RNAi as a treatment for HIV-1 infection

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doi 10.2144/000112167

Human immunodeficiency virus type 1 (HIV-1) was the first primate virus shown to be inhibited by RNA interference (RNAi). Early studies used both synthetic and promoter expressed small interfering RNAs (siRNAs) or expressed short hairpin RNAs (shRNAs) to demonstrate that this virus was susceptible to RNAi. In addition to targeting the virus itself, RNAi-mediated down-regulation of cellular targets that encode receptors required for viral entry also proved to be effective. The power of RNAi as an anti-HIV agent has propelled development of RNAi-based gene therapy approaches for the treatment of HIV infection in humans. Nevertheless, extensive in vitro experimentation has revealed potential problems of viral escape mutants and other toxicities caused by the si/shRNAs. This review covers the progress and problems in the development of RNAi for the treatment of HIV infection. Potential modalities for clinical application of RNAi in the treatment of HIV-1 infection are also described.

RNA INTERFERENCE IN MAMMALIAN CELLS

The formal description of RNA interference (RNAi) as a biological response to double-stranded RNA resulted from a desire to understand a number of intriguing observations arising from the use of antisense RNAs in Caenorhabditis elegans (1). This led ultimately to the discovery that worms could be programmed to silence genes by exposing animals to homologous double-stranded RNAs (dsRNAs; termed triggers) (1). It is now clear that an RNAi pathway is present in many if not most eukaryotes (2). A biochemical understanding of the RNAi pathway was crucial to realizing that dsRNAs shorter than 30 bp could be used to specifically trigger an RNAi response in mammals. Tuschl and colleagues demonstrated that transfection of mammalian cells with small interfering RNAs (siRNAs) could specifically induce RNAi and thus cracked the barrier to the use of RNAi as a genetic tool in mammals (3). It took a remarkably short period of time for siRNAs to be adopted as a standard component of the molecular biology toolkit.

The introduction of siRNAs into mammalian cells can be achieved through standard transfection. The strength and duration of the silencing response is determined by several factors. On a population basis, the overall efficiency of transfection is a major determinant, which must be addressed by optimizing conditions. In each individual cell, silencing depends upon a combination of the amount of siRNA that is delivered and upon the potential of that individual siRNA to suppress its target (the potency). Even a relatively poor siRNA can silence its target provided that sufficient quantities are delivered. However, essentially “forcing” the system with such reagents is likely to lead to numerous undesired effects (4).

The discovery of the endogenous triggers of the RNAi pathway in the form of microRNAs (miRNAs) that are encoded in the genome suggested that RNAi might be triggered in mammalian cells by providing synthetic genes that express mimics of such endogenous triggers. A number of laboratories simultaneously took related approaches to this goal by expressing mimics of miRNAs in the form of short hairpin RNAs (shRNAs) from RNA polymerase III or RNA polymerase II promoters (5–8) or expressed separate sense and antisense transcripts from Pol III promoters (9,10). The shRNAs themselves varied in size and design, with stems ranging from 19–29 nucleotides in length and with various degrees of structural similarity to natural miRNAs or siRNAs. All of these approaches were effective to varying degrees, and indeed, at present, no real consensus has developed on the most effective way to present expressed siRNAs into the RNAi pathway, although most investigators utilize short hairpins of 19–25 bp in length transcribed by Pol III promoters.

Since these triggers are encoded by DNA vectors, they can be delivered to cells in any of the innumerable ways that have been devised for delivery of DNA constructs that enable ectopic messenger RNA (mRNA) expression. These include standard transient transfection, stable transfection, and delivery with viral vectors ranging from retroviruses to adenoviruses. Expression can also be driven by either constitutive or inducible promoter systems (11–13).

RNAi AND HUMAN IMMUNODEFICIENCY VIRUS THERAPEUTICS

Human immunodeficiency virus (HIV) was the first infectious agent targeted by RNAi, perhaps because the life cycle and pattern of gene expression
of HIV is well understood. Synthetic siRNAs and expressed shRNAs have been used to target virtually all of the HIV-encoded RNAs in cell lines, including tat, rev, gag, pol, nef, vif, env, vpr, and the long terminal repeat (LTR) (10,14–17). Subsequent work showed a host of other viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV), poliovirus, respiratory syncytial virus (RSV), and others, were targetable by RNAi (recently reviewed in Reference 18).

