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OPEN ACCESS JOURNALS

Journal of Biomedical Research & Innovation

Perspective | Vol 2 Iss 1

Alternative Latency Reversal Agent Targets Identified in the gp120-to-CCR5 Induced Cellular Signaling Pathways

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Received: May 18, 2021; **Accepted:** May 26, 2021; **Published:** May 31, 2021

Abstract

The latent reservoir of HIV-infected CD4$^+$ T-cells presents challenges for finding an effective strategy to eradicate HIV. HIV latency occurs after the reverse transcription and integration of HIV-DNA into the host cell genome. After HIV-DNA integrates, the proviruses may produce trans-activator of transcription proteins (Tat) that bind to the promoters located in the long terminal repeats (5'LTRs), initiating HIV-DNA transcription. In contrast, when Tat is absent, the CD4 T-cells enter a state of latency without HIV-DNA transcription. Latent-reversal agents (LRAs) can "shock and kill" infected and latent CD4 T-cells but without fully reactivating and eliminating all dormant cells. Many LRAs fail to eliminate the latent reservoir because deficient levels of Tat reduce LRA potency. LRAs that could modify the epigenetics of HIV genomic promoters, located in LTR regions, may not reduce latency due to the unstable availability of Tat, which produces "bipolar" LTRs. Because many existing LRAs fail to reduce HIV latency, we need to identify alternative novel LRAs. It may be possible to find novel LRAs through examining potential LRA targets in cellular signaling pathways initiated by HIV viral entry. Currently, we know the binding between HIV-gp120 and the CD4-T-cell CCR5 co-receptor induces cellular signaling pathways that differ from signaling cascades stimulated by conventional CCR5 ligands. However, further research is needed to fill this gap in the literature, concerning the early impact of viral entry on HIV latency. To enhance our understanding of HIV latency this commentary recommends exploring the cellular signaling pathways formed by HIV viral entry.

Keywords: HIV Latency; Latency reversal agents; HIV genome; Transactivation

1. Introduction

The Human Immunodeficiency Virus or HIV is a retrovirus that infects CD4$^+$ T cells [1]. The virus enters the cell, and then the HIV-RNA is reverse transcribed into DNA that becomes integrated into the host cell genome. Once the HIV-DNA integrates, there are two possible end results available. One is the HIV may begin replication and then kill the infected cell, producing many viruses. Secondly, the HIV virus can begin and enter latency, which is a stage of dormancy where there is no viral gene-expression [1]. The destruction of CD4$^+$ T cells causes the Acquired Immune Deficiency Syndrome or AIDS. The

latency of HIV is the greatest challenge for completely eradicating HIV in a patient [1]. Infected cells are mainly resistant to antiretroviral drugs, and these infected cells store reservoirs of HIV. When a patient stops his or her antiretroviral treatment, the virus returns to its pre-treatment state and phase. HIV infects activated $CD4^+$ T cells that transitions into a memory T cell resting phase. While in this resting state, the T cells have lesser amounts of cellular factors responsible for viral replication and inhibiting HIV gene expression.

HIV proviral DNA integrates into the human genome, and then the HIV Long-Terminal Repeat (LTR) promoter interacts with the Tat protein, which is needed for viral replication. Tat works to initiate transcription using the HIV LTR promoter and this forms an essential positive feedback cycle or loop that turned on early in the HIV infection [1]. The Tat protein combined with the positive feedback loop can efficiently activate the viral cell-fate decision [1]. The positive feedback loop and genetic circuit causes a bimodal expression and allocation of Tat. This bimodal distribution of Tat is characterized such as high amounts of Tat switch the genetic circuit of HIV transcription on while a low level of Tat turns this positive genetic feedback circuit off. Therefore, the use of latent reversing agents (LRAs) may become less potent because of the everchanging dynamics of Tat availability that controls the genetic circuits of the HIV proviral genome via the Tat-to-LTR interaction. Due to there being a reduced number of potent and effective LRAs present to reduce the size of the HIV-1 latent reservoir, an identification of more LRAs is needed. In this commentary, it is suggested that additional cellular targets, located in the host $CD4^+$ T-cell, need identification to develop more potent LRAs. More cellular targets may be found in analyzing the unique cellular pathways activated by the HIV-1 gp120-to-CCR5 co-receptor interaction.

