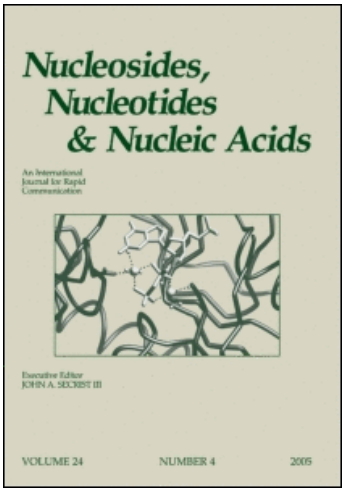

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Lentiviral-Mediated Delivery of Combined HIV-1 Decoy TAR and Vif siRNA as a Single 1RNA Molecule that Cleaves to Inhibit HIV-1 in Transduced Cells

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LENTIVIRAL-MEDIATED DELIVERY OF COMBINED HIV-1 DECOY TAR AND Vif siRNA AS A SINGLE RNA MOLECULE THAT CLEAVES TO INHIBIT HIV-1 IN TRANSDUCED CELLS

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▫ *RNA interference (RNA_i) silences gene expression via short interfering 21–23 mer double-stranded RNA (siRNA) segments that guide cognate mRNA degradation in a sequence-specific manner. On the other hand, HIV-1 decoy TAR RNA are known to competitively interact with the HIV-1 Tat protein, to downregulate the enhanced gene expression from the long terminal repeat (LTR) promoters. Here we report that a novel expression construct, encoding both HIV-1 decoy TAR and Vif siRNA, as a single RNA substrate, was expressed under the control of the human U6 promoter, and later the TAR and siRNA were cleaved into their respective separate RNA by the endogenous RNase III-like enzyme. Each of the cleaved HIV-1 anti-genes then synergistically contributed toward enhancing the inhibition efficacy (>80%) of HIV-1 replication in transduced Jurkat cells. These results suggest that targeting HIV-1 mRNA with simultaneously expressed intracellular decoy TAR and Vif-siRNA could lead to an effective gene therapy strategy for the control and management of HIV-AIDS.*

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INTRODUCTION

The role of double-stranded RNA (dsRNA) as a potent silencer of homologous genes in the nematode *Caenorhabditis elegans* was unveiled through the pioneering work of Fire et al.[1] in 1998. This technique, termed as RNA interference (RNAi), has proven to be a powerful tool and has been used to disrupt the function of genes in both plants and animals. Recently, the use of RNAi has been extended to differentiated cultured mammalian cells.[2] Importantly, siRNA expressed from DNA templates are able to silence gene expression as effective as exogenously introduced synthetic siRNA. RNAi and other gene therapy strategies have been effectively used to inhibit the replication of several different pathogenic viruses including HIV-1, by targeting the Gag-Pol, Env, Vif, and the small regulatory proteins, such as Tat and Rev, in culture.[3] In this study, we have combined the RNAi and HIV-1 decoy TAR RNA mechanisms via a single RNA molecule, delivered for intracellular expression by a novel lentiviral-based vector construct (CS-Vif siRNA-TAR) under the control of the human U6 promoter, which was later cleaved in the cells by the endogenous RNase III-like enzyme. The dual HIV-1 anti-genes efficiently inhibited HIV-1 replication in a dose-dependent manner. They further mediated a substantial down-regulation of the HIV-1 viral Vif mRNA and the reporter gene (EGFP) expression in transduced Jurkat cells. Our results have provide clear evidence that targeting the HIV-1 genes with simultaneously expressed intracellular dual HIV-1 anti-genes, such as Vif siRNA and decoy TAR RNA, could be a promising gene therapy approach for HIV-1.

RESULTS AND DISCUSSION

To evaluate the enhancement of siRNA in the sequence-specific inhibition of HIV-1 replication, we constructed the U6 vectors (Figure 1A) by linking the HIV-1 Vif siRNA with the decoy TAR, and assessed the mRNA expression in HeLa $CD4^+$

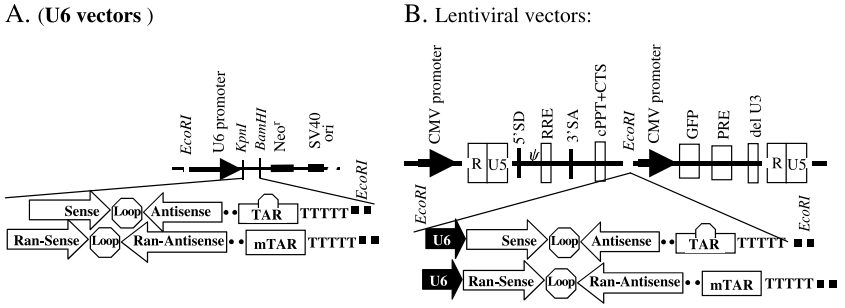

FIGURE 1 Construction of the U6 plasmid and lentiviral vectors. A. Sense and antisense strands of the synthetic DNA oligonucleotides were annealed at 95°C for 5 min, and cloned into the KpnI and BamHI cloning sites in the U6 vector. B. The EcoRI sites upstream from the U6 promoter and downstream from the terminating sequence of the generated U6 vectors were digested, and the fragments were cloned into the CS-CDF-CG-PRE vector to generate the lentiviral vectors.

