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Blocking interaction of viral gp120 and CD4-expressing T cells by single-stranded DNA aptamers

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ABSTRACT

To investigate the potential clinical application of aptamers to prevention of HIV infection, single-stranded DNA (ssDNA) aptamers specific for CD4 were developed using the systematic evolution of ligands by exponential enrichment approach and next generation sequencing. In contrast to RNA-based aptamers, the developed ssDNA aptamers were stable in human serum up to 12 h. Cell binding assays revealed that the aptamers specifically targeted CD4-expressing cells with high binding affinity (K_d = 1.59 nM), a concentration within the range required for therapeutic application. Importantly, the aptamers selectively bound CD4 on human cells and disrupted the interaction of viral gp120 to CD4 receptors, which is a prerequisite step of HIV-1 infection. Functional studies showed that the aptamer polymers significantly blocked binding of viral gp120 to CD4-expressing cells by up to 70% inhibition. These findings provide a new approach to prevent HIV-1 transmission using oligonucleotide aptamers.

1. Introduction

Aptamers are short, single-stranded (ss) oligonucleotides that have emerged as a new class of small molecule ligands with potential broad application (Hu and Zhang, 2013; Liu et al., 2011; Ni et al., 2011; Song et al., 2012; Strehlitz et al., 2012; Thiviyanathan and Gorenstein, 2012; Wang and Ray, 2012). As molecular ligands, oligonucleotide aptamers can specifically recognize and bind to a variety of targets with high affinity, including ions, toxins, drugs, low molecular weight molecules, peptides, proteins, cells, and tissues (Ni et al., 2011; Sefah et al., 2010; Thiel et al., 2012; Tombelli and Mascini, 2010). Although aptamer applications have been investigated in biomedical research, the use of aptamers in clinical applications remains less explored (Cheng et al., 2013; Schmidt et al., 2004).

Recent advances in antiretroviral therapy have significantly reduced the morbidity and mortality associated with infection by the human immunodeficiency virus type 1 (HIV-1) (Andrieux-Meyer et al., 2012; Hatano, 2013; Scanlon and Vreeman, 2013). Despite these advances, elimination of the virus with antiretroviral therapy is not yet possible due to harmful side effects and the development of resistance (Fidler and Bock, 2013; Moss, 2013). In addition, an effective vaccine for prevention of HIV-1 transmission is not yet available (Dhalla and Poole, 2013). Indeed, the continued emergence of drug-resistant HIV-1 strains and the high mutation rate of viral antigens necessitate the search for new therapeutics and, more importantly, better prevention approaches (Cahn and Wainberg, 2010; Peeters et al., 2010). One attractive and unique approach for prevention of transmission is to target and disrupt the viral entry mechanism into host cells (Teissier et al., 2010; Didigu and Doms, 2012; Gibson and Arts, 2012). Since HIV-1 transmission requires direct interaction of the viral gp120 and CD4 receptors on host cells, viral gp120 has been studies as a target for blocking HIV-1 transmission (Flores and Quesada, 2013). As the reverse transcriptase of HIV-1 is error prone, the hypervariable regions of gp120 mutate readily. Additionally, the hypervariable loops are highly glycosylated, forming what is often referred to as a 'glycan shield' that masks the conserved core binding regions (Castro et al., 2008). Thus, owing to its variability and shielded structure, the viral gp120 is a challenging target for prevention and/or therapy.

To overcome these technical obstacles and address clinical needs, we reasoned that an alternative entry inhibition strategy could be based on blockade of host CD4 receptor rather than viral gp120. To this end, ssDNA-based aptamers specific for cell-surface CD4 receptor were developed using a hybrid enrichment protocol (Hicke et al., 2001; Mann et al., 2010; Sefah et al., 2010). The developed aptamers significantly blocked the interaction between viral gp120 and CD4-expressing cells, suggesting a new approach to prevention of HIV-1 transmission.

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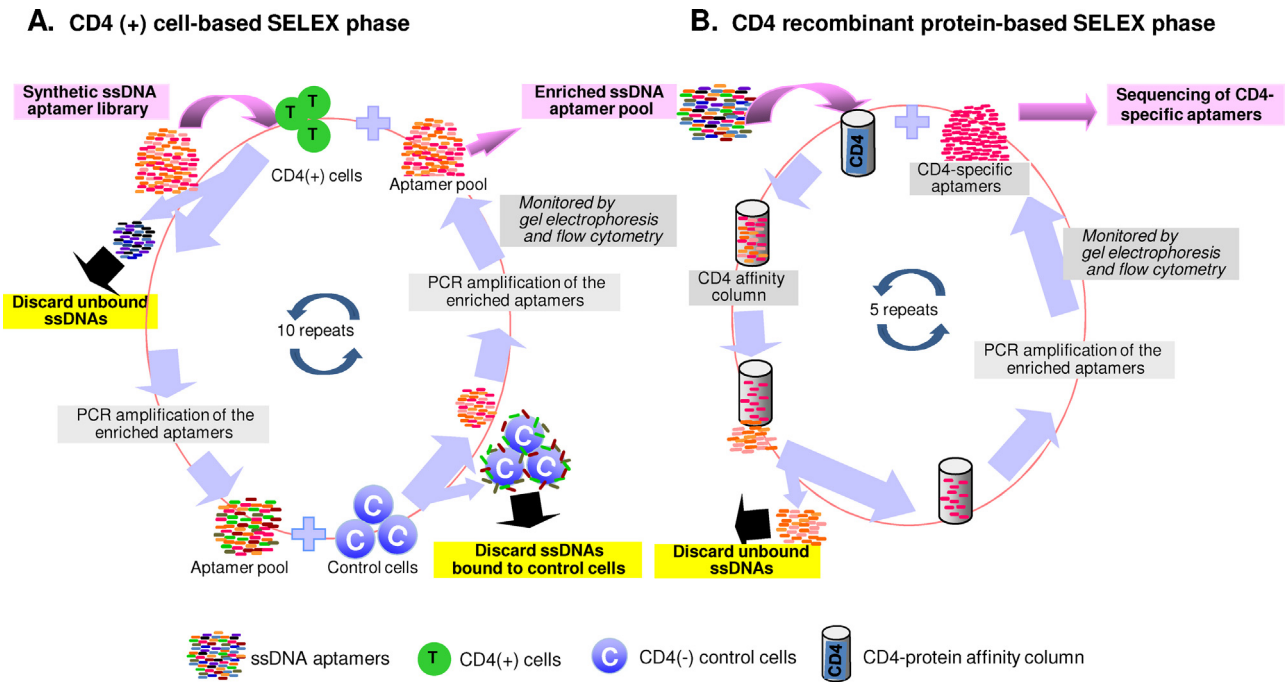

