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Proceeding Paper

New Compound Combining an Integrase-Targeting Aptamer and a Small Interfering RNA Targeting the Trans-Activation Response/Poly A Region of HIV-1 Potently Suppresses HIV-1 Replication †

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Abstract: We have developed a novel aptamer-based siRNA delivery system for HIV therapy. Apsi510 was obtained by chemical conjugation of an anti-HIV integrase aptamer and an siRNA sequence targeting the HIV-1 TAR/poly A regions to a dendron [2-((4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)amino)acetaldehyde]. Apsi510 activity against HIV-1NL4.3 was evaluated in two experimental systems using HeLa CD4+ and TZM-bl cells. Apsi510 activity was dose-dependent and inhibited >95% of viral replication at 50 nM. Apsi510 inhibited HIV-1 replication to a similar extent as siRNA alone, indicating efficient intracellular release of the siRNA molecule. Apsi510 is a promising drug candidate for the treatment and prevention of HIV.

Keywords: HIV integrase aptamer; TAR siRNA; Apsi510 compound; antiviral activity

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1. Introduction

The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), a disease that remains incurable and requires lifelong treatment. The best way to end the HIV/AIDS pandemic by 2030 is to cure the infection. Stem cell transplantation has already cured four HIV patients. Simpler, more accessible therapeutic strategies to cure HIV infection have been explored, but none have yet reached the clinic [1].

RNA interference (RNAi) is a natural antiviral defense mechanism found in plants, fungi and invertebrates [2]. It is mediated by small interfering RNA (siRNA) and microRNA (miRNA), which degrade or repress messenger RNAs (mRNAs), thereby silencing the expression of specific genes. Several studies have reported using siRNA agents for silencing key HIV-1 genes and inhibiting HIV-1 replication [3,4]. HIV has a high rate of mutation; therefore, effective siRNAs should target conserved sequences [4]. Naito et al. described a highly effective targeting of the trans-activation response (TAR) element and the poly A region in the HIV-1 LTR [5]. Nevertheless, the lack of efficient delivery systems and low biostability challenge the potential of siRNA-based therapies. Several strategies may improve siRNA therapeutic efficacy, including the use of chemically modified siRNAs and novel siRNA delivery vehicles such as polymers, lipids, peptides, antibodies, nanoparticles, dendrimers and aptamers [6]. Aptamers (Ap) are short (15–80 nucleotides, DNA or RNA)

single-stranded oligonucleotides capable of binding specific molecules (small molecules, proteins, nucleic acids) with high specificity and affinity [7]. They have been used in various biomedical applications including aptamer–drug conjugates as a simple and effective way to deliver therapeutic agents to specific targets. However, concerns have been raised about the clinical effectiveness of the first generation of aptamer–drug conjugates (ApDC) due to their low circulating half-life and low stability [6]. Circulating half-life can be increased by chemical modifications, such as locked nucleic acids and the conjugation of high-molecular-weight polymers [6]. Reversible or irreversible covalent bonds (e.g., amides, imines or disulfides) have been used for conjugation, increasing the stability of ApDCs and allowing drug release in target cells [8].

The HIV integrase enzyme is highly conserved and is crucial for proviral DNA integration [9]. Several successful aptamers with anti-integrase activity have been identified, including T30695—an aptamer consisting of a repeating motif of a d(GGGT)4 forming a parallel-strand G-quadruplex [10]. In humanized mice, dendrimer-siRNA nanoparticles targeting both cellular and HIV-1 (tat/rev) transcripts led to a significant viral suppression [11].

In this study, we developed and evaluated the anti-HIV-1 activity of a novel T30695 aptamer-siRNA510 conjugate, named Apsi510. siRNA510 targets the TAR/poly (A) regions in the HIV-1 LTR promoter, which is the binding site for the Tat protein and many cellular factors involved in transcription initiation [12]. Disruption of the TAR/poly A regions disturbs the recruitment of these viral and cellular factors, affecting the production of new virus particles.

2. Materials and Methods

2.1. Apsi510 Synthesis

The inner core of Apsi510 is the linker 2-((4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)phenyl)amino)acetaldehyde, which is conjugated with an Ap targeting the HIV-1 integrase protein (T30695) [13] and one siRNA sequence. The synthesis of this linker (**1**) was attained following the methodology depicted in Figure 1. From commercially available 1-fluro-4-nitrobene (**2**, mono ApDnC) and aminoacetaldehyde dimethyl acetal, intermediate **3** was obtained in good yields by nucleophilic aromatic substitution. After the introduction of the acetal side chain, the nitro moiety was subsequently reduced to amine and reacted with maleic anhydride (**4**). After ring closure to form the maleimide intermediate (**5**), acid hydrolysis of the acetal gave the final aldehyde side chain in **1**.

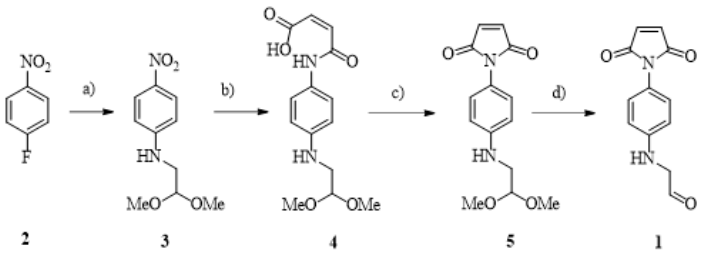

Figure 1. Synthesis of the linker (**1**) for the aptamer and siRNA510. (**a**) Aminoacetaldehyde dimethyl acetal, DMSO, overnight, r.t; (**b**) (**1**) Pd/C 10%, Et3SiH, MeOH, 1 h, r.t.; (**2**) maleic anhydride, DCM, 3 h, reflux; (**c**) acetic anhydride, sodium acetate, 1 h, r.t.; (**d**) H_2O:TFA (90:10), 10 min, r.t.

