The Therapeutic Potential of RNA Interference in Controlling HIV-1 Replication

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) has long been a major problem to handle. Its existence is incurable (yet) and has reached pandemic proportions despite strictly-controlled epidemiological surveillance. The current treatment regimen involves the use of multiple antiretroviral agents (known as HAART) is very complex and may harm patients through its serious risk of toxicities. Moreover, the continuing emergence of drug resistance further threatens the future therapy, thereby necessitates another treatment strategy i.e. specific and efficient with low or minimal toxicity. RNAi is a potent candidate for the future treatment of HIV-1. It involves an immune-based silencing mechanism (post transcriptional gene silencing/PTGS) that uses small sequence of RNA (21-25 nucleotides in length) to inhibit almost every genes expression, including HIV-1 RNA and its mRNA byproducts. Since RNAi uses sequence of base pairs, it can be designed very specific and homologues to silence the genes in favor. RNAi works either through binding with HIV-1 to inhibit provirus integration into cellular genome or with mRNA products to inhibit certain genes expression (e.g. p24/Gag, Vif, Rev) that plays an important role in HIV-1 infectivity to knockdown its virulence capacity. Given the need for a treatment modality that are sequence-specific and able to overcome the highly mutation rate of virus like HIV-1, also by its enormous power to inhibit HIV-1 expression through various target sites, it is considered essential to discuss the molecular mechanism of RNAi, progresses that have been achieved, and future directions for its use in clinical settings.

Key words: RNAi, HIV-1, PTGS, antiviral agent.

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) belongs to the retroviral family which causes an indolent-type of infection and terminally ended to AIDS.1,2 It has reached pandemic proportions and currently infects more than 42 million individuals worldwide with an estimated 14,000 new infections occurring daily.2,3 In the absence of effective anti-retroviral therapy, CD4+ T lymphocytes may continue to decrease and results in weakened immune system, arising a vast majority of opportunistic infections.4 Yet, the use of antiretroviral regimens in treating HIV/AIDS (also known as HAART [Highly Active Antiretroviral Therapy]) may potentially develop secondary problems including the complexity of these regimens, long-term and immediate risk of toxicities (i.e. hepatotoxicity, pancreatitis, nephrotoxicity, Stevens-Johnson syndrome, acute renal failure, liver steatosis), and the most threatening event—the continuous emergence of drug resistance variants.5,6 Given these evidences, it is important to consider alternative strategies to overcome viral burden in HIV-1 patients in a highly specific manner with minimal risk of toxicity.

RNA interference (RNAi) provides a strong justification to become a novel but remarkable therapeutic strategy in HIV-1 infection. RNAi is an immune-based technology in which it uses a short strand of RNA, so-called short interfering RNA (siRNA) with approximate length of 21-25 nucleotides (nt) that works in a highly sequence-specific manner to inhibit the target gene in favor.7,9 RNAi can be designed to be absolutely homologous to a certain base pair sequence possessed by HIV-1 RNA.10,11 It is then transfected into target cells which have already been infected with HIV-1.12,13 The binding between siRNA and HIV-1 genome
or its mRNA products will block the integration with the host genome or inhibit their translational process in the ribosome.\(^{14,15}\) HIV-1 mRNA products encoded from important genes (like Nef, Vif, Gag, and Ref) which function to enhance their infectious capacity will be degraded by cleavage soon after the binding process takes place.\(^{16,17}\)

RNAi also works very efficient since only a few molecules of dsRNA (will be discussed in next chapter) were needed to effect gene silencing because a catalytic or amplification machinery is involved.\(^{18}\) To date, the use of RNAi in the treatment of broad-type of diseases (age-related macular degeneration, various types of carcinomas, hepatitis B and C, human papilloma virus, Rous sarcoma virus, herpesviruses, etc)\(^{19,20}\) yield no serious adverse effects or toxicity.\(^{21}\) Given the enormous capacity and benefits of RNAi in controlling HIV-1 replication, it is considered essential to briefly discuss the related issues and future development of this technology-engineered, immune-based therapeutic science.

**MOLECULAR PRINCIPLES OF RNAI-INDUCED GENE SILENCING**

The silencing capability of RNAi is initiated by the introduction of double-stranded RNA (dsRNA), synthetic siRNA, or short hairpin (shRNA) into cellular target. shRNA is a combination of RNAi effector molecules with plasmids or viral vectors (commonly used are adeno associated virus [AAV] and lentiviral [LV] vectors) encoding small stem-loop RNAs (Figure 1).\(^{22-26}\) Vector-based RNAi is unique because it has U6 or H1 promoters (RNA polymerase III). The first model of promoter (U6) contains siRNA that the sense and antisense are expressed from different cassettes aligned in tandem in the same construct.\(^{27}\) On the other hand, in the H1 promoter, the sense and antisense are expressed as a connected ribonucleic acid with several intermediate bases which form a stem loop structure (shRNA).\(^{28}\) There are 2 short duplex stems with one stem connected to a loop sequence, and the other ending with 6 or more thymidines (T) as the termination signal.\(^{29-31}\) This loop structure contains a crucial sequence for effective

**Figure 1.** The molecular pathway of RNAi silencing mechanism
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TARGETING CD4+ T LYMPHOCYTES WITH RNAI

The very beginning site of RNAi action is to inhibit genes encoding cell surface receptor either in resting or active CD4+ T cells. Novina et al. has successfully repressed CD4 expression in those cells through RNAi machinery. Silencing CD4 receptor was proven to inhibit viral entry, syncytia formation, and viral load.

