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Recent advances in RNAi-based strategies for therapy and prevention of HIV-1/AIDS☆

Manjunath N. Swamy *, Haoquan Wu, Premlata Shankar *

Center of Emphasis in Infectious Disease, Department of Biomedical Sciences, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, TX 79905, USA

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ABSTRACT

RNA interference (RNAi) provides a powerful tool to silence specific gene expression and has been widely used to suppress host factors such as CCR5 and/or viral genes involved in HIV-1 replication. Newer nuclease-based gene-editing technologies, such as zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, also provide powerful tools to ablate specific genes. Because of differences in co-receptor usage and the high mutability of the HIV-1 genome, a combination of host factors and viral genes needs to be suppressed for effective prevention and treatment of HIV-1 infection. Whereas the continued presence of small interfering/short hairpin RNA (si/shRNA) mediators is needed for RNAi to be effective, the continued expression of nucleases in the gene-editing systems is undesirable. Thus, RNAi provides the only practical way for expression of multiple silencers in infected and uninfected cells, which is needed for effective prevention/treatment of infection. There have been several advances in the RNAi field in terms of si/shRNA design, targeted delivery to HIV-1 susceptible cells, and testing for efficacy in preclinical humanized mouse models. Here, we comprehensively review the latest advances in RNAi technology towards prevention and treatment of HIV-1.

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1. Introduction

Despite the availability of effective treatment, HIV-1 still remains a global epidemic, responsible for considerable morbidity and mortality. Although highly active antiretroviral therapy (HAART) can suppress viral replication to undetectable levels, it also has limitations, including high cost, patient compliance issues, the side effects of long-term therapy, as well as the emergence of drug resistance [1]. Moreover, although HAART extends the life of HIV-1-infected individuals, it does not offer a permanent cure, as interruption of therapy leads to rapid rebound of viremia from latent reservoirs [1]. Therefore, there is a need to develop more effective countermeasures against HIV-1 infection. Gene therapy has been thought to provide an attractive method for deriving HIV-1-

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* Corresponding authors.
E-mail addresses: Manjunath.swamy@ttuhsc.edu (M.N. Swamy), Premlata.shankar@ttuhsc.edu (P. Shankar).

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resistant cells (reviewed in [2–4]). Interest in this field started following the identification of CCR5 as a major co-receptor for HIV-1 [5,6] and the naturally occurring homozygous *CCR5*-Δ32 mutation (a 32-bp deletion in the single coding exon of the gene resulting in a frame-shift mutation that disrupts CCR5 expression on the cell surface) in humans, confers resistance to HIV-1 infection without other deleterious effects [7,8]. The remarkable success in treating the so-called "Berlin Patient" has led to a resurgence of interest in gene therapy. In that case, an HIV-1-positive patient with lymphoma who had been transplanted with bone marrow from a *CCR5*-Δ32 homozygous donor became HIV-1 free, with no demonstrable virus 7 years after transplantation, showing the potential benefits of *CCR5* disruption [9,10]. However, due to the low frequency of *CCR5*-Δ32 homozygotes in the general population and the difficulties of identifying suitable HLA-matched donors, alternative methods to artificially disrupt *CCR5* are being pursued. RNA interference (RNAi) and other gene-editing approaches provide a way to suppress *CCR5* as well as viral genes. (See Table 1.)