2. The Latent Phase

2.1 The process of HIV-1 latency

Reverse transcription of HIV-RNA into HIV-DNA is the most significant step of the HIV-1 life cycle, which is followed by the proviral genome integrating into the host cell genome [2]. HIV-1 depends on the viral protein Tat when it is in an exponential growth state where Tat is needed for a rapid transcriptional process of the integrated proviral DNA [2]. Tat is 14 kDa in size with about 101 amino acids that becomes expressed from spliced full-length transcripts [2]. Without the Tat protein, transcription is induced at the long terminal repeat (LTR) promoter; however, only short and ending viral transcripts are produced because of RNA polymerase II (RNAPII) ceasing transcription after passing the promoter. Tat regulates transcription by controlling RNAPII elongation via Tat's interaction with the Transactivation Response Element (TAR) RNA [2]. TAR RNA is an RNA stem loop complex that quickly configures in the first 59 nucleotides of the viral transcripts. Tat interacts with the positive transcription elongation factor b (P-TEFb), which consists of Cyclin T1 and Cyclin-dependent kinase 9 (CDK9). When Tat is absent, P-TEFb is present at high levels in the host cell where P-TEFb is a large dormant or inactive structure consisting of 7SK snRNA and MAQ1/HEXIMI proteins [2]. Tat recruits P-TEFb that then activates more phosphorylation processes produced by CDK9. CDK9 stimulates the conversion of a paused elongation process into a higher frequency of transcription.

2.2 The epigenetics of latency

The CDK9 phosphorylates Serine (Ser) 2 a part of the RNAPII C-terminal domain (CTD) heptapeptide repeats, which triggers the interaction of more productive transcription factors. After these post-translational modifications, a high synthesis of full-length HIV-viral transcripts are formed. The promoter activity of the provirus becomes regulated by the local chromatin of the

proviral DNA. LTR activity is inhibited by histone post-translational modifications such as phosphorylation, methylation, acetylation, ubiquitination, and Nuc-1 that is a nucleosome located downstream of the transcription start site. Epigenetic modification of chromatin at the nucleosomes located near the 5'LTR ceases transcription levels [2]. Common epigenetic modifications are deacetylation and methylation of the N-terminal ends of histones. Low quantities of Tat or of P-TEFb causes HIV-1 latency, producing the early termination of RNA transcripts [2]. Histone Deacetylase 1 or HDAC1, histone methyltransferase Suv39H1, and heterochromatin protein HP1 are all recruited to the locations of chromatin near HIV-1 LTR, which causes the latency phase. Modifications in local chromatin induces the reactivation of transcription, which is dependent on recruiting Tat, other chromatin remodeling factors, the histone acetyl transferase called hGCN5 that works to change the effects of histone deacetylation, p300/CBP associated factors (PCAF), and histone acetyl transferases (HATs) such as CREB binding proteins (CBP) and p300 [2]. PCAF and p300, respectively, acetylate the Lys28 and the Lys50 of Tat. After Lys28 is acetylated, Tat binds to P-TEFb while the acetylation of Lys50 leads to Tat/PTEFb dissociating from Transactivation Response Element (TAR).

2.3 Latent reversal agents (LRAs)

The "shock and kill" approach has been used to eliminate the HIV-1 latent reservoirs. "Shock and kill" can reactivate these resting reservoirs of HIV infected cells by triggering transcription of the proviruses and then eliminate the reactivated host cells by using the host immune response. The "shock and kill" strategy is a well- known and highly discussed approach [3]. Many latency-reversing agents or LRAs have been found and are currently being clinically tested in humans, but these LRAs have produced inconclusive results that are mixed or insignificant [3]. Many LRAs are highly toxic, lack target specificity, and some LRAs induce drug resistance [3]. Most LRAs cannot target HIV-1 infected cells, nor can they target the HIV-genome. Therefore, more novel and innovative strategies are needed to eliminate many of these issues [3]. There are no current latency-reversing agents that are safe and effective for eradicating HIV-1 reservoirs, so there is a gap in the research and a need for more new and novel latency reversing agents and methods [3].

3. Limitations of LRAs Targeting Tat and the LTR

3.1 High mutation rate of Tat

Tat produces large values of viral transcription while traversing the HIV viral life cycle [4]. Tat induces transcription after binding to the viral 5' LTR promoter, regulating RNA polymerase II or RNAPII elongation. Tat binds to the hairpin of TAR in an RNA transcript, and then PTEFb composed of CyclinT1 (CycT1) and cyclin-dependent kinase 9 (CDK9) that phosphorylates the C-terminal domain of RNAPII altogether force transcriptional elongation to occur by increasing activity at the promoter site. Therefore, a lack of Tat or a defective Tat affects viral replication and the overall expanse of the latent reservoir [4]. However, Tat is increasingly polymorphic as compared to the HIV proteins such as Env, Nef, and Vpu [4]. Tat polymorphisms are caused by viral escape mutants from immune responses [4].

