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Small RNA Molecules as Therapeutic Agents for Viral Infectious Diseases

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Abstract: The potential of using small RNA molecules as therapeutic agents has been extensively explored, antisense RNA, ribozyme, aptamer, decoy and more recently siRNA have been demonstrated to be highly efficient in inhibiting a number of pathogenic viruses including human immunodeficiency virus, hepatitis B and C virus, poliovirus and influenza virus. The specificity and potency of the sequence-specific agents such as antisense, ribozyme and siRNA in particular, imply that these strategies will prove to be promising therapeutics for treating viral infections, although the antiviral efficacy may be limited by emergence of escape variants. Distinct from the reagents targeting viral RNA, decoy and aptamer inhibit viral replication by binding and thus inactivating the viral component such as regulatory gene product and viral enzyme. This review provides an up-to-date overview of the progress and problems in small RNA-based antiviral approaches, with a focus on their therapeutic utility, delivery and unwanted side effects.

Key words: Antisense RNA, antiviral approaches, ribozyme, aptamer, decoy, siRNA, therapeutics utility

INTRODUCTION

Despite much effort towards preventing viral infectious disease, chronic infection with viruses such as Human Immunodeficiency Virus (HIV), hepatitis B and C viruses (HBV and HCV) have been increasing, remaining serious worldwide health problems. Additionally, the emerging of avian influenza virus that can infect humans and the outbreak of the Severe Acute Respiratory Syndrome (SARS) caused by SARS coronavirus imply the threat of a global virus pandemic. The approaches to combat viral infections include vaccine and drugs that are targeted to specific viral enzymes or other proteins. One unavoidable problem is selection of resistant mutants during long-term treatment and multiple targets are generally required to prevent the emergence of mutant variants.

The concept of using RNA molecules as therapeutic agents for viral infection has aroused increasing interest in the recent decade. Antisense strategies, which encompass antisense oligonucleotides, ribozymes and small interfering RNA (siRNA), involve small nucleic-acid-based molecules that inhibit viral replication in a sequence-specific manner. The antisense reagents can be designed to target any viral RNA provided its sequence is known, making them theoretically ideal antiviral therapeutics. However, because of the strict sequence-specific property intrinsic in these approaches, the possibility of emergence of escape mutants upon persistent treatment is concerned. Other RNA-based strategies for combating viral disease include apatmer and decoy, which inhibit viral replication by binding and consequently inactivating the viral component such as regulatory gene product and viral enzyme.

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Antisense RNAs

Antisense oligonucleotides are single-strand DNA or RNA oligomer of 18-25 nucleotides in length, designed to bind to its target RNA via Watson-Crick base pairing. Depending on the type of antisense oligonucleotides used, there are two different action modes involved in gene knockdown. Conventional oligonucleotides such as phosphodiesters and phosphorothioates, recruit cellular RNase H to the duplex to cleave the target RNA; later-generated oligonucleotides including the modified oligonucleotides derivatives such as morpholinos, locked nucleic acids and peptide nucleic acids, do not active RNase H but inhibit translation by steric hindrance instead. In addition to their application in analysis of gene function, intensive studies have been conducted to explore the potential of antisense oligonucleotides as therapeutic modalities for diseases caused by the expression of deleterious genes, especially in the field of cancer and viral infections.

Viruses with the RNA genomes are particularly well suited to be targeted by antisense oligonucleotides, because both the mRNA and genomic RNA can be targeted, destruction of viral RNA could eliminate not only viral protein synthesis but also viral replication. On the other hand, in the case of DNA viruses or retroviruses, only mRNA can be targeted by antisense nucleotide, making virus clearance theoretically impossible and persistent treatment necessary. The first attempt employing antisense nucleotides as the viral therapeutic was published in 1978, which reported an inhibitory effect of oligodeoxynucleotides on replication of Rous-Sarcoma virus (Zamecnik and Stephenson, 1978). Since then, a lot of studies have demonstrated that antisense oligonucleotides are effective in fighting many pathogenic viruses. Theoretically, antisense oligonucleotides can be designed to target any region of viral RNAs, however, in view of the genetic diversity among viral isolates, it is necessary to target viral RNA sequences that are conserved and normally invariant among different strains. Actually, the 5'-untransalted region (UTR) is one of the most highly conserved regions in the HCV genome and has most frequently been targeted with antisense oligonucleotide. The 5'-UTR region, however, constitutes the internal ribosome entry site (IRES) capable of initiating cap-independent translation of the viral protein. Highly ordered RNA structures and multiple sites participating RNA-protein interaction within 5'-UTR have been documented to be crucial for translation and/or replication, both of which make the RNA inaccessible by oligonucleotides, extensive efforts were made to identify effective target sites.