However, CD4 may not be a good therapeutic target since it plays a critical role in many binding activities. Another studies were performed through silencing of the two main co-receptors which are involved in the HIV-1 cellular uptake, CCR5 and CXCR4 (Figure 2). It has been shown that in vitro knockdown of these co-receptors by using siRNA confers long term protection from HIV-1 without any serious deleterious effects in immune function.

TARGETING HIV-1 WITH RNAI.

Various researches have proven the efficacy of employing RNAi to inhibit several HIV-1 genes expression, thereby reducing their capacity to replicate and infect new cells. One of the potential targets for siRNAs is the viral genomic RNA upon viral entry and uncoating. HIV-1 possesses a single-stranded RNA genome that are reverse transcribed and integrated into the host chromosome. The integrated provirus is organized as a eukaryotic transcriptional unit with the 5’-long terminal repeat (LTR) containing a strong enhancer/promoter region and the 3’-LTR encoding a polyadenylation site.

During the viral life cycle, viral RNA (shielded by nucleocapsid proteins) is transported to the nucleus since it plays a critical role in many binding activities. However, CD4 may not be a good therapeutic target because these genes are essential for subsequent infection, indicating that incoming RNA can effectively be degraded using RNAi. This contradictory finding is apparently due to differential sequence targeting within HIV-1 genome, since not all RNA regions are shielded by viral nucleocapsids.

Targeting several HIV-1 genes which are involved in the early infection (HIV-1 long terminal repeat, vif, nef, tat and rev) also become effective targets for siRNAs because these genes are essential for subsequent expression of HIV-1 structural genes (Gag, Pol, and Env). (Figure 2) For instance, Nef protein is a non-

184,24,25,31,33,35,41,42 This structure consists of a guide (or antisense) strand that is complementary to the target mRNA, and a passenger strand. siRNA unwinds, the guide strand is incorporated into the RNA-induced silencing complex (RISC), whereas the passenger strand is discarded. shRNA is then cleaved by RNase III enzyme (Dicer) to become 21-25 nt long siRNA. This has been made possible because the 5’-end of antisense strand is thermodynamically unstable when compared to the passenger strand. RISC – together with guide strand – begin to search for any target mRNA that shall be degraded. It contains the endonuclease Argonaute 2 (Ago2) which composed of two domains: PIWI and PAZ that binds to 5’ and 3’-ends of the guide strand, respectively. PIWI domain functions as the target recognition and site of cleavage because it has nucleotides 2-8 (so-called seed sequence) which are able to drive the initial pairing with the target mRNA. If the mRNA is not complement to seed sequence, then the binding process fail to occur. Vice versa, once bound to its target in the setting of perfect pairing, Ago2 cleaves the mRNA between the nucleotides complementary to bases 10-11 of the guide strand, independent of the total length of this short RNA. The mRNA fragments generated are in turn degraded by different exonucleases. However, if there is imperfect binding between guide strand and mRNA (as occurs with missense mutation or mismatch in the 3’ half beyond nt-11, the cleavage process will slow down until finally stop. The mismatched mRNA is then converted into processing bodies (P-bodies) and undergo destruction, therefore halting the gene expression by translational arrest instead of Ago2-directed cleavage. After successfully destroys the target mRNA, the guide strand-RISC machinery then begin to find new targets and repeat the previous steps.

18,49,50 The very beginning site of RNAi action is to inhibit genes encoding cell surface receptor either in resting or active CD4+ T cells. Novina et al. has successfully repressed CD4 expression in those cells through RNAi machinery. Silencing CD4 receptor was proven to inhibit viral entry, syncytia formation, and viral load. However, CD4 may not be a good therapeutic target since it plays a critical role in many binding activities. Another studies were performed through silencing of the two main co-receptors which are involved in the HIV-1 cellular uptake, CCR5 and CXCR4 (Figure 2). It has been shown that in vitro knockdown of these co-receptors by using siRNA confers long term protection from HIV-1 without any serious deleterious effects in immune function.

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structural protein which functions to modulate CD4+ T cells and MHC class I. It decreases CD4+ concentration, which in turn preventing the T cell activation by blocking the receptor expression or by transducing inappropriate signals. The RNAi mechanism that inhibits Nef expression at 3’termini will reduce the viral transcript significantly since Nef s located at the end of the HIV-1 provirus.\textsuperscript{66} The siRNAs blockade activity results in pre-integrated HIV-1 RNAs degradation as well as a marked reduction of viral load.\textsuperscript{55}

RNAi also targets Rev (regulator of viral expression) protein to breakdown HIV-1 infectivity. Rev is a small protein (116 amino acids) which plays a crucial role in nuclear exports and final expression of HIV-1 mRNA. Rev interacts with β importin (β IMP) and translocates to the nucleus where they form GTP Ran complex that will induce Rev to multimerize target RNA to recruit Crm1.\textsuperscript{67} This complex is then transported to cytoplasm to initiate HIV-1 translational process. RNAi specifically designed to inactivate Rev will prevent HIV-1 mRNA late transport. When Rev is silenced, HIV-1 mRNA will be unable to translocate out from the nucleus, thus no translation of HIV-1 components will occur. Lee et al.\textsuperscript{17} generated a mammalian Pol III promoter system capable of expressing functional double-stranded siRNAs following transfection into human cells. Cotransfection with the HIV-1 pNL4-3 proviral DNA and the rev siRNA-producing constructs induced a marked reduction (≤4log10) in virus production.

