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RNAi-based treatment of HIV/AIDS: Current status and perspectives

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The global HIV epidemic kills around 3 million people annually and attempts to control further spread have been of limited success. The efficacy of antiviral drug therapy has been limited by toxicity and viral resistance. Thus, alternative therapies need to be explored. Recently RNA interference (RNAi) has been shown to efficiently inhibit gene expression in a sequence-specific manner. Therefore, RNAi mediated treatment of HIV is a promising alternative for future therapy of HIV/AIDS.

Introduction

Because of the success of highly active antiretroviral therapy (HAART) the need for continued research on gene therapy for AIDS has been questioned. Although HAART effectively reduces the viral load in the peripheral blood to undetectable levels, resulting in an increase in peripheral $CD4^+$ T cells and an improved clinical status, current regimens are expensive, not curative, and accompanied with important toxicity for the patient. Moreover, drug-resistant HIV-1 strains are more frequently isolated [1], even in the lymph nodes of patients who receive HAART having undetectable HIV-1 levels in the blood [2]. The search for novel therapeutic approaches should, therefore, never cease. The major aim of gene therapy is to provide for the cell an intrinsic advantage for survival by blocking virus replication. One can target both viral and cellular functions. Targeting highly conserved, host-encoded functions that are important for viral replication, but not essential for the survival of the host cell, has a theoretical advantage with regard to preventing resistance.

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Over the recent years justified excitement about the clinical prospects of RNA interference (RNAi) has developed in the scientific community, and antiviral therapy is likely to be one of the first applications. This review aims to summarize recent RNAi-based therapeutic approaches against HIV. Progress in achieving more potent gene silencing and better delivery as well as the careful design of the antiviral approach will have important implications for future antiretroviral strategies.

Strategies to modulate gene expression

Within the course of only six years, RNAi has grown from a molecular curiosity to a major technological breakthrough with applications in target validation and drug therapy. Its great potential is illustrated by the exponential increase in the number of scientific publications – from approximately 15 in 1998 to over 10,000 in 2005. The discovery of RNAi revealed an important role for small RNAs in regulating gene expression. This can be achieved by both perfect base pairing (post-transcriptional gene silencing) and by imperfect base pairing of the single-strand guided RNA (translational repression) (Box 1 and Fig. 1)

RNAi-mediated inhibition of HIV-1 replication

A strategy to achieve potent inhibition of HIV-1 replication *in vivo* should address the following aspects: (i) the cells to be targeted; (ii) the viral vector to be used; (iii) should the HIV-1 genome or the cellular co-factors important for HIV-1

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Box 1.

Originally described in plants, as 'post-transcriptional gene silencing' (PTGS) [56], RNA interference (RNAi) is now recognized as a nucleic-acid based defence mechanism against viruses, transgenes and transposons [57]. Triggered by double-stranded RNA (dsRNA), RNAi results in the sequence-specific degradation of a target mRNA [58]. In eukaryotic cells, long dsRNAs are processed by Dicer, an RNAse III-like protein, into small RNA duplexes of 21 to 23 nucleotides, denominated siRNAs [59,60]. One strand of this siRNA duplex is then selectively incorporated into a large protein complex termed RNA induced silencing complex or RISC [61]. Upon perfect base-pairing of the single-stranded guide RNA with the target mRNA, cleavage of the target mRNA results in post-transcriptional gene silencing [62,63].

A second species of small RNA of 18–25 nts, referred to as microRNA (miRNA), has been identified to interfere with gene expression [64]. miRNAs originate from the transcription of a large, highly-structured primary miRNA (pri-miRNA) of approximately 70 nts encoded by a cellular gene. In contrast to siRNA, perfect complementarity is not required for miRNA-based gene silencing. As a result one miRNA is able to target a variety of transcripts. Although both siRNA and miRNA utilize the same machinery (RISC) for gene silencing, the fates of their target mRNAs differ considerably. siRNAs regulate gene expression via mRNA degradation at a post-transcriptional level, whereas miRNAs silence their target genes through translational repression.

replication be targeted and (iv) the risk of HIV-1 escaping from the RNAi strategy.