In addition to mediating degradation of the target RNA, antisense oligonucleotides have been designed to inhibit essential processes of viral life cycle by steric blockade. For example, chimeric 2-*O*-methyl/LNA oligoribonucleotides against trans-activation response element (TAR) of HIV, which interacts with Tat trans-activator protein and cellular factors to stimulate transcriptional elongation, were reported to block HIV transcription by steric hindrance of the tat-TAR interaction (Arzumanov *et al*., 2001). Additionally, it was reported that antisense RNA targeting the splice donor-packaging signal of HIV inhibited viral replication via inhibition of both viral protein synthesis and virion RNA packaging (Chadwick and Lever, 2000).

Antisense oligonucleotides are usually delivered with cationic lipid reagents that are positively charged and capable of neutralizing the negative charge of the oligonuleotides. One of the major challenges of antisense oligonucleotide approach is the stabilization of oligomers, since single-stranded nucleic acid molecules are unstable and are degraded in blood stream with a few hours. A number of chemically modified nucleotides have been employed to enhance nuclease resistance. Phosphorothioates are the major representatives of first generation of modified oligonucleotide. Phosphorothioates are advantageous over unmodified oligonucleotides in resistant to nucleolytic degradation, but they also have undesirable feature such as decreased binding affinity to target RNA and propensity to bind to various protein, which may result in toxic side effects (Levin, 1999). Second generation oligonucleotides represented by 2'-*O*-methyl and 2'-*O*-methyoxy-ethyl RNA were developed to solve these problems, they are less toxic and have enhanced affinity with target RNA,

but the unsatisfied feature is that they cannot activate RNase H to cleave the target RNA. More recently, novel chemically modified nucleotides, so-called third generation oligonucleotides, have been developed. Most of them exhibit improved properties such as enhanced stability, higher target affinity and lower toxicity. Further, gapmers with a stretch of unmodified or phosphorothioate DNA monomers in the center of the oligonucleotide are widely used to overcome the shortcoming of inability to activate RNase H.

Although nearly 20 antisense oligonucleotides have progressed to the stage of clinical trials, only one drug was approved by the Food and Drug Administration, which is used to treat cytomegalovirus-induced eye infection in AIDS patients. Most clinical trials were interrupted because of the unsatisfactory effectiveness.

Ribozymes

Ribozymes, catalytic RNAs that are capable of cleaving target RNA, are another important category of sequence-specific gene-silencing molecules. Since the discovery of the first group I nitron ribozymes in the early 1980s, a variety of ribozymes, including hammerhead and hairpin ribozymes, have been developed. Ribozymes have a catalytic domain that is flanked by sequences complementary to the target RNA. The hammerhead ribozyme was first isolated from viroid RNAs, it can be transformed from a *cis*-cleaving molecule into a target-specific *trans*-cleaving enzyme by dissecting the catalytic and substrate strands of the ribozyme. Hammerhead and hairpin ribozymes are the most intensively studied ribozymes. Similar to those for antisense nucleotides, the problems that should be cleared in developing therapeutic ribozymes are: 1) accessible target sites have to be selected; 2) the oligoribonucleotides have to be stabilized against nucleolytic degradation and 3) the ribozymes have to be delivered to target cells.

Hammerhead ribozyme consists of two substrate binding arm and a catalytic core cleaving any NUH triplets (where N can be any ribonucleotide and H can be any ribonucleotide except guanosine) with AUC and GUC triplets being processed most efficiently. Hairpin ribozymes usually cleave after BNGUC (where B can be any nucleotide except adenosine). Because of secondary and tertiary structures of the target RNAs, not all sequences that are theoretically cleavable by ribozymes can be served as the target sites for efficient cleavage. Computer predictions of the secondary structure of the target RNA and systemic experiments with a number of antisense oligonucleotides or ribozymes have been made to identify accessible target sites. Another approach to facilitate the access and subsequent cleavage of the ribozyme was reported by Taira and coworkers, who developed novel ribozymes with the ability to access any target site regardless of the secondary structure by combining with the unwinding activity of the endogenous RNA helicase eIF4AI (Kawasaki and Taira, 2002). In addition to the secondary structure, cellular compartmentalization of target RNAs is also thought to influence their susceptibility to ribozyme cleavage. Enhanced cleavage efficacy was reported by co-localization of the viral RNA and ribozyme tethered with the retroviral packaging signal (Sullenger and Cech, 1993).