3.2 LTR is "bipolar"

Because HIV LTR is autoregulated by Tat, HIV LTR differs from cellular promoters [5]. From 25 years of intense and detailed research, it has been shown that Tat triggers transcription and elongation of HIV proviral DNA [5]. The P-TEFb, a protein

kinase complex, triggers HIV elongations by stimulating negative and positive factors of elongation. When the negative elongation factor (NELF) is phosphorylated, the blockage of elongation is removed. Phosphorylating the C-terminal domains (CTD) of the RNAIIP and the Spt5 activates and increases polymerase activity [5]. Because Tat stimulates an enormous level of transcription while its decline limits transcription, the HIV-LTR promoter site is highly "bipolar" [5]. Weinberger et al. and Burnett et al. found that stochastic fluxes of the gene expression of Tat can be like a molecular switch [5]. Changing the rates of initiation for NF-κB and Sp1 binding sites can limit and block Tat production that increases the rate of infected cells entering latency [5]. This molecular switching is controlled by Tat autoregulation. Expressing Tat at high levels, while it uses an ectopic promoter, the HIV proviruses remain active where they do not enter a state of latency or dormancy. The LTR structure is mostly conserved [6]. However, a small quantity of mutations such as a single point mutation can either increase positive fitness in one environment of the host cell or form a negative fitness effect in another cellular environment.

3.3 The Tat-dependency

Replication of HIV-1 depends on the activation of a promoter present in the long terminal repeat (LTR) area of the HIV-1 genome, consisting of sites where the HIV-1 transactivator protein called Tat can bind. Many other transcription factors can also bind to the LTR. Tat transactivation in the LTR is monitored by lysine acetylation [7]. Acetylating lysine k28 is monitored by the host cell histone acetyltransferases (HATs) p300 that works to fortify binding of Tat to the transactivation response (TAR) RNA complex in the LTR [7]. This interaction amplifies transcription. However, acetylating K50 via the p300 allows the dissociation of the Tat-P-TEfb-TAR complex that then orients the Tat to bind the RNA Pol II for elongation. Therefore, the strength of a latency reversal agent (LRA) that works to produce HIV viruses occurs when Tat is present [7]. LRAs may enhance Tat with posttranslational modifications [7]. As a result, Tat expression during the reversal of latency should be further researched to allow the full activation of the HIV replication cycle that can increase LRA potency [7]. Without Tat, latency will be maintained, and HIV proviruses will remain dormant in resting $CD4^+$ T-cells, so as a result, Tat is not present during the preliminary reactivation of the HIV provirus. This will reduce the potency and strength of some LRAs, such as HDACi that will not initiate splicing of viral transcripts when Tat is absent [7].

4. CCR5 and HIV-1 latency

G protein signaling induced by HIV gp120 binding may help us better understand the pathogenesis of HIV replication [8]. CCR5 and CXCR4 are co-receptors called G protein-coupled receptors (GPCR). CCR5 and CXCR4 trigger signaling pathways by heterotrimeric G proteins [8]. These signals are also induced by HIV gp120 binding to these co-receptors, but the HIV gp120 stimulates signaling pathways that are different from the normal binding of chemokine ligands to the CCR5 and CXCR4 co-receptors where this may be a result of the differing binding sites between ligands and the HIV gp120 binding affinities [8]. Currently, we know that G protein signaling plays a major and pertinent role in organizing, triggering, and driving HIV infection. A method that can eliminate latency of infected cells by inducing transcription of HIV proviral DNA is found in latency reversing agents (LRAs) where many potent, safe, and viable LRAs are becoming increasingly in high demand. One such LRA could be maraviroc. Maraviroc (MVC) initiates the phosphorylation of Nf-kB and enhances amplification of HIV-RNA transcription in resting CD4 T cells. Maraviroc, a clinically approved CCR5- inhibitor, can increase HIV transcription in vitro, acting as a novel LRA [9]. More studies of MVC as an LRA are required *in vivo* [9].

