagnosis (Chaulk et al., 2011; Zhao et al., 2013).

miRNA and Cancer Progression

A series of studies published during the last 2-3 years have displayed the association of miRNA expression with opposed outcome in a variety of human tumors. The profiles of miRNA expression have been linked to aggressive cancers with advanced disease detected at diagnosis (Tie et al., 2009). The study of miRNAs and cancer progression has focused on 3 major biological pathways: cell adhesion, angiogenesis, and cell matrix digestion and signaling. The transcription factor E2F1 exhibits dual properties, acting as a tumor suppressor and oncogene (Knoll et al., 2013). Cellular stress such as DNA damage or mitogenic signaling leads to the activation
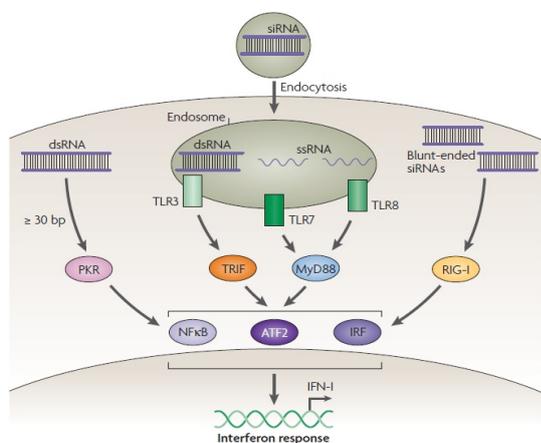
of E2F1 as a mediator of apoptosis in the context of a conserved cellular anti-tumorigenic protects mechanism. However in highly aggressive chemo resistant tumors like malignant melanoma and prostate/bladder cancer it switches off this role and acts as promoter of cancer progression (Leite et al., 2013). Possible reasons for E2F1 mediated aggressiveness are defects in cell death pathways caused by epigenetic inactivation of important tumor suppressor genes, which often occur in late stage cancer and contribute to chemo resistance (Tchernitsa et al., 2010). Nevertheless exact mechanisms underlying E2Fs role in intrusiveness and metastasis are largely unknown. Different reports intimate towards the existence of feedback loops between E2F1 and microRNAs. miRNAs are activated by E2F1 and either the transcription factor itself or cellular genes necessary for the growth regulating function of E2F1 are inhibited by different miRNAs (Kim et al., 2013; Knoll et al., 2013; Paterson et al., 2013). This interactive regulation possibly impacts the balance between E2F1s proapoptotic in contrast to prosurvival function. In the following we will summarize some miRNA E2F1 interactions contributing to a complex regulatory network (Figure 6) (Knoll et al., 2013).

miRNA as a Target for Cancer Treatment

Given the aforementioned associations of miRNAs with cancer development and progression, it is now held that these small regulatory RNAs could serve as targets of anticancer gene therapies (Avci et al., 2013). The antisense technology based on antagomiRs is the major method used to reduce unwanted miRNA expression in tumor cells. AntagomiRs have been progressively improved by chemically modified oligonucleotides, providing more stability and affinity to the target miRNA and, consequently, more efficiency than their natural counterparts (Connelly et al., 2012). Antisense molecules can inhibit the activity of oncogenic miRNAs, and these have been tested for their efficacy in a strategy of reducing miRNA activity on reporter genes bearing miRNA-binding sites (Krutzfeldt et al., 2005). Antagomirs represent a new class of oligonucleotides designed to antagonize specific