As the delivery of ribozymes, chemically synthesized ribozymes can be exogenously introduced into the target cells using the reagent as described for antisense oligonucleotides. However, stabilization of ribozymes is more difficult than that of antisense oligonucleotides, since introduction of modified nucleotides most likely causes conformational changes that attenuate its catalytic activity. Indeed, a number of attempts exploiting uniform structural modifications to enhance nuclease resistance of ribozymes were demonstrated to be infeasible due to the reduced catalytic activity (Paolella *et al*., 1992; Pieken *et al*., 1991; Shimayama *et al*.,1993). A systemic study of a variety of modified hammerhead ribozymes led to the identification of a consensus ribozyme motif with enhanced nuclease resistance while maintaining the catalytic activity by keeping the 5 purine ribonucleotides in the catalytic core unmodified (Beigelman *et al*., 1995). In addition to directly introducing the synthesized one, ribozyme can also be endogenously expressed from plasmids inside the target cells, which can

elicit constant and long-lived ribozyme expression. Because RNA polymerase III (pol III) promoter is highly productive and capable of generating complex RNA structures with high integrity, pol III promoters such as the tRNA promoters are widely used to direct the expression of both hammerhead and hairpin ribozymes (Medina and Joshi, 1999; Yamada *et al*., 1994).

Ribozymes have widely been used to inhibit virus replication. Combined with retrovirus system, hammerhead and hairpin ribozymes direct target various HIV-1 regions were demonstrated to be effective in inhibiting viral replication (Zhou *et al*., 1994; Ojwang *et al*., 1992). Another ribozyme-based anti-HIV approach was accomplished by cleavage of the chemokine receptor CCR5 or CXCR-4 and thus perturbing their coreceptor function (Goila and Banerjea, 1998). Further, the protective effect of ribozymes against HIV-1 infection has also been demonstrated *in vivo*. Using the SCID-hu mouse *in vivo* human thymopoiesis model, $CD34^+$ hematopoietic progenitor cells transduced by retrovirus expressing anti-tat-rev and -env ribozymes and Rev aptamers were showed significantly resistant to HIV-1 infection upon challenge (Bai *et al*., 2002).

In addition to HIV-1, the potential to use ribozymes as tools to control HCV infection has also been studied. Extensive knowledge of IRES structure and high conservation among HCV genotypes have rendered the IRES element attractive as the target for ribozymes. Chemically synthesized hammerhead ribozymes targeting the conserved sites of HCV IRES significantly inhibited (>90%) the replication of HCV/poliovirus chimera (Macejak *et al*., 2000). Adenoviral vectors have been considered to be an attractive candidate to deliver anti-HCV ribozymes because of the hepatotropic property, it was reported that adenovirus-delivered anti-HCV ribozymes were effective at eliminating HCV RNA in infected primary human hepatocytes (Lieber *et al*., 1996). Simultaneous expression of multiple ribozymes targeting different conserved HCV RNA region from a single vector was attempted to circumvent the emergence of resistant viral mutants (Welch *et al*., 1998).

Encouraged by successful inhibition of viral replication in cell culture and *in vivo*, several ribozymes have subsequently been tested in clinical trials. The first clinical trials was conducted with retroviral-delivered hammerhead and hairpin ribozymes against HIV-1 RNA and another one used chemically synthesized hammerhead ribozyme targeting HCV (Hepatazyme), but unfortunately, both of which had to be quitted because of the poor therapeutic efficacy (Michienzi *et al*., 2003; Peracchi, 2004).