Despite the early successes of RNAi-mediated inhibition of HIV-encoded RNAs in cell lines, targeting the virus directly represents a substantial challenge for clinical applications, because the high viral mutation rate will lead to mutants that can escape being targeted (19–22), although a clever recent strategy takes advantage of escape mutants in critical genes by targeting the mutants directly (23). The problem of viral resistance mutants to RNAi is not limited to HIV, as other RNA viruses with RNA-dependent RNA polymerases or reverse transcriptases also share this propensity to produce populations of mutants during replicative cycles (21,24–26). An alternative approach to avoid this problem is to target cellular transcripts that encode functions required for HIV-1 entry and replication. To this end, cellular cofactors such as NFκB, the HIV receptor CD4, and the co-receptors CCR5 and CXCR4 have all been down-regulated with the result of blocking viral replication or entry (15,16,27–29). The macrophage-tropic CCR5 co-receptor holds particular promise as a target. This receptor is not essential for normal immune function, and individuals homozygous for a 32-bp deletion in this gene are resistant to HIV infection, whereas individuals who are heterozygous for this deletion have delayed progression to autoimmune deficiency syndrome (AIDS) (30–32). Andersen and Akkina (27) used a lentiviral vector to transduce a combination of anti-CCR5 and CXCR4 shRNAs in human lymphocytes. Down-regulation of these receptors resulted in virtually complete inhibition of viral infectivity relative to controls. However, since CXCR4 is essential for hematopoietic stem cell homing to marrow and subsequent T cell differentiation (33–35), targeting this receptor is not a good choice for an anti-HIV therapy nor is targeting the essential CD4 receptor, with the exception of dendritic cells where the DC-SIGN receptor can be targeted.

Figure 1. Proposed multiplexing RNA interference (RNAi) with ribozymes and decoys for the treatment of human immunodeficiency virus (HIV) infection. HIV-1 binds to the CD4 receptor and CCR5 co-receptor, which triggers entry and uncoating. The proviral RNA is reverse transcribed into DNA, which integrates randomly in the host chromosomes. Different stages of the HIV-1 replicative cycle can be attacked using RNAi and combinations of other RNA-based inhibitors. The scissors represent either small interfering RNAs (siRNAs) or ribozymes, and the decoy for binding viral Tat or Rev is depicted as a barrel in the nucleus. The virus may be exposed to RNAi or ribozymes at the preintegration step blocking proviral DNA formation and integration. Postintegration, siRNAs targeting all classes of HIV transcripts can be used. The CCR5 co-receptor RNA is targeted by either an siRNA or a ribozyme in this depiction. mRNA, messenger RNA.
Figure 2. Overall scheme for delivery of viral vector-encoded anti-human immunodeficiency virus (HIV) short hairpin RNAs (shRNAs) and other inhibitory RNAs to autologous blood cells in HIV-infected patients. Two approaches are presented. Top left approach uses peripheral blood mononuclear cell isolation and selection for CD4+ lymphocytes. These are stimulated to divide and are transduced and expanded ex vivo prior to infusion. The right hand panel depicts hematopoietic stem cell differentiation into mature hematopoietic cells. The autologous hematopoietic stem cell approach requires stimulation of stem cells into the peripheral circulation via multiple courses of granulocyte colony stimulating factor injections. This is followed by collection of CD34+ hematopoietic stem cells by an antibody column. The collected cells are then transduced with a viral vector harboring the anti-viral shRNAs and reinfused into patients. For the scheme shown, the patients are autoimmune deficiency syndrome (AIDS) lymphoma victims that are treated for their lymphoma by autologous stem cell transplant with full marrow chemoablation (52). Other possibilities are partial or no ablation of the marrow for nonlymphoma patients. The stem cells engraft in the ablated marrow and differentiate into all of the lineages shown in the top right panel.