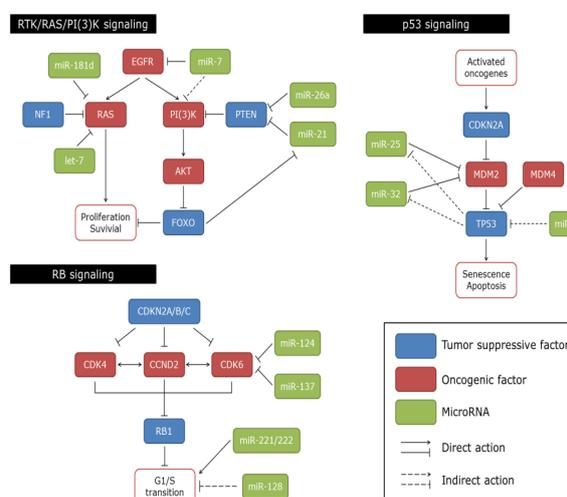
Figure 5. Immunostimulatory Effects of RNA Interference (Daniel et al., 2007). MyD88, myeloid differentiation primary response gene 88;TRIF, TLR-signalling adaptor protein

Figure 6. Role of miRNAs in Cell Signaling (Sihan et al., 2013)

miRNAs. miRNAs that function as tumor suppressors can also be lost in cancer, as well as overexpressed. Thus, alternative approaches toward the targeting of miRNAs in novel cancer therapies would be the use of viral vectors or small molecules or compounds to restore the expression of these lost miRNAs and simultaneously repress the expression of the corresponding target gene (Krutzfeldt et al., 2007). For example, in a recent study, inhibition of miR-200c using an anti-miRNA 2'-0-methyl oligonucleotide resulted in an increase in expression of one of the targets, the transcription factor TCF8. In 2008, the first clinical trial using miRNA-based therapy began. A phase I trial was initiated to treat hepatitis C using a LNAoligonucleotide complementary to miR-122 to inhibit the expression of this oncogenic miRNA, which normally facilitates virus replication (Scherr et al., 2010). In 2010, the success of this clinical trial and the beginning of phase II was announced .Despite the fact that this is the only study addressing the manipulation of a specific miRNA expression, several other clinical trials are currently being performed, aiming at the treatment of several diseases, such as asthma, leukemia, and other cancer types (Castaneda et al., 2011; Ye et al., 2013; Zhu et al., 2013).

Antiviral Mechanism of RNAi

Infection by all RNA viruses, except retroviruses, generates dsRNAs during the virus life cycle. DNA viruses also make dsRNAs by transcription. In plants and invertebrates, the aim is viral mRNAs and genomic RNAs by RISC binding and their cleavage and degradation, thus blocking virus replication (Figure 7) (Bagasra, 2005).

Plant and Invertebrate Viruses Can Block Antiviral RNAi

The tomato bushy stunt virus p19 protein inhibits RNAi through direct binding of 21 bp dsRNAs with 2-nt 39 overhangs, thus stopping the siRNAs (Akashi et al., 2004). The cucumber mosaic cucumo virus 2b protein

is able to inhibit RNAi by interfering with RISC, by inhibition of the RdRP-derived viral siRNAs and signaling (Matthew, 2004). Some insect viruses like the flock house virus (FHV) B2 protein, inhibits RNAi by direct binding of dsRNAs and also block viral siRNA by direct action with the viral RdRP in viral RNA synthesis (Dzianott et al., 2012).

Mammalian Cells Have an Antiviral RNAi Response

Cloned small RNAs from cell lines infected with a range of viruses including Kaposi's sarcoma-associated herpesvirus (KSHV), mouse g-herpesvirus 68 (MHV68), human cytomegalovirus (hCMV), human immunodeficiency virus (HIV-1), YFV, and HCV. They show that HIV-1, YFV, and HCV produce neither viral miRNAs nor siRNAs in infected cells (Mueller et al., 2010). Similar small RNA cloning, reported a similar absence of viral siRNAs in cells infected by HIV-1, human T-cell leukemia virus type I (HTLV-I), HCV, hCMV, murine cytomegalovirus (mCMV), EBV, and influenza virus (Bagasra, 2005).

RNAi Play a Role as an Innate Antiviral Immunity

Antiviral RNAi was first identified in Drosophila cell culture, where the beetle Nodamura virus FHV (flock house virus) acts as both an initiator and a target of the viRNA pathway. It was later mentioned, using the natural host–virus combination of O'nyong-nyong virus (alphavirus; Togaviridae) and Anopheles gambiae mosquitoes (Merkling et al., 2013).

