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Inhibition of HIV-1 Transcription Through Disruption of TAT-PP1 Interaction as a Novel Target for Functional HIV Cure

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Opinion

Although combination antiretroviral therapies (cART) effectively suppress HIV-1 replication, viral rebound occurs shortly after therapy cessation. The major barrier to HIV-1 cure is a pool of latently infected CD4+ T cells, called the latent reservoir. Activation of viral transcription from integrated provirus is a key point for both viral latency and active HIV-1 replication. Here we discuss novel small-molecule inhibitors of HIV-Tat interaction with host protein phosphatase 1 as potential "block and lock" strategy for functional HIV cure.

Although combination antiretroviral therapies (cART) effectively suppress HIV-1 replication, viral rebound occurs shortly after therapy cessation. The major barrier to HIV-1 cure is a pool of latently infected CD4+ T cells, called the latent reservoir. Latency is established early during infection. Latently infected CD4+ memory cells are harboring integrated replication-competent provirus and have a long half-life. HIV-1 is not eliminated from latent reservoir during treatment because cART targets only actively replicating virus and does not target HIV-1 transcription. Also, the emergence of drug-resistant HIV-1 mutations in latently infected T-cells and macrophages allows virus to escape treatment.

Currently two strategies for HIV cure are under investigation: "shock-and-kill" and "block and lock". The "shock-and-kill" strategy aims to reduce the reservoir by activating HIV-1 provirus in the latently infected cells thus unmasking the reservoirs and eliminating the infected cells. The "block and lock" strategy aims to block activation of HIV-1 provirus permanently. Development of small-molecule compounds is a leading approach for both strategies. Latency reversal by inhibitors of chromatin-remodeling enzymes has been studied extensively during recent years in an attempt to eliminate HIV latent reservoir but still has not achieved successful elimination of latent reservoir in clinical trials [1]. On the other hand, the full complexity of latent HIV infection is still not fully understood thus slowing the development of effective small-molecule compounds that may permanently silence HIV-1 provirus.

Activation of viral transcription from integrated provirus is a key point for both viral latency and active HIV-1 replication. Current cART treatment does not include inhibitors of viral transcription. Therefore, targeting HIV-1 transcription with a novel anti-HIV drug may help in preventing HIV-1 reactivation and facilitate permanent HIV-1 suppression. Similar to other viruses with relatively small genome, HIV-1 uses multiple host cell proteins during replication including transcriptional activators, transcription suppressors and epigenetic transcription factors. The virus-encoded early gene product Tat activates transcription of the viral genome and promotes exponential viral production. The unique mechanism of Tat transcriptional activation was elucidated over the past 20 years [2,3]. Unlike other known transcriptional activators, Tat primarily induces transcriptional elongation by recruiting the cellular positive transcriptional elongation factor b (P-TEFb) containing CDK9/cyclin T1 to HIV TAR-RNA [4,5]. Inability of Tat to recruit CDK9/cyclin T1 to TAR RNA may contribute to the establishment of latency [6]. Therefore permanent inhibition of viral transcription through blocking of Tat action may facilitate functional cure of HIV-1 infection and thus presents a novel anti HIV-1 therapeutics. In contrast to cART that combines drugs developed against viral proteins, drugs used for "lock and block" strategies are developed primarily against the host proteins. CDK9 is one of the targets for HIV-1 transcriptional inhibition. Most kinase inhibitors block enzymatic activity and potentially may impact cell functions. Phosphorylation dynamics is supported by kinases and proteinases activities.