cells (3×10^5) transfected with the Lipofectamine 2000 reagent. Northern blot analysis of total RNA extracted with Trizol showed an in vivo cleavage activity of the RNA molecule 72 h post-transfection in the cells. Further in-vitro cleavage assays using recombinant human dicer proved that the Vif siRNA-decoy TAR RNA substrate was mostly cleaved into its separate components, as the Vif siRNA and decoy TAR RNA, respectively (data not shown).

We then examined the dose-dependent anti-HIV-1 efficacy of the RNA substrate by co-transfecting various amounts (0.1, 1.0, and 3.0 μg) of U6 vector DNA and 0.2 μg of HIV-1 plasmid DNA into HeLa $CD4^+$ cells, and measuring the HIV-1 gag p24 antigen production level from the cell-free culture supernatant by a chemiluminescence enzyme-linked immunosorbent assay system (CLEIA) after 72 h, as an index for inhibition. The highest inhibition was observed at a concentration of 3 μg U6 vector DNA, while at the 1 μg U6 vector DNA concentration, there was still an appreciable level of inhibition. The decoy TAR U6 vector alone at 3 μg DNA did not mediate as much inhibition as that of the Vif siRNA U6 vector alone at 3 μg DNA. Our results therefore suggest that the TAR component of the U6 Vif siRNA-decoy TAR RNA molecule only complemented the inhibition efficacy in the co-transfected cells. The observed inhibitions correlated with the down-regulation of the HIV-1 viral mRNA (data not shown).

We further elucidated the inhibitory capacity of the RNA substrate, by constructing the lentiviral versions of the U6 plasmid Vif siRNA-TAR and Vif Ran-mTAR vectors (Figure 1B). 293T cells were transfected by the calcium phosphate precipitation method, and the viral titers of transduced MT-4 cells (3×10^5) were calculated using the expressed EGFP from FACS analysis. Jurkat cells (5×10^5) were then transduced with 10 MOI of the lentivirus expressing the Vif siRNA-TAR, including its random siRNA-mutant TAR version in addition to mock transduced Jurkat cells as control,[4] and were finally challenged with 0.02 MOI of HIV-1_{NL4-3}.

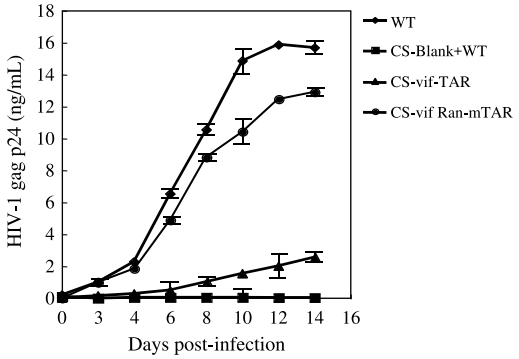

FIGURE 2 Evaluation of the inhibition efficacy of the trans-genes. Transiently transduced Jurkat cells expressing the Vif-TAR, Ran-Vif-mTAR and empty lenti-vectors were challenged with 0.02 MOI of HIV-1_{NLE} and cultured over a period of 14 days. Cell-free supernatants were sampled over the period and analyzed for HIV-1 *gag p24* antigen production by CLEIA. Data represent the mean values in the supernatants ± SD of three independent experiments.

Cell-free culture supernatants were sampled over a period of 14 days and evaluated for HIV-1 gag p24 antigen production, to determine the sustainable inhibition efficacy of the lentivirus-delivered dual HIV-1 anti-genes. The results indicated the sustained inhibition of HIV-1 replication by the lentivirus-mediated Vif siRNA-TAR RNA molecule, compared to the lentivirus-mediated random siRNA-mutated TAR version (Vif Ran siRNA-mTAR), the positive controls (lentivirus-mediated CS-empty vector + HIV-1_{NL4-3}. (wt)), and the mock infected, empty vector transduced Jurkat cells (negative control) over the same period of 14 d (Figure 2). In conclusion, our findings suggest that targeting the HIV-1 genes with the intracellularly expressed HIV-1Vif siRNA and decoy TAR RNA, as a single RNA substrate, enhances both delivery efficiency to the target cells and the inhibition efficacy on HIV-1 replication. This strategy will be a promising tool for HIV-1 gene therapy.

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