Viral Suppression and Evasion of the viRNA Pathway

The Hc-Pro protein of plant Poty viruses and the 2b protein of plant cucumo viruses were identified as VSRs (Mallory et al., 2001). The first VSR identified in an animal virus was the B2 protein of the beetle virus FHV, which was introduced to block RNAi in plants, before being studied to play the same role in insect (Pandya et al., 2013).
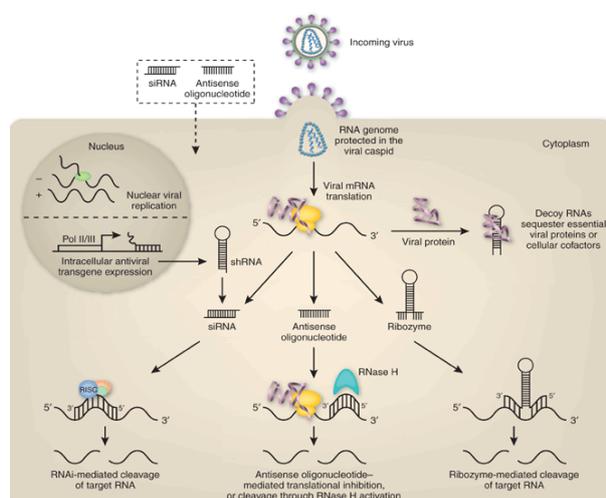
Table 2. Pre-miRNA Encoded by Viruses

Virus Family Subfamily/Genus	Name	Host	No. of pre-miRNAs
Herpesvirus			
α/Simplexvirus	HSV-1, HSV-2	Human	6,3
α/Mardivirus	MDV-1, MDV-2	Avian	14 ,17
β/Cytomegalovirus	hCMV, mCMV	Human,Murine	11 ,18
γ1/Lymphocryptovirus	EBV	Human	25
γ2/Rhadinovirus	rLCV, KSHV	Simian,Human	16 ,12
Polyomavirus	SV40,	Simian,	
	BKV,	Human,	
	JCV	Human	1
Adenovirus	hAV	Human	1

*As each pre-miRNA may give rise to one or two mature miRNAs, this table enumerates the pre-miRNAs encoded by each virus. Only herpesviruses have so far been shown to encode more than one pre-miRNA. Several other viruses, including human papillomavirus,HCV, YFV, HIV-1, and HTLV-I, have been reported to lack miRNAs

Figure 7. Role of RNAi as an Antiviral (Haasnoot et al., 2007)

Viral mRNA Targets of Viral miRNAs

miRNAs have several usages, first of all they are target specific genes. Second, the evolution of a miRNA to a new target gene is more easily than the regulatory protein. Third, miRNAs are small for the tight space to viral genomes. Finally miRNAs are not antigenic (Umbach et al., 2009). All herpesviruses, human adenovirus, Heliothis virescens ascovirus (HvAc), and several members of the polyomavirus family encode miRNAs (Gorbatyuk et al., 2010). Interestingly, most of the viral mRNA aiming viral miRNAs are targets of the host immune response and/ or viral regulatory proteins. For example, SV40 miR-S1 shuts down production of the viral T antigen (TAg) as an early protein, which regulates viral transcription and DNA replication. During late viral replication, TAg can provoke a cytotoxic T-cell (CTL) response against SV40-infected cells. The miR-S1 locating antisense to the TAg mRNA cleaves and degrades TAg mRNA (Cantalupo et al., 2005). The genomic location of SV40 miR-S1 is conserved in other members of the polyomavirus family, such as human viruses JCV and BKV, mouse polyomavirus (mPy), and the primate SA12 and Merkel cell virus (MCV) and suppress TAg expression in their similar viruses (Broekema et al., 2013).