Which cells to target?

The major host cells for HIV replication are of lymphoid ($CD4^+$ lymphocyte) or myeloid (macrophage, monocyte, microglia, dendritic cell) origin. Because pluripotent hematpoeitic stem cells (HSCs) generate cells of both lymphoid and myeloid origin, all HIV host cells could be made resistant to replication if antiviral genes could be efficiently transferred into their stem cells. An alternative cellular target for gene therapy of AIDS is $CD4^+$ lymphocytes. $CD4^+$ cells can be easily isolated, transduced, expanded and selected in culture, before re-infusion. The obvious disadvantage is the temporal benefit of this strategy due to the limited lifespan and growth potential of $CD4^+$ cells *in vivo* (for a recent review, see [3]).

Lentiviral vectors as best choice for shRNA delivery

Lentiviral vectors are capable of transducing nondividing cells and long-term expression of the transgene seems to be guaranteed [4]. To prevent the formation of replication competent viruses through recombination, sequences originating from the viral genome have been reduced to the minimum. It should be highlighted that the currently used lentiviral vectors have self-inactivating (SIN) LTR promoters [5], and only transcripts of genes inserted within the body of the vector are made. Unlike retroviruses, such as Moloney murine leukemia virus, lentiviruses tend to integrate distally from promoters, potentially limiting their overall oncogenicity [6]. Because lentiviral vectors have the potency to transduce nondividing cells, they are suitable to transduce primitive, quiescent HSCs, avoiding the engraftment defect seen with HSCs manipulated in cell culture before transduction with oncoretroviral vectors [7]. The vast majority of studies evaluating lentiviral vector transduction of HSC is aimed at gene transfer with minimal *in vitro* culturing to reduce negative effects on the survival and perhaps the long-term *in vivo* repopulation ability of stem cells [8]. Current data suggest that lentiviral vectors express transgenes at higher levels and expression of lentiviral vectors might be less prone to gene silencing [8]. Moreover, complex regulatory gene sequences can be easily incorporated [9]. Finally, lentiviral vectors can be pseudotyped to target specific cell types [10] or alternatively designed with a receptor-ligand bridge to target specific cell types.

Targeting the HIV-1 genome

The use of RNAi has reshaped recent advance in the field of gene therapy of AIDS [11]. Theoretically, the most direct approach targets the viral RNAs by RNAi. In the early stage of HIV-1 infection, the RNA genome can be targeted before reverse transcription in the cytoplasm. In later steps of infection, RNAi can target the newly synthesized viral mRNAs and the genomic RNA. Sequences that have been targeted so far encode the structural proteins Gag [12–15] and Env [15], the reverse transcriptase [13], the regulatory proteins Tat [11,16,17] and Rev [11,16], as well as the two accessory proteins Nef [18–20] and Vif [18] (Fig. 2 and Table 1). Nontranslated sequences in the long terminal repeats, that contain important regulatory elements, have been targeted as well [14,18]. As described above, RNAi therapy may be confounded by escape mutations in the targeted portion(s) of the viral genome. To overcome this problem lentiviral vectors have been constructed encompassing three or more hairpin sequences, targeting different sequences in the HIV genome. Although it is not trivial to compare the efficacy of individual transgenes or classes of transgenes certain target sequences seem more efficient than others. As shown in Table 1, comparison of the target genes tested is exceedingly difficult or even impossible as there are differences in the chosen promoters, the delivery vehicles, the challenge conditions and the cell types used. Translation from the *in vitro* to the *in vivo* situation is difficult particularly because only a few lab-adapted viral strains are usually tested and clade-dependent inhibition is known to exist [21]. Still, some general principles can be put forward. RNAi directed against HIV genes works best when directed against highly conserved HIV sequences, although these remain prone to escape mutations [22]. Combination of shRNAs or siRNAs targeting distinct steps of the HIV-1 replicative cycle is more likely to be effective than silencing of individual genes [23]. Because of the overlap of coding sequences in the HIV-genome, targeting of genes that code for two proteins acts synergistically and is more potent, as shown by the targeting