5. Conclusion and Perspectives

Future studies are needed to identify more potent LRAs. Because Tat binds to HIV-1 LTRs to activate transcription of proviral HIV-DNA, a few research studies have suggested creating Tat-like small molecules that can reverse latency by these small molecules binding to the LTR, inducing HIV proviral transcription. However, Tat highly and quickly mutates, simultaneously, as HIV viruses mutate to avoid the immune response. Other researchers have promoted the idea of reversing latency by modifying the epigenetics at the binding sites of promoters located in the LTR. Modifying the epigenetics at the LTR would still be arduous due to the slight but still present mutations in the LTR caused by the post-transcription modifications of Tat, and by the presence or absence of Tat, which makes the LTR "bipolar." The current LRAs lose their potency due to the low numbers of Tat present during the LRAs initial activation of HIV proviral transcription. Therefore, a new view and perspective of latency reversal is currently needed. Studying and re-analyzing how the binding of CCR5 to HIV-1 gp120 creates new and more unique cellular pathways and networks than compared to the normal binding of chemokine ligands may direct us into finding more LRA targets.

It is suggested in this commentary that there is a need to identify all the signaling events that are organized and mediated by CCR5 where these signaling pathways are necessary for better understanding HIV pathogenesis [10]. Studying this interaction of the initial HIV-1 entry into CD4 T-cells may enhance our understanding of how HIV viral entry affects latency, CCR5's role in HIV-1 latency, and the overall scheme of HIV pathogenesis. Many new therapeutic targets may be found in HIV's gp120 unique activation of signaling pathways to interrupt the HIV life cycle with less side effects. Maraviroc, which is a CCR5 inhibitor and antagonist, was found to have LRA type of qualities by initiating the phosphorylation of Nf-kB to amplify HIV- RNA transcription in dormant and infected $CD4^+$ T-cells. More LRA targets are needed other than the two known main targets we presently recognize such as the transcription factor known as Tat and the HIV genomic regions including LTRs. More LRA targets may be found in the cellular pathways and networks activated by the HIV-1gp120-to-CCR5 co-receptor interaction of $CD4^+$ T-cells.

6. Authors and contributors

The Author contributed to the investigation and methodology of this work.

7. Conflicts of Interest

The author declares that there are no conflicts of interest.

8. Funding Information

This work received no specific grant from any funding agency.

9. Ethical Approval

There were no experiments with humans or animals.

10. Consent for Publication

This publication contains no details, images, or videos relating to an individual person.

11. Acknowledgements

Many thanks are given to my mentors and their staff.

REFERENCES

1. Singh A. Stochastic analysis of genetic feedback circuit controlling HIV cell-fate decision. IEEE Conference on Decision and Control (CDC). 2012:4918-23.
2. Mousseau G, Valente S. Strategies to block HIV transcription: focus on small molecule Tat inhibitors. Biology. 2012;1(3):668-97.
3. Wang P, Qu X, Wang X, et al. Specific reactivation of latent HIV-1 with designer zinc-finger transcription factors targeting the HIV-1 5′ -LTR promoter. Gene Ther. 2014;21(5):490-95.
4. Kamori D, Ueno T. HIV-1 Tat and viral latency: what we can learn from naturally occurring sequence variations. Front Microbiol. 2017;8:80.
5. Karn J. The molecular biology of HIV latency: breaking and restoring the Tat-dependent transcriptional circuit. Curr Opin HIV AIDS. 2011;6(1):4-11.
6. van Opijnen T, Jeeninga RE, Boerlijst MC, et al. Human immunodeficiency virus type 1 subtypes have a distinct long terminal repeat that determines the replication rate in a host-cell-specific manner. J Virol. 2004;78(7):3675-83.
7. Khoury G, Mota TM, Li S, et al. HIV latency reversing agents act through Tat post translational modifications. Retrovirology. 2018;15(1):36.
8. Juno JA, Fowke KR. Clarifying the role of G protein signaling in HIV infection: new approaches to an old question. AIDS Rev. 2010;12(3):164-76.
9. Symons J, vanLelyveld SFL, deSpiegelaere W, et al. The antiretroviral CCR5-inhibitor maraviroc effectively reverses HIV latency by phosphorylation of Nf-kappa B. J Int AIDS Soc. 2018;21(S6):29-30.
10. Paruch S, Heinis M, Lemay J, et al. CCR5 signaling through phospholipase D involves p44/42 MAP-kinases and promotes HIV-1 LTR-directed gene expression. FASEB J. 2007;21(14):4038-46.