In EBV the first target of viral miRNA is mRNA encoding the EBV DNA polymerase, BALF5, which is blocked by EBV miR-BART2. The miR-BART2 is antisense to BALF5, and thus acts as a siRNA cleaving the target transcript in the center of binding site (Malterer et al., 2011). In the insect virus HvAc produces a miRNA that shuts down the viral DNA polymerase. Unlike EBV miR-BART2, this HvAc miRNA is not antisense to the polymerase and binds the target (Mierlo et al., 2011). The miRNA for hCMV is miR-UL112-1 that can target the viral gene IE1 which is an immediate-early gene product requiring for activating transcription of hCMV early genes. When miRUL112- 1 expressed early in hCMV infection, a decrease in viral replication was observed (Weiwei et al., 2009).Herpes Simplex Virus Type 1 (HSV-1) causes long term latent infections in neurons. HSV-1 express two miRNAs in latently infected neurons which caballing of ICP0 and ICP4 down-regulating, mmediate early proteins (Hill et al., , 2009). The miR-H2, is transcribed antisense to ICP0, while a second HSV-1 miRNA, miR-H6, targets ICP4 mRNA. The miR-H3 and miR-H4, are both transcribed antisense to a viral mRNA encoding ICP34.5, which is a neurovirulence factor (Lukiw et al., 2010; Munson, 2012). HSV-2 miR-H3 and miR-H4 are similar to HSV-1 miR-H3 and miR-H4i and inhibit ICP34.5 expression (Bagasra, 2005). HSV-1 miR-H6, which is expressed during latency, suppress ICP4, a second immediate–early protein required for viral replication, suggests that these viral miRNAs are critical for HSV-1 and HSV-2 latency *in vivo* (Hong et al., 2006; Munson, 2012).

Cellular Targets of Viral miRNAs

It is possible for a single viral miRNA to block both viral and cellular mRNAs. For example hCMV miR-UL112-1 suppresses both viral IE1 expression and cellular MICB expression. The region of miR-H2 in HSV is conserved and it is possible that this viral miRNA down-regulate both viral ICP0 mRNA and a similar set of cellular transcripts (Leucci et al., 2010; Malterer et al., 2011). In apoptosis regulators include pro apoptotic factor (PUMA), which is a target of EBV miR-BART5 (Chatterjee et al., 2013). Suppression of PUMA by miR-BART5 may protect EBV infected cells from virus-induced apoptosis. BCLAF1, another apoptotic factor is a cellular target of the KSHV miRNAs miR-K5, miR-K9, and miR-K10 which raises the levels of BCLAF1 and decreased virus production after induction of viral replication (Tian et al., 2009; Weiwei et al., 2009). THBS1 is blocks by multiple KSHV miRNAs, including miR-K1, miRK3- 3p, miR-K6-3p, and miR-K11 may help KSHV-infected cells in avoiding detection by the host immune system (Malterer et al., 2011; Wu et al., 2011). Another example of immune regulator is chemokine CXCL-11, which is a target of EBV miR-BHRF1-3 might allow infected cells to avoid T-cell detection and killing (Malterer et al., 2011). MICB is a stress-induced cell surface ligand, and the hCMV miRNA miR-UL112-1 was reported by Stern-Ginossar et.al to inhibit the expression of MICB by binding sites within the 39UTR of this mRNA. So, down-regulation of MICB by miR-UL112-1 may allow hCMV to escape NK cell immune surveillance (Malterer et al., 2011; Qiu et al., 2011; Weiwei et al., 2009).

Antiviral Cellular miRNAs and Viral Tropism

Mammalian cells might inhibit virus infection by targeting viral transcripts with cellular miRNAs. This is very difficult for a cellular miRNA to stop a virus effectively, given that a single nucleotide mutation in the region of the viral mRNA target has the potential to block down regulation. For example some RNA viruses such as HIV-1 and HCV which are 'pseudo species'' (Zhou and Rossi, 2011). In mice was demonstrated a reduced level of Dicer activity become hyper susceptible to infection by vesicular stomatitis virus (VSV) due to reduced levels of cellular miR-24 and miR-93, which blocks VSV protein expression (Hosono et al., 2008).