RNA Interference

In the past few years, research in the antisense field was revolutionized by discovery of RNA interference (RNAi). RNAi is an evolutionarily conserved phenomenon of posttranscriptional gene silencing that has been described in plants, invertebrates and vertebrates. When double-stranded RNAs (dsRNAs) are introduced into these organisms, they are cleaved into small interfering RNAs (siRNAs) of 21-23 nt by the endonuclease Dicer, followed by incorporation of siRNA into a RNA-Induced Silencing Complex (RISC), which unwinds the duplex and uses the antisense strand as a guide to seek and degrade homologous RNA. In mammals, however, introduction of long dsRNA (>30 bp) induces systemic, nonspecific inhibition of translation due to activation of the interferon response. A breakthrough was achieved by the finding that specific gene silencing in mammalian cells can be mediated by siRNAs of 21 nt, which can bypass dsRNA-induced nonspecific interferon response (Elbashir *et al*., 2001). This finding triggered numerous studies using siRNA in mammalian cells. RNAi is the most potent antisense reagent discovered thus far, it was reported that siRNA-mediated gene silencing is about 100-1000 fold more efficient than that by antisense oligonucleotides (Bertrand *et al*., 2002).

Similar to ribozymes, siRNA can be either introduced as synthetic short dsRNA molecules or intracellularly transcribed from plasmids. siRNA is of relatively high stability and efficient siRNA delivery and silencing can be achieved by use of the cationic lipid reagent, but the silencing effect

mediated by exogenously introduced siRNA is short-lived. When longer lasting gene silencing is desired, plasmids or viral vectors are employed to deliver siRNA expression cassette. The pol III promoters of small nuclear RNA U6 and the H1 RNA component of RNase P have been widely used to direct the siRNA expression. Double-stranded RNA molecules can be expressed separately as sense and antisense RNA using two promoters or transcribed as short hairpin RNAs (shRNAs) which are then processed to give siRNAs. Inducible knockdown of gene expression was achieved by incorporated the doxycycline-responsive element into pol III promoter (Van de Wetering *et al*., 2003) or by coupled with Cre-loxP recombination system (Kasim *et al*., 2004). Additionally, it was reported that transport of shRNAs from the nucleus to the cytoplasm is likely to be an event involving Exportin-5, a karyopherin participating in the nuclear export of pre-microRNA, thus efficient nuclear export could be obtained by artificial modification to render the loop sequences of shRNA analogous to that of pre-microRNA (Yi *et al*., 2003).

The first study using siRNA as a antiviral reagent was reported by Bitko and Barik (2001), who demonstrated an inhibitory effect of synthetic siRNAs direct target viral polymerase and fusion protein F on respiratory syncytial virus. Afterwards, many studies have described RNAi-mediated inhibition of a large variety of viruses. One common approach in siRNA-based antivial strategy is to directly target key RNA sequences within viral genome. Inhibition of HIV-1 has been achieved by siRNAs directed against tat and rev (Coburn and Cullen, 2002; Lee *et al*., 2002), reverse transcriptase (Surabhi and Gaynor, 2002), trans-acting response region (TAR), 3'-UTR and vif (Jacque *et al*., 2002). Similarly, replication of HCV replicon RNA was suppressed by siRNAs targeting the capsid and nonstructural protein (NS) 4B (Randall *et al*., 2003), NS3 and NS5B (Wilson *et al*., 2003; Kapadia *et al*., 2003), 5'-UTR (Yokota *et al*., 2003). Besides these two viruses, siRNA-based antiviral strategy has also been successfully applied to other pathogenic viruses, including poliovirus, dengue virus, influenza virus, SARS coronavirus and many others. In addition to direct targeting viral sequence, another approach is to target host proteins considered to be crucial for the life cycle of viruses. It was reported that siRNA targeted to HIV-1 receptor CD4 (Novina *et al*., 2002), or co-receptor CCR5 (Qin *et al*., 2003) block the entry and replication of HIV-1. Also, evidence obtained from our group and those from others showed that knock down of cellular co-factors polypyrimidine tract binding protein, La antigen and human VAMP-associated protein of 33 kDa inhibits HCV RNA replication (Zhang *et al*., 2004; Domitrovich *et al*., 2005; Gao *et al*., 2004). Cellular genes are less prone to mutation and antiviral approach by knock down host factors is therefore less likely to allow viral escape of silencing. However, the unintended side effects of knocking down cellular gene are concerned and must be addressed thoroughly prior to therapeutic application.