Fig. 1. Hybrid SELEX approaches to develop CD4-specific aptamers. The hybrid SELEX protocol included a two-step enrichment process that combines CD4-positive T-cell-based enrichment with biomarker-based selection. (A) CD4-binding aptamers were selected from a synthetic single strand DNA library with CD4-positive T cells. After PCR amplification of the enriched aptamers, negative selection, using CD4-negative control cells, was carried out and aptamers that non-specifically bind to cellular components were discarded. Seven rounds of positive and three negative selections with PCR amplification were performed. (B) Aptamers able to bind CD4-postive cells were then subjected to further enrichment with protein-based selection. Aptamers were added to a CD4 affinity column and unbound ssDNAs are discarded. PCR amplification of the bound aptamers was then performed. Protein-based selection was repeated 5 times. The final aptamer pool was amplified, sequenced and consists of CD4-specific aptamers.

2. Materials and methods

2.1. Reagents and cell lines

The CD4-positive cell line Karpas 299 was used to select ssDNA aptamers with high affinity for CD4-positive cells. CD4 recombinant protein (CD4-IgG2), obtained from the NIH AIDS Research and Reference Reagent Program (Germantown, MD), was used to further select DNA specific for the CD4 receptor at the protein level. Recombinant human CD4 protein with a polyhistidine tag at the C-terminus (Sino Biological Inc., Beijing, China) was used for aptamer affinity assays. The CD4-positive cell lines used included H9, SupT1, and U937 (ATCC, Manassas, VA). The CD4-negative cell lines tested were: B lymphoma cell lines CA46, JeKo-1, and Maver-1; breast cancer cell lines SKBR-3, MDA-MB-468, and T-47D; ovarian cancer cell lines HEY and A2780; and prostate cancer cell lines LN4, LNCaP, and PC3 (ATCC, Manassas, VA). All suspension cells were cultured with RPMI1640 medium with 10% FBS. All adherent cell lines were cultured with DMEM with 10% FBS.

2.2. Hybrid selection

The DNA library used for aptamer selection consisted of a central, continuous stretch of 45 randomized sequences flanked by PCR primer sequences (5′-ATCCAGAGTGACGCAGCA-45N-TGGACACGGTGGCTTAGT-3′) (Fig. 2A) (Sefah et al., 2010). Cy5-labeled 5′ primer (5′-Cy5-ATCCAGAGTGACGC AGCA-3′) and biotinylated 3′ primer (5′-biotin-ACTAAGCCACCGTGTCCA-3′) were used in the initial PCR. DNA library and primers were purchased from Integrated DNA Technologies (Coralville, IA).

The first phase of aptamer development began with cell-based enrichment performed on the CD4-positive cell line Karpas 299 as previously described (Sefah et al., 2010). After 7 rounds of positive selection and 3 rounds of counter selection with the CD4-negative cell line CA46, the enriched DNA pool was used in sequential protein-based selection by incubating with CD4-IgG2 protein. The entire protein selection process was repeated five times. After a total of 15 rounds of cell- and protein-based selection, binding affinity and specificity of the selected pool was determined by flow cytometry (LSRII, BD Biosciences, San Jose, CA). The final selected pool was sequenced using second-generation sequencing (LC Sciences, Houston, TX).

2.3. Protein binding assay

To identify which aptamers had higher affinity for CD4 protein, we used the BLItz system (ForteBio, Inc., Menlo Park, CA) to test aptamer binding ability. Prior to starting the experiment, the anti-His biosensor was equilibrated in PBS buffer with 0.5% BSA for 10 min. The biosensor was then incubated with 50 μg/ml CD4 recombinant proteins (Sino Biological Inc., Beijing, China) for 10 min. After washing with PBS for 5 min, 10 nM CD4 DNA aptamers #1, #10 and #11 were incubated with the biosensor for another 5 min while the instrument recorded the kinetic binding ability of the aptamers.

2.4. Cell binding assay

Flow cytometry was used to monitor the enrichment of pools during the selection and to determine the binding affinity and specificity of the synthesized aptamers. Cell staining by aptamers and detection by flow cytometry were carried out as previously reported (Zhao et al., 2013). Based on fluorescent intensity, the binding affinity of aptamers was calculated by the reference method (Meng et al., 2012). The equilibrium dissociation constant (K_d) of the fluorescent ligand was obtained by fitting a plot of the specific intensity (Y) *versus* the aptamer concentration (X) to the equation $Y = B_{max}X/(K_d + X)$ using SigmaPlot (Jandel, San Rafael, CA).