Several cellular miRNAs, including miR-28, miR-125b, miR-150, miR- 223, and miR-382, are able to inhibit HIV-1 replication by binding sites located within the viral genome (Vlachakis et al., 2013). Cellular miRNAs can affect viral tropism is provided by HCV which contains two adjacent binding sites for cellular miR-122 within its 59UTR. Unlike 39UTR miRNA-binding sites, which inhibit translation of target transcripts, binding of miR-122 to the viral 59UTR helps HCV replication (Shwetha et al., 2013).Base on miR- 122 expressing in the liver the primary replication site of HCV, suggests that this cellular miRNA plays a main role in determining the tropism of HCV for this tissue (Shwetha et al., 2013).

KSHV encodes a viral miRNA, miR-K11 that not only has the same seed as miR-155, but also down-regulation many cellular mRNAs. An accidental result of the virus-induced expression of miR-155 is that EBV-, KSHV-, or MDV-1-infected cells may cause oncogenic

transformation (Eichelser et al., 2013; Ferrajoli et al., 2013).

RNAi Against Hepatitis B and Hepatitis Delta Viruses

Hepatitis B virus (HBV) is one of the major causes of liver disease worldwide, and chronic HBV infection can progress to cirrhosis and hepatocellular carcinoma .About 5% of the global population (350 million persons), are currently infected with HBV(Afshar and Mollaie, 2012b). Chronic HBV can be treated with a nucleoside analogue, lamivudine, which in the short term can inhibit HBV replication by blocking the viral polymerase activity (Afshar and Mollaie, 2012a).

Transfection of siRNA against hepatitis B surface antigen (HBsAg) region into HepG2.2.15 cells reduces more than 80 per cent of HBsAg and HBeAg secretion in the culture medium and also in a mouse model, which produces HBV particles on injection with HBV plasmid, co-administration of the plasmid and siRNA blocked the virus specific transcripts, antigens and DNA in mouse liver and sera (Huang et al., 2005; Jiang et al., 2010). HBV viral replication inhibited by synthesized siRNA in a human epatoblastoma cell line, producing infectious HBV particles, The secretion of HBsAg into culture media measured and shows that target selection plays an main role in the success of RNAi process; but no systematic method is available yet to predict which targets would be more effective than the others (Konishi et al., 2003; 2006). Four candidate shRNA plasmids significantly block HBV genotypes A, B, C, D and I *in vitro* and *in vivo*. A combination strategy of various siRNA in a single transcript done to improve efficacy and also delay the rise of viral escape mutants (He et al., 2006).

In a study used siRNA against the core region of HBV co-transfected with the full-length HBV DNA into Huh-7 and HepG2 cells, demonstrated that HBeAg levels in the cell culture medium fell about 5-fold. Also, a Southern blot for the levels of replication elements showed a decrease when compared to control siRNAs against GFP (Konishi et al., 2003; Yang et al., 2010). RNAi sequences can be designed to get effective reduction in cell and animal models. Different constructs with the same RNAi sequences had different capacity in their activities *in vitro* and *in vivo*. Sequence, stability, ways of delivery is all important (Sun et al., 2010).

Plasmid-based RNAi expressed under the control of H1 RNA promoter from a plasmid against HBV Core and X genes. Using the core and X gene expression vectors along with the RNAi construct and a significant reduction in the levels of these proteins reported by Western blots (Shlomai et al., 2004; 2009). When HBV vector was co-injected with siRNAs against either the core gene or the surface protein gene, the HBsAg levels or the HBeAg levels were reduced (Han et al., 2011). SiRNAs targeted the S gene of HBV are effective against HBV both in cell culture and in mouse models in vivo as evaluated by HBV antigen and DNA levels (He et al., 2010). Delta antigen mRNA can be aimed by siRNAs in cell culture. However, the genomic and anti-genomic RNAs are resistant to siRNA action (Tang et al., 2008).