By demonstrating that a great variety of viruses can be successfully targeted by siRNAs, it is conceivable that these powerful antisense molecules can be used to target any preexisted or newly emerging human pathogenic virus. However, siRNA-mediated antiviral technology faces several important challenges that must be circumvented. Like other sequence-specific antisense reagents, one outstanding drawback of the approach using siRNA direct against viral genome is that emergence of siRNA-resistant variants. In fact, it was shown that a single point mutation in the siRNA target region conferred escape in poliovirus (Gitlin *et al*., 2002) and more recently HIV-1 was shown to elude siRNA targeting by the evolution of an alternative structure in RNA genome (Westerhout *et al*., 2005). Additionally, increasing evidence showed that viruses counteract RNAi effect by encoding viral proteins that act as the suppressor of RNAi pathway (Bennasser *et al*., 2005; Li *et al*., 2002). Several strategies may be useful to prevent the escape of mutant variants, for example, 1) simultaneous expression of multiple anti-viral siRNAs targeting different conserved viral sequences, 2) combination with siRNA against cellular co-factors indispensable for viral replication. Indeed, Schubert *et al*. (2005) reported that the silencing effect of the vector doubly expressing two different siRNAs was maintained even after artificially introducing a point mutation that disabled the respective mono-expression vector.

Although siRNAs are considered to be of high specificity for their targets, unwanted off-target effects may occur by siRNA recognition of other mRNAs with partially complementary sequence. Moreover, siRNAs can act like microRNAs to inhibit translation if there is a consecutive base pairing between siRNA and mRNA. Therefore, to minimize potential off-target effects, it is important to carefully comparing the candidate siRNA sequences with mRNAs in the human genome to avoid long stretches of homology.

Another potential toxicity of siRNA is that endogenous RNAi pathway, which is important in regulating the expression of developmentally essential genes, may be competitively inhibited by exogenously introduced siRNA, because the RNAi machinery such as Dicer and RISC may be limit in amount (Bitko *et al*., 2005). This is especially in the cases that high siRNA dose is administrated or multiple siRNAs are simultaneously expressed to avoid escape viral mutants.

As mentioned above, outstanding progress has been made since RNAi was shown to work in mammalian cells three years ago. The first phase I clinical trials targeting the VEGF angiogenic pathway in age-related macular degeneration have begun. Although the issues such as delivery and unwanted side effects are still the problems to be further cleared to turn RNAi from an effective functional genomics tool into a potent antiviral therapy, the prospects for overcoming these are good as we improve our understanding of the RNAi mechanism.

RNA Aptamers and RNA Decoys

RNA aptamers are short RNA ligands with binding and inhibitory activity to small molecule or protein targets. The screening and identification of such molecules with unique binding properties from very large random RNA libraries are generally accomplished by a technology termed systemic evolution of ligands by exponential enrichment (SELEX) (Tuerk and Gold, 1990). The SELEX process starts with a large library of randomized RNA sequences containing 10^{14}-10^{15} different RNA species. The library is incubated with the target protein of interest and the RNAs that bind the protein are separated, amplified, cloned and sequenced. The high affinity and specificity of aptamers make them attractive therapeutic agents and increasing evidence indicated that the use of SELEX technique could generate aptamers to many disease relevant targets. The therapeutic utility of aptamers has been studied in a variety of human maladies including cancer, cardiovascular disease and infectious diseases.

In the case of using aptamers for antiviral purpose, the proteins essential for viral replication such as regulatory protein and viral enzyme are good target. Indeed, it was reported that RNA aptamers selected by SELEX against HIV-1 Tat (Yamamoto *et al*., 2000) or reverse transcriptase (Joshi and Prasad, 2002) inhibited HIV-1 replication by up to 99%. Similarly, RNA aptamers specific for HCV NS3 (Hwang *et al*., 2004; Nishikawa *et al*., 2003) or RNA-dependent RNA polymerase (Biroccio *et al*., 2002; Vo *et al*., 2003) were shown to block the replication of HCV replicon by inhibiting the viral enzymatic activities. In addition to vial proteins, antiviral aptamers were also selected to target key viral RNA sequences. Nishikawa and coworkers reported an inhibitory effect on IRES-directed translation by RNA aptamers binding to HCV IRES sequences (Kikuchi *et al*., 2005).