Novina et al\textsuperscript{55} and Jacque et al\textsuperscript{64}, together with a parallel publication by Lee et al\textsuperscript{17}, also demonstrated that siRNA had an effect at a later step in the HIV-1 life cycle, namely a post-integration degradation of HIV-1 RNA transcripts. Jacque et al. reported similar results by utilizing intracellular T7 transcription from a plasmid DNA template to produce siRNAs in the cytoplasm of mammalian cells. Utilizing this system to produce shRNAs targeting HIV-1 Vif, they were able to suppress virus production to a 20–30-fold and confer intracellular immunity in resting and active CD4+ T cells, in part by blocking reverse transcription of genomic RNA into proviral DNA.

![Figure 2. The molecular depiction of HIV-1 target site inhibition by RNAi](image-url)
MAJOR OBSTACLES AND FUTURE DEVELOPMENT

Although transfected siRNAs can effectively inhibit HIV-1 replication, the effects gained are largely transient.\textsuperscript{58} It only persists not more than 4 days post-administration.\textsuperscript{60} However, recent insights have made possible to create an engineered DNA vector capable of long term production of siRNAs or shRNAs.\textsuperscript{27,28} One example for this is the utilization of lentiviral vectors. They are highly effective for shRNAs delivery because they can transduce both dividing and non-dividing cells and provide long-term gene expression.\textsuperscript{13,69} Transfection of cells by lentiviral vector controlled by H1 promoter to drive the shRNAs expression demonstrated a suppressed target proteins for 5 to 7 days after 10 rounds of cell division.\textsuperscript{70} It has also been demonstrated that RNAi is actively repress HIV-1 genes expression in non-dividing macrophages for over two weeks, suggesting the longer durability of RNAi protection in resting cells.\textsuperscript{56}

Interferon-\(\gamma\) as a host’s defense mechanism against viral infection is activated by long dsRNA more than 30 to 500 bp.\textsuperscript{18,31} Several studies have documented that siRNAs as well as shRNAs expressed from DNA vectors can trigger the activation of interferons. However, introduction of shorter siRNAs (<30 bp) are shown to effectively degrade target genes without activating the interferon response.\textsuperscript{21} Even it will, there is no evidence that the activation of interferons by short RNAs influences the degree or specificity of RNA silencing.\textsuperscript{18}

Another concern is the likelihood of HIV-1 RNA to emerge a rapid mutation, rendering the virus resistant to RNAi.\textsuperscript{71} The most common etiologies of HIV-1 mutation are nucleotide substitutions or deletions in or near the targeted sequence. The activity of siRNA that contained one or more mismatches relative to the target RNA sequence showed that a mismatch might be sufficient to reduce the silencing effect.\textsuperscript{64} To counteract this weakness, co expression of multiple siRNAs that target different HIV-1 RNA sequences could reduce the emergence of single siRNA-resistant virus with a comparable effect to that achieved by three to four anti-HIV drug combinations.\textsuperscript{65} However, there are a growing attention that overtransducing RNAi machinery externally may harm the body’s homeostasis since it uses RNA polymerase III which is also used by the native cells to regulate microRNA (miRNA) that control endogenous genes expression.\textsuperscript{72} This problem is overcome by several strategies, i.e. the use of second-generation siRNAs that target specific escape variants, the use of long hairpin RNAs.\textsuperscript{73,74} Whereas the latest and most effective strategy was done by Liu et al.\textsuperscript{75} through expressing multiple antiviral siRNAs from a single polycistronic miRNA transcript, such as a natural genomic miRNA cluster that uses RNA polymerase II promoter. By using a polycistronic transcript, a single administration of an agent can express four anti-HIV siRNAs simultaneously and are being processed by RNA polymerase II, thus reducing the risk of saturation because different pathway (not RNA polymerase III) is used.

CONCLUSION

RNAi is a well-known immune-based silencing technology which gains increasing attention ever since the awarded 2006 Nobel Prize. It has an enormous capacity to silence almost every genes expression, including HIV-1 RNA genome and its byproduct (mRNA’s gene transcription) through a sequence-specific and highly efficient manner. Several gene targets have been studied intensively in accordance with the observation of HIV’s life cycle. To date, the efficacy of RNAi in controlling HIV-1 expression has been well-documented. A further clinical translation from basic science to phase I/II human trial(s) shall be conducted immediately, given the continuing emergence of HIV-1 drug resistance phenomena.

REFERENCES