RNAi Against Hepatitis C Virus

HCV is divided into six major genotypes and more than 80 subtypes. 30-50% variation among genotypes and 15-30% among subtypes while 1-5% variation in nucleotide sequence from a single HCV infected patient. The hypervariable region is in E1 and E2 and the lowest variability is found in the 5' untraslated region (UTR) containing RNA secondary structures which are required for replication and translation functions (Keyvani et al., 2012). HCV RNA sub genomic replicon can be used that has a Neomycin resistance gene for selection and a luciferase gene for monitoring the levels of replicon expression. A fall (85-90%) in the levels of luciferase was detected if cells were transfected with siRNAs specific for either the 5'-UTR or the luciferase, whereas nonspecific control siRNAs or siRNAs with three nucleotide mismatch to the luciferase target failed to show any reduction. To verify that siRNAs do not cause cellular toxicity, they also studied cellular ATP levels and found them unchanged between transfected and mock-transfected cells (Seo et al., 2009). siRNAs can be used as a potent approach to decrease HCV replication in a sequence-specific manner. Six siRNAs targeting regions of HCV non-structural genes reported and examined their effect on viral replication (Jahan et al., 2011). HCV NS3si-229 and NS5Bsi-241 showed greater than 50% inhibition in viral titer, while NS2si- 241 exhibited only 27% reduction in viral titer. Effect of siRNAs against HCV non- structural genes on HCV viral titer reduction is possibly because of the simultaneous degradation of HCV genomic RNA (Idrees et al., 2013). A sub genomic replicon system derived from HCV genotype 1b and siRNAs can be used against to NS3 and NS5B, showed 5.7- and 8.3-fold reduction, respectively, as reported by real-time PCR 2 days after transfection. The levels of NS3 and NS5B proteins were unchanged after 2 days by Western Blot analysis. They also compared the HCV RNA replication inhibition by RNAi and IFN treatment and found that siRNAs did 3-fold better than IFN and that the antiviral effect of siRNA is independent of IFN (Takigawa et al., 2004; Konishi et al., 2006). Hepatitis C virus replicon system supports HCV replication but does not produce infectious virus in Huh-7 hepatoma cells. siRNA transfection of these cells decrease the HCV specific RNA synthesis by 80-fold and cured >98 per cent of cells (Trejo-Avila et al., 2007). A similar RNA replicon system, demonstrating 5-fold decrease in 12 h and an 80-fold decrease in 96 h in HCV RNA levels detected by real-time PCR when siRNAs against 5'-UTR were used (Wang et al., 2006). The siRNA against NS5B about 90% reduction in HCV RNA levels 72 h post-transfection using Northern blot analysis. Also the nonstructural proteins NS3 and NS5B were not in high levels as measured by immunoblotting (Wilson, 2006).

In a study was choose five targets against 5'-UTR and showed siRNA-331 suppressing HCV replication by 81% at a concentration of 2.5 nM and the suppression rate increased to 94% at 125 nM. The siRNA-expressing vectors suppressed HCV replication, but the stem-loop

type was more efficient than the tandem type (Yokota et al., 2007).

RNAi Against Hepatitis A Virus

Hepatitis A virus (HAV) is a major cause of acute liver difunction in children in the world. HAV RNA replication in HuhT7 cells was inhibited by siRNA. Combinations of siRNAs directed against two different genes were more useful and these treatments did not change expression of endogenous cellular genes. The viral infectivity shut downed after using a siRNA targeting hairpin structure. This siRNA is a therapeutic tool for severe courses of HAV infection. In addition, the results show new insight into the structural bases for sequence specific RNAi (Wu et al., 2004).

RNA Interference and Inhibition of Viruses

JC virus, a member of the genus polyomavius, causes progressive multifocal leukoencephalopathy.Human astrocyte cells transfected with siRNAs directed against T antigen or Agno protein coding mRNAs. Individually, siRNAs were partially effective but combined treatment of both siRNAs completely revoked JC virus capsid protein production. Similarly, siRNAs can cause inhibition of viral capsid protein VP1 and Agno protein by JC virus production in human glial cells (Cantalupo et al., 2005). The effect of RNAi on JCV production based on hemagglutination activity of JCV-infected SVG-A cells 36 h after siRNA transfection. The hemagglutination activities of cells transfected with Ag122, VP274, or both of these siRNAs were 6.7, 9.3, and 4.1%, respectively. Thus, siRNAs that target Agno protein or VP1 block JCV production in infected cells. The results may have important suggestion for the development of a new approach to the treatment of PML (Suzuki et al., 2010; Broekema et al., 2013).