Another approach using small RNAs to target pathogenic protein is RNA decoys. RNA decoys are small RNA molecules analogous to *cis*-acting element, which can compete with the corresponding endogenous sequences for *trans*-factors binding, thereby attenuating the authentic *cis-trans* interaction. Since the concept of using decoys as therapeutic agents emerged 15 years ago, increasing evidence has shown that the decoy approach may be useful in the treatment of a variety of human disease. The first study performed to determine if an RNA decoy could be used to inhibit the activity of a pathogen protein was published by Sullenger *et al*. (1990) who demonstrated cells over expressing TAR decoy are highly resistant to viral replication. Later, same group reported that over expression of RRE-derived decoys inhibited HIV-1 replication by preventing the binding of Rev protein to the vial RNA (Lee *et al*., 1992). Also, it was demonstrated that RNA decoys mimicking the Stem-Loop (SL)

structures of HCV 5'-UTR inhibited IRES-dependent translation (Ray and Das, 2004). Additionally, considering that the SL structures in the NS5B coding region were demonstrated to function as *cis*-replicating elements (CREs) and replication of HCV is likely initiated by interaction between replicase complex and SL structures containing CREs, we explored the possibility of using RNA species corresponding such SL structures as antiviral decoys and provided the evidence showing that pol III-directed over expression of SL RNA inhibited HCV replication by sequestering the replication complex and preventing its binding to the physiological target in the viral RNA (Zhang *et al*., 2005).

Distinct from sequence-specific antisense reagents, the efficacy of antiviral approach by aptamers and decoys may be less affected by the extensive variability encountered among viral isolates. Aptamer resistance may be less of a problem because RNA-protein interactions are not easily disrupted by mutations in the protein. Indeed, the effective cross-clade inhibition of HIV-1 by gp120 aptamers (Khati *et al*., 2003) and to a lesser extent by reverse transcriptase (Joshi and Prasad, 2002) was documented. Additionally, because the interaction between *cis*-acting element and *trans*-acting factor is usually essential for viral replication and mutation in the *trans*-acting factor that blocks its binding to RNA decoy also blocks its binding to the authentic target in viral RNA. To circumvent RNA decoy-mediated inhibition, double mutation, one in *trans*-acting protein and another compensatory one in the *cis* element is simultaneously required, thus making the chance of emergence of escape mutants lower.

To provide the resistance to nuclease degradation in biological fluids, aptamers are routinely selected with amino-or fluoro-modifications at the 2' position of pyrimidines which are prone to nuclease attack. Further stabilization of selected RNA aptamers includes *O*-methyl-substitutions in purine nucleotides, which requires chemically synthesis of modified RNA molecules. An alternative to chemical modification is the application of L-nucleic acids during and after *in vitro* selection. Modified aptamers can be delivered with the same reagents used for antisense oligonucleotides, the half-life of aptamers in the plasma is increased by coupling with high molecular linkers such as PEG. Similar to ribozymes and siRNAs, both aptamers and decoys can also be intracellularly expressed from plasmid-or vector-delivered expression cassette. Intracellularly expressed aptamers (intramers) and decoys could elicit long-term and stable effect, which is particularly essential for antiviral purpose.

Despite of the high binding specificity and affinity, the efficiency of aptamer-or decoy-mediated inhibition may be not efficient enough in some cases. This can be improved by combination with other antisense modalities. Because aptamers and decoys aim at inhibition of protein function, whereas ribozyme and siRNA destroy target protein-encoding mRNA or viral RNA; so the additive or synergistic antiviral effect can be expected by combining these two conceptually different reagents.

Another issue that limits the therapeutic use of aptamers is relatively high cost for aptamer manufacturing and delivery of aptamers and decoys faces the same problems as with other antisense reagents. Further progress in aptamer and decoy therapeutic field will depend on the breakthrough of such problems.

CONCLUSION

Small RNA-based antiviral approach represents a useful alternative to small molecular compounds for combating viral infection. Antisense oligonucleotides and ribozymes have been used for many years to inhibit viral replication. The therapeutic efficiency of antisense oligonucleotides and ribozymes was far from satisfied. The more recently developed RNAi strategy, however, is obviously advantageous over the older generation antisense reagents due to the high efficiency in knockdown gene expression. RNAi field is moving at an unprecedented speed and the first clinical trials using RNAi have already commenced. The problem for emergence of siRNA resistant variants can be dissolved either by simultaneously targeting different conserved region or combination with other small RNA-based modalities such as aptamer and decoy, which bind and subsequently inactivate target protein. With the

advances in developing efficient delivery systems, one could expect that RNAi and other small RNA-based approaches can become realized as effective therapies to treat viral infections in the near future.

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