The maleimide group in **1** allowed the irreversible covalent conjugation of the thio-modified-HIV-1-integrase-targeting T30695 aptamer. Finally, the acid-labile imine-linker was synthesized by reaction of the commercial amino-modified siRNA510 with the aldehyde side chains to form the acid-labile linkage. The C6-amino-modified siRNA is placed in the 5′-terminus of the sense strand. The imine-acid-labile linkers are expected to release the siRNA content in the acidic endosomal compartments, after cell internalization. Purification of Apsi510 was performed by reversed-phase HPLC.

2.2. Production of Virus and Antiviral Assays

HeLa cells were co-transfected with pNL4.3 or pNL4-3.Luc.R-E- DNA (0.5 µg) and treated with different concentrations of siRNA510 (5–30 nM), Ap(T30695) (25–50 nM) or Apsi510 (25–50 nM). For experiments with pNL4.3, the media were collected after 48 h and virus production in the supernatant was assessed by measuring replication in TZM-bl cells. TZM-bl cells express the luciferase gene under the control of the HIV LTR promoter, which is activated upon HIV infection. For assays with NL4-3.Luc.R-E-virus, firefly luciferase activity was directly measured in HeLa cells 48 h post transfection.

3. Results

3.1. Production of Apsi510

We synthetized the aptamer conjugate (Apsi510) combining a branched aromatic inner core (dendron) and the commercial HIV-1 integrase Ap (T30695) attached by a thioether linkage. This ensemble allowed the attachment of one acid-triggered (acid-labile imine)-releasing linker containing siRNA510.

3.2. Activity of siRNA510, Ap(T30695) and Apsi510 against HIV-1

The activity of different concentrations of siRNA510, Ap (T30695) and Apsi510 was evaluated against the subtype B reference isolate HIV-1NL4.3 in HeLa-CD4+ and TZM-bl cells (Figure 2). siRNA510 and Ap(T30695) (50 nM) alone inhibited >80% of HIV-1 replication in TZM-bl cells (Figure 2A). On the other hand, Apsi510 exhibited a dose-dependent antiviral activity with 50 nM, inhibiting >95% of HIV-1 replication in both experimental systems (Figure 2A,B).

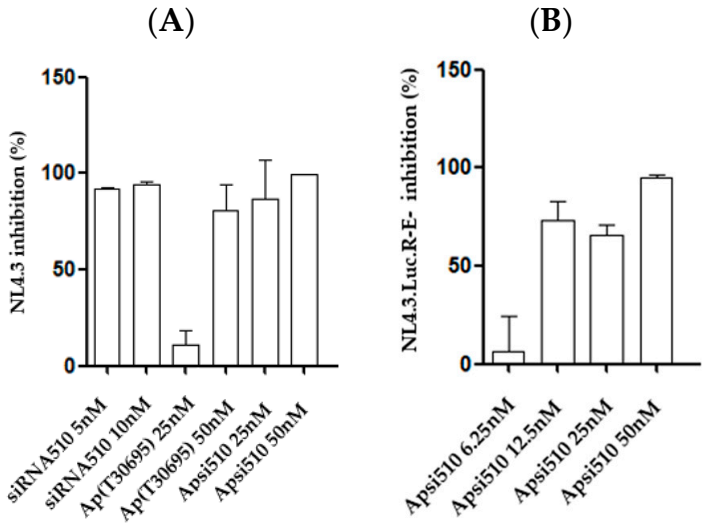

Figure 2. Apsi510 potently suppresses HIV-1 production. (**A**) HeLa cells transfected with pNL4.3 plasmid were treated with different concentrations of siRNA510, Ap(T30695) or Apsi510. Supernatants collected after 48 h were used to infect TZM-bl cells and 48 h after infection, luciferase activity was quantified. (**B**) HeLa cells were transfected with pNL4-3.Luc.R-E-plasmid and treated with Apsi510 (6.25–50 nM). Luciferase activity was quantified 48 h after transfection.

4. Discussion

We successfully synthesized Apsi510—an HIV-1-specific integrase aptamer conjugated to a dendron bound to HIV-1 TAR/poly A-specific siRNA510. Conjugation of siRNA510 with the dendron molecule was expected to protect the siRNA from ribonuclease degradation and to promote cell uptake via endocytosis, thereby increasing the antiviral activity of Apsi510. Apsi510 was a very potent inhibitor of HIV-1 replication in two different experimental systems. The antiviral activity of Apsi510 was higher than those of siRNA510 and Ap(T30695) alone, suggesting an additive effect. A similar inhibitory activity has been obtained with

other aptamer-siRNA-based compounds. For example, an anti-gp120 aptamer-anti-tat/rev siRNA chimera had potent anti-HIV activity in vitro and in vivo [14]. Likewise, siRNA aptamers against the HIV protease and the CCR5 co-receptor inhibited HIV-1 replication and cell entry in vitro and in vivo [15]. As siRNA510 has the potential to block RNA expression, Apsi510 may promote HIV latency by the block and lock strategy [1]. On the other hand, as Ap(T30695) inhibits HIV-1 integration, Apsi510 may help to decrease the size of the HIV reservoir and be useful in a shock-and-kill strategy for an HIV cure [1]. These potential activities are being investigated in cell lines that model the HIV reservoir and HIV latency, such as ACH-2 and J-lat E-6. In conclusion, Apsi510 has shown potent activity against HIV-1 and may be useful for the treatment, prevention and cure of HIV infection.

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