Foot and mouth disease virus (FMDV) is an animal virus. Transfection of siRNA expressing plasmid showed 80-90 per cent fall in expression of VP1 protein of FMDV in BHK-21 cells. Subcutaneous injection of this plasmid in suckling mice also protected the mice against lethal FMDV challenge (Ferrer et al., 2009).

Poliovirus Escapes from RNA Interference by a single substitution in viral RNA is enough to submit siRNAs ineffective within only a few replication cycles, even at highly conserved regions (Gitlin et al., 2005). SiRNA can stop gene expression to make a technology for antiviral gene therapy. There are RNAi against Coxsackievirus B3 (CVB3) infection and evaluated the effects of RNAi on viral replication in HeLa cells and murine cardiomyocytes by using five CVB3-specific siRNAs targeting regions of the viral genome (Yuan et al., 2010). There is evidence that siRNA can knock-down human cytomegalovirus (HCMV) genes in various cell culture systems. This involved RNA transfection, even in growth-arrested primary human fibroblasts (Bagasra, 2005). In Herpes Simplex Virus Type 1 (HSV-1) siRNA can inhibit glycoprotein E expression and function during active infection in vitro and established RNAi as a genetic tool for the study of

HSV and provided novel antiviral therapy (Prakash et al., 2010). The inhibition of murine herpesvirus 68 replication by siRNAs against sequences encoding Rta, an immediate-early protein, as a conserved viral protein. The results show that RNAi can block gamma herpesvirus replication (Kayhan et al., 2007). Specific siRNAs for influenza genomic regions were used in cell culture, eggs and in infant mice. Of the several siRNAs investigations, those specific for nucleoprotein (NP) and polymerase acidic (PA) genes of influenza virus could inhibit both, PR8 and WSN strains of influenza virus. The same siRNAs can stop influenza virus in embryonated eggs. The NP and PA siRNA treatment reduced virus titer in lung and saved the mice against lethal virus challenge (Barik, 2010).

The NP- or PA-specific siRNA interfered with NP- or PA-specific mRNA, and NP- or PA-specific cRNA and vRNA. when siRNA give to mice either before or after virus infection, siRNA decrease influenza virus activity in the lungs (Truong et al., 2013). Sequence-specific M1 siRNA could be used to inhibit viral protein synthesis and viral replication. It suggests that lentivirus vector expression of siRNA is useful for targeting the complex interaction of viral and cellular regulatory proteins (Hui et al., 2004). The silencing capacity of seven synthetic siRNAs against SARS-CoV leader, TRS, 3'-UTR and Spike coding sequence to prevent SARS-CoV infection, that siRNAs directed against Spike sequences and the 3'-UTR can block the replication of SARS-CoV in Vero-E6 cells (Wu et al., 2005). In adeno virus siRNAs by improving suppressed adenovirus-mediated cytotoxicity against two late genes with the siRNA against the E1A early gene. The RNAi-based inhibition strategies can be used by co-silencing of early and late adenoviral genes, with down regulation of the E1A (Cruz et al., 2007).