Our previous studies demonstrated that HIV-1 Tat interaction with cellular protein phosphatase 1 (PP1) is critical for the HIV-1 transcription activation [7-9]. PP1 is dimer of a catalytic subunit (PP1α, PP1β/δ or PP1γ) and a regulatory subunit that binds to the catalytic subunit through one or more short motifs (RVxF, SILK and MyPhoNE) presenting in majority of regulatory subunits [10,11]. Recent studies have identified a highly diversified PP1 interactome. PP1-interacting proteins function as targeting

subunits, substrates, inhibitors and activators of PP1. As targeting subunits, PP1 binding proteins determine cellular location and substrate specificity of PP1. PP1 catalyzes the majority of eukaryotic protein dephosphorylation reactions in a highly regulated and selective manner. Although phosphatase-interacting proteins often contain variants of the same PP1 binding motifs, they differ in the number and combination of docking sites. Major PP1 regulators, such as NIPP1 (Nuclear Inhibitor of PP1) and PNUTS (Phosphatase Nuclear Targeting Subunit), bind PP1 with nanomolar affinity and modulate the dephosphorylation of a wide range of PP1 substrates. We demonstrated that PP1 enters the viral transcription through direct recruitment by the 35QVCF38 motif of Tat. To develop a novel small molecule inhibitor we used the complex of PP1γ with RRVSFA peptide for docking experiments. About 300,000 compounds from the Enamine (Kiev, Ukraine) stock collection were virtually screened for binding to PP1 RVxF binding site. We selected 262 candidate compounds and tested them for inhibition of Tat-dependent HIV-1 transcription using *in vitro* reporter assay.

Sixty compounds that inhibited HIV-1 transcription by at least 80% at 25 μM concentration were further selected. These 60 compounds were further analyzed to determine the IC50 for the inhibition of HIV-1 transcription. This analysis identified eight compounds that inhibited HIV-1 transcription at IC50s below 15 μM. The initial hit compound 1H4 was selected from 8 compounds because on its low cytotoxicity. We applied an iterative procedure (design-synthesis-evaluation) to the modification of the structure of 1H4 in order to improve inhibition of HIV-1 transcription without increasing cellular toxicity. Through the sequential analysis of several libraries, we obtained HIV-1-inhibitory compounds with higher inhibitory properties and lower toxicity comparing to the initial 1H4 compound. 1H4 and its cyclopentan quinolone derivative with improved activity, 1E7-03, bind non-competitively to PP1 *in vitro* without affecting PP1 enzymatic activity and prevent the interaction of HIV-1 Tat protein with PP1 [12,13]. 1H4 and 1E7-03 did not affect PP1 binding to NIPP1 and PNUTS [12]. It is important to note that 1H4 and 1E7-03 inhibitors don't block function of cellular PP1 but disrupt the interaction of PP1 with Tat, thus reducing amount of PP1 available for the recruitment to HIV-1 promoter and increasing epigenetic repression of the HIV promoter. In HIV-1-infected humanized mice, 1E7-03 significantly reduced plasma HIV-1 RNA levels [14].

Thus, inhibition of Tat-PP1 interaction may present a novel approach for "block and lock" strategy. Future experiments are needed to determine whether long-term transcription inhibition will induce permanent latency of HIV-1 provirus. cART efficiently inhibits viral replication, therefore addition of novel transcriptional inhibitors to current formulation is not warranted. A cell niche is important determinant in the activation of latency as tissue-specific environments may impact the mechanisms of HIV activation. A recent study demonstrated that levels of total transcripts per provirus were significantly lower in CD4+ T cells in rectum comparing to CD4+ T cells in blood [15]. The lower levels of HIV transcripts per provirus suggest that this tissue site is enriched in cells with a "deeper" state of latency. In addition to CD4+ T cells, HIV reservoirs also persist in tissue macrophages as an unintegrated transcriptionally active DNA form [16].

Organ-specific physiological factors determine activation of HIV. We demonstrated that high oxygen environment induced HIV-1 transcription and replication [17]. We speculated that addition of transcriptional inhibitors to the organs with high oxygen levels like lung and brain may be beneficiary for "block and lock" strategy. In conclusion, it is clear that complete eradication of HIV-1 requires combinations of various effective therapeutic strategies tailored for specific viral niche.

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