RNA interference (RNAi) is a process in which small (~21 nt in length) double-stranded RNAs, either produced endogenously within mammalian cells (microRNA, also known as miRNA) or introduced exogenously into cells (small interfering RNA or siRNA), mediate sequence-specific suppression of gene expression (reviewed in [11–13]). The small RNA in the cytoplasm associates with a multiprotein complex called the RNA-induced silencing complex (RISC), consisting of Argonaute family (Ago1–4) and other proteins [14–16]. Upon RISC binding, one of the two strands of the small RNA is removed (by cleavage and degradation for Ago2-bound siRNA or unwinding by other Ago proteins) and the other so-called the "guide" strand directs the RISC to the corresponding messenger RNA (mRNA). If there is complete homology between the guide strand and the target mRNA, as is generally the case with siRNA, the small RNA-bound Ago-2 protein (which is the only Ago protein with slicer activity) cuts the mRNA at a site corresponding to positions 10–11 from the 5′ end of the guide strand [17,18]. The mRNA is then rapidly degraded by exonucleases, leading to post-transcriptional gene silencing (PTGS). On the other hand, if there is only partial homology between the guide strand and the target mRNA as in miRNAs (generally between positions 2–8 at the 5′ end of the guide strand, which is called the "seed" sequence), the Ago proteins binding the mRNA and miRNA recruit a protein called GW182 (also known as TNRC6a, b, and c), which then leads to the recruitment of a series of proteins, including the Caf1–Ccr4 and Pan2–Pan-3 deadenylase complexes and the Dcp-1, Dcp-2 decapping complex, which eventually destabilize the mRNA by inducing deadenylation and decapping, resulting in PTGS (reviewed in [19]). Although siRNA is endogenously produced in plants and worms, mammalian cells generally only produce miRNAs and not siRNAs. The miRNAs (there are over 500 in humans) serve to rapidly regulate gene expression in response to environmental cues, which is important in almost every aspect of cellular physiology. Although siRNAs are not produced endogenously, artificial introduction of siRNAs has gained great attention because of their potential in therapeutics to silence selected cellular or viral genes in many diseases [20]. siRNAs can be either chemically synthesized and transfected into cells or they can be expressed within the cells using a DNA template to transcribe what is called a short hairpin RNA (shRNA, encoding the two complementary siRNA strands, which are separated by a non-homologous stem loop), generally under the control of Pol III promoters, which produce small RNAs like small nucleolar RNA (snoRNA). The shRNA is transcribed in the nucleus as stem loop RNA and exported to the cytoplasm to be further processed by Dicer into siRNA. The only difference between miRNA and shRNA biogenesis is that the former involves an additional step: the miRNA is transcribed as a long primary RNA (pri-miRNA), which is first processed by Drosha-DGCR8 in the nucleus into a pre-miRNA (with a stem loop structure resembling shRNA), and this molecule is then exported to the cytoplasm for Dicer processing into mature miRNA (Fig. 1). While exogenously introduced siRNA gets rapidly diluted, particularly in dividing cells, endogenously synthesized shRNA is more stable, particularly if expressed via genome-integrating lentiviral vectors [21]. Thus, for a

Table 1
RNAi approach tested for HIV inhibition in vivo.

Host/viral RNAi targets	Vehicle	Model	Route of delivery	Result	Reference
HIV tat/rev siRNA, TNPO3 siRNA and CD4 siRNA	Cationic poly (amidoamine (PAMAMM) dendrimers	HIV infected Rag2 −/− γc −/− humanized mice	Intravenous	Reduction in plasma viremia, inhibition of CD4 decline	[118]
HIV-gag/c-myc, MDM2 and VEGF siRNAs	HIV-envelope antibody F105 fused to Protamine	WT mice with HIV-env expressing B6 melanoma tumors	Intratumoral/Intravenous	Reduction in tumor size	[119]
HIV tat, Vif siRNA and CCR5siRNAs	CD7scFv/9R	HIV infected BLT mice	Intravenous	Reduction in plasma viremia, inhibition of CD4 decline	[120]
CCR5 siRNA	LFA-1 antibody coated nanoparticles	HIV infected BLT mice	Intravenous	Reduction in plasma viremia, inhibition of CD4 decline	[121]
HIV tat/rev siRNA	Gp120 aptamer/siRNA chimera	HIV infected Rag2 −/− γc −/− humanized mice	Intravenous	Reduction in plasma viremia, inhibition of CD4 decline	[125]
HIV gag/vif siRNA	CD4 aptamer/siRNA chimera	HIV infected BLT	Intravaginal	Reduction in viremia	[127]
CCR5 shRNA LTR shRNA	Lentiviral transduction of HSPC	HIV infected BLT mice	HSPC transduction	Reduction in plasma viremia, inhibition of CD4 decline	[134]
CCR5 shRNA and C46 peptide	Lentiviral transduction of HSPC	HIV infected BLT mice	HSPC transduction	Reduction in plasma viremia, inhibition of CD4 decline	[135]
CCR5 shRNA, TRIM5α and TAR decoy	Lentiviral transduction of HSPC	Rag1 −/− γc −/− mice	HSPC transduction	Inhibition of CD4 decline	[137]
LTR shRNA	Lentiviral transduction of PBMC	Hu-PBL mice	Transplantation of transduced PMBC	Reduction in plasma viremia, inhibition of CD4 decline	[139]
7 ShRNAs targeting CCR5 and HIV Gag, Env, Tat, Pol, Vif	Lentiviral transduction of PBMC	Hu-PBL mice	Transplantation of transduced PMBC	Reduction in plasma viremia, inhibition of CD4 decline	[107]
shRNA targeting an overlapping region in Tat and Rev., TAR decoy RNA and CCR5 Ribozyme	Lentiviral transduction of HSPCs	Pilot human clinical trial NTC00569985	HSPC transfer	Gene marking in PBMCs for up to 6 months	[99]