Dengue virus replication and transmission was decreased by expressing RNAi in Aedes aegypti mosquitoes. Studies to develop transgenic mosquitoes that express dengue virus specific siRNA in targeted tissues are ongoing with final aim to reduce vector competence for dengue virus transmission (Idrees et al., 2013). In Measles Virus can be used siRNAs against N, P, and L, hemagglutinin (H) and fusion (F) mRNAs and reduced the cell-cell fusion. This siRNA-mediated knockdown of the matrix (M) protein not only enhanced cell-cell fusion but also increased the levels of both mRNAs and genomic RNA by a factor of 2 to 2.5 so that the genome-to-mRNA ratio was constant. Studies indicate that M acts as a negative regulator of viral polymerase activity, affecting mRNA transcription and genome replication to the same extent (Otaki, 2007). It is also possible to target siRNAs to selectively reduce cellular mRNAs, proteins of which interact with viral proteins or in some ways are vital for virus replication (Bagasra, 2005). Cellular CCR5, a chemokine receptor, acts as a co-receptor for majority of primary HIV-1 isolates. Lentivirus vector was used for stable intracellular expression of siRNA against CCR5. These cells were survived. Some HIV-1 use CXCR4 as co-receptor. Transfection of siRNA against CXCR4 suppress CXCR4 expression on cell surface (Boutimah et al., 2013). The siRNA targeted against HIV tat protein, a trans activator, was tested to protect against HIV-1. It was

found that antiviral activity of short hairpin siRNA against tat was revoked subsequently because of quasispecies by point mutation in target sequences. This show that siRNA should be directed against multiple targets and against conserved sequences for inhibitory activity. This is true for mutating viruses like HIV that make escape mutants (Zhou, 2012; Vlachakis et al., 2013).

Conclusions

The discovery of the miRNA and siRNA pathways has begun to change our knowledge of virus–host cell interactions. Although the role of miRNAs and siRNAs in virally infected cells is not complete, it is clear that both viruses and host cells using these systems play an important role in both host innate antiviral defense and virus replication. Future studies of these interactions in plants, invertebrates, and mammals will continue to increase our understanding of the viral interaction with the host antiviral innate immune response (Morris et al., 2006; Jankovic et al., 2009). This review shows that RNAi can be used against different viruses. Both cell culture experiments and *in vivo* studies support this matter. While synthetic siRNAs have some effects, the plasmid-based siRNAs have been shown to induce stronger response. According to studies, no IFN-related side effects have been reported in vivo in animal that got RNAi. Suitable levels of interferon system activation in a therapeutic setting should be decided and the lowest effective dose of siRNA should be used because nonspecific induction of IFN by RNAi depends on the quantity of siRNA (Ma et al., 2005; Wang et al., 2013). Like other therapeutic strategy, effective delivery presents a major impediment before RNAi can be adapted to clinical trials. Chemical components of siRNAs may need to increase their half-life. Another aim would be to mix the siRNAs with liposomes along with a small peptide that can bind to a specific receptor.RNAi is sensitive to mismatches in the target regions. As low as a single-base mismatch can abolish RNAi activity. It is an issue when RNAi is used as a therapy because viruses may become resistant by altering their nucleotides in the target region. This is especially true in some viruses where the RNA-dependent RNA polymerase (RdRP) lacks proofreading activity and it causes large number of errors during viral replication. This problem of escape viruses has been observed in the case of poliovirus and more recently in the case of HIV (Gitlin et al., 2005).

The theory is raised that mutations happen for escaping viruses when the suppression against viral levels is not complete. For example, in the case of chronic hepatitis C where the viral levels are between 105 and 107 genome copies/ml of blood becoming resistant to therapy by acquiring mutations in the target region. Thus, it is necessary to target more than one region by using a mixture of different siRNAs or combine different techniques such as ribozymes and antisense oligonucleotides along with RNAi as a combined therapeutic approach to fight the virus (Konishi et al., 2006; Jahan et al., 2011). RNA interference is an extremely potent and skilled tool to reduce expression of targeted genes. Using this technology has moved from *in vitro* cell culture studies to *in vivo*

administration in mammals. It is becoming clear that especial controls should be performed when using siRNAs *in vivo* to prevent the risk of misinterpreting results due to unplanned stimulation of the innate immune system or wrong target effects. Measurement of serum IFN-a, TNF-a, and possibly IL-12 levels should be thought, especially when systemic administration methods are used. Some researchers have reported success administration of naked siRNAs, a greater number of studies reported that using some kind of delivery system improved results. Different ways of administration and their selection of best delivery tool will be important to success.

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