Viral targets should be sequences that are highly conserved throughout the various clades to ensure efficacy against all viral strains and to minimize emergence of viral mutants resistant to RNAi. Multiplexing shRNAs targeting several sites in the virus is an option that should be fully explored and carefully examined for efficacy, inhibition of viral mutants, and potential toxicity. Since the shRNA pathway impinges on the endogenous miRNA pathway, there is ample opportunity for off target effects and competition with miRNAs for loading into the RNA-induced silencing complex (RISC). An additional potential concern is the putative inhibition of RNAi via HIV Tat and TAR. HIV-1 Tat has been demonstrated to bind and inhibit DICER (40), although most investigators do not see inhibition of RNAi in targeting HIV, suggesting that this is a minor concern for therapeutic applications. TAR also binds TRBP, which is a DICER cofactor and is a component of RISC (41). Moreover, unlike other components of the RISC complex, TRBP is made in limited amounts in the cell, and hence binding to the TAR RNA could sequester TRBP from interacting with RISC and perhaps limit the effectiveness of an RNAi-based therapy. Binding of TRBP by TAR may also be a factor in the observed changes in miRNA profiles in HIV-infected cells (42). A few early reports showed that both siRNAs and shRNAs induced type
I interferons and interferon-regulated gene expression, suggesting that small RNAs could activate proteins such as PKR and 2′-5′ OAS (43,44). Other potential toxicity issues reside around the ability of some siRNAs to activate the Toll-like receptors in immune cells. This is a sequence-specific effect (45,46) and clearly a problem when siRNAs are delivered by lipid vehicles, but has not yet been shown to be a problem with expressed shRNAs.

An alternative approach to relying solely upon RNAi as an anti-HIV approach is mixing a single shRNA with other antiviral genes to provide a potent combinatorial approach. This has been successfully accomplished by co-expressing an anti-tat/rev shRNA, a nucleolar-localizing TAR decoy, and an anti-CCR5 ribozyme in a single vector backbone (47). An example of how HIV-1 can be targeted by this approach is shown in Figure 1. A somewhat different combination used an shRNA with a dominant negative Rev M10 protein in a co-expression system (48). Perhaps other, more potent combinations of shRNAs with mixtures of non-shRNA antivirals will be developed in the near future for testing in preclinical settings.

STEM CELLS AND VIRAL VECTORS

Delivery of siRNAs or shRNAs to HIV-1-infected cells is also a challenging problem. The target cells are primarily T lymphocytes, monocytes, and macrophages. Since synthetic siRNAs will not persist for long periods in cells, they would have to be delivered repetitively for years to effectively treat the infection. Systemic delivery of siRNAs to T lymphocytes is probably not feasible. Using viral vectors to deliver anti-HIV encoding shRNA genes is also problematic. Systemic delivery is not yet feasible since the immunogenicity of the vectors themselves precludes performing multiple injections, which would be necessary to maintain protection. Therefore a potential method is to isolate T cells from patients, followed by transduction, expansion of the transduced cells, and reinfusion. In an ongoing clinical trial, T lymphocytes from HIV-infected individuals are transduced ex vivo with a lentiviral vector encoding an anti-HIV antisense RNA (49). The transduced cells are subsequently expanded and reinfused into patients (Figure 2). This type of therapeutic approach would also be applicable to vectors harboring genes that encode siRNAs. A different approach is to transduce isolated hematopoietic progenitor, or stem, cells with vectors harboring the therapeutic genes (Figure 2). This approach has the advantage that all the hematopoietic cells capable of being infected by the virus are transduced. Hematopoietic stem cells are mobilized from the patients and transduced ex vivo prior to reinfusion. Two clinical trials with retroviral vectors expressing ribozymes were transduced into hematopoietic stem cells have demonstrated the feasibility of this approach (50,51). Since RNAi is more potent than ribozyme or antisense approaches, movement of this technology to a human clinical trial for HIV treatment is expected to take place in the near future.

SUMMARY

RNAi for the treatment of HIV-1 infection was first demonstrated in 2002. In just 4 years there have been dozens of publications testing various siRNAs and shRNAs against different strains of the virus. Viral vector-mediated delivery to hematopoietic cells, including stem cells, is a feasible approach for shRNA gene delivery. Clearly, the barriers that initially confronted therapeutic applications of RNAi for HIV infection are rapidly being broken down, and one can expect to see this powerful cellular process applied clinically to HIV-1 infected patients within the year.

ACKNOWLEDGMENTS

J.J.R. was supported by National Institutes of Health (NIH) grant nos. AI29329, AI42552, AI061389, and HL07470.

COMPETING INTERESTS STATEMENT

The author declares no competing interests.

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