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Feasibility of Using Gag Proteins to Reawaken Dormant HIV Infection Based on Bioinformatics Analysis

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Abstract: Recent studies revealing varied responses of infected cells to LRAs underscore the limited effectiveness of these agents and emphasize the wide array of determinants contributing to the heterogeneity of reservoirs, including virus genetic background, cell model, cell type, silencing mechanisms, tissue reservoirs, integration sites, patient, and gender specific factors. The enhancer region of the HIV-1 LTR contains two adjacent NF-κB binding sites that play a central role in mediating inducible HIV-1 gene expression. Beyond the involvement of various transcription factors, such as NF-κB, epigenetic constraints also play a pivotal role in suppressing the initiation of latent HIV transcription. Consequently, even latent viruses containing functional NF-κB sites remain unresponsive to drugs that activate NF-κB. Thus, it is evident that the activation of NF-κB alone does not suffice to trigger latent HIV, contradicting the central hypothesis of this study. The author used bioinformatics methods to analyze the viral proteins and their primer binding sites. The results show that the amino acid sequence ARG of Gag proteins of HTLV-1, HTLV-2, STLV-1 and STLV-2 match their primer binding site GGGGGCTCG in the 3'-to-5' direction and that the amino acid sequence SPR of Gag proteins of HIV-1, HIV-2, SIV and FIV match their primer binding site GGCGCCCGA in the 3'-to-5' direction. Related studies have shown that the genomic Gag/Gag-Pol complex recruits the LysRS/tRNA complex. The selective packaging of the tRNA primer requires HIV-1 Gag and Gag-Pol, and an interaction between LysRS and Gag is observed in vitro. In HIV-1, Gag/LysRS interaction depends on Gag sequences within the CTD of CA around amino acids 283-363 and motif 1 of LysRS around amino acids 208-259. It should be noted that the amino acid sequence SPR of the Gag protein is located at amino acids 148-150 within the NTD of CA, specifically at the NTD-NTD interface 1. Although this research is purely bioinformatics analysis, the relevant studies have demonstrated that Gag proteins match the HIV-1 primer binding site and possess the potential to directly activate dormant retroviruses.

Keywords: HIV; HTLV; NF-κB; LysRS; Gag

Introduction

Acquired immunodeficiency syndrome (AIDS) is a disease caused by human immunodeficiency virus (HIV). The virus attacks immune system cells in the body and then uses their machinery to make copies of itself. However, some HIV-infected immune cells enter a state in which they do not produce new virus, which is called the resting or latent state. These cells form a latent HIV reservoir in which HIV can hide for years, resulting in the avoidance of HIV therapy. At any time, these cells can become active again and start to make more copies of the virus. [1]

A significant challenge in curing HIV infection is the virus's ability to stay dormant within specific immune cells like CD4 cells, sometimes for years. During latency, the immune system remains unaware of the virus, rendering antiretroviral therapy (ART) ineffective. Latency-reversing agents (LRAs) are being explored to reactivate latent HIV in cluster of differentiation 4 (CD4) cells, enabling ART and the immune system to combat the virus. Currently, LRAs still have not been approved by the FDA. [2]

Scientists have used this opportunity to develop methods to target these latent reservoirs and make them active such that they can be identified and targeted by HIV therapy. However, scientists at Johns Hopkins reported compounds they hoped would 'wake up' dormant reservoirs of HIV inside

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the immune system, but T cells have failed to achieve this effect in laboratory tests using white blood cells collected directly from patients infected with HIV. [3]

Hence, further investigation is needed to determine the applicability of this method. In this study, the author analyzed whether LRAs can be used to activate dormant virus reservoirs and proposed the use of Gag proteins to reawaken dormant HIV infection.

Methods

HIV latency is a complex phenomenon where the virus remains dormant within cells, evading the immune system and antiretroviral drugs. The promoter-proximal (enhancer) region of the HIV-1 long terminal repeat (LTR) contains two adjacent NF-κB binding sites that play a central role in mediating inducible HIV-1 gene expression. [4–6] This region is pivotal in mediating the inducible expression of the HIV-1 gene when the virus is activated.

Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) is a transcription factor that plays a significant role in regulating immune responses and inflammation, it controls the activation of various genes involved in these processes. [7] In the context of HIV-1, the presence of NF-κB binding sites in the promoter-proximal region of the LTR is significant for several reasons.

The adjacent NF-κB binding sites allow NF-κB transcription factors to bind to the HIV-1 LTR. [8] When immune responses or other stimuli activate NF-κB, it can bind to these sites and initiate the transcription of the HIV-1 gene. This activation leads to the production of viral RNA and eventually new virus particles.

By having NF-κB binding sites in the promoter-proximal region, the virus gains a mechanism to control its replication in response to changes in the host environment. [4] This allows the virus to remain dormant when conditions are not favorable for replication, and to become active when immune responses or other factors signal an appropriate environment for replication.

The presence of NF-κB binding sites in the promoter-proximal region presents a potential target for therapeutic interventions. If researchers can manipulate NF-κB activation, they might be able to control the expression of the HIV-1 gene and potentially develop strategies to suppress viral replication or reactivate latent virus for elimination.

However, clinical trials of LRAs within the "shock and kill" strategy have so far produced unconvincing results. [9] Recent studies revealing varied responses of infected cells to LRAs underscore the limited effectiveness of these agents [10–12] and emphasize the wide array of determinants contributing to the heterogeneity of reservoirs, including virus genetic background [13], cell model [14], cell type [15–17], silencing mechanisms [18,19], tissue reservoirs [18–20], integration sites [21–23], patient [19,24], and gender [25] specific factors. Furthermore, some studies show conflicting observations on the impact of LRAs on natural killer cell [26] and cytotoxic T-cell lymphocyte [27] activity, indicating either an immunosuppressive effect or a reduced influence of LRA activity on cells sensing HIV-1 reactivation [10,27–30].

Beyond the involvement of various transcription factors, such as NF-κB, epigenetic constraints also play a pivotal role in suppressing the initiation of latent HIV transcription. Epigenetic modifications involve changes to the structure of DNA and its associated proteins, affecting gene expression without altering the DNA sequence itself. In the context of latent HIV, these modifications create a repressive environment that prevents the virus from becoming active. This repression is maintained through various mechanisms, including DNA methylation and histone modifications.

Even if the NF-κB transcription factor becomes activated, as it normally triggers immune responses and gene expression, it doesn't necessarily overcome the epigenetic barriers present in latent HIV. This means that even if the latent HIV viruses have functional NF-κB binding sites, they may remain unresponsive to drugs that specifically activate NF-κB. Essentially, the epigenetic modifications create a "brake" on the transcriptional machinery that even NF-κB activation might not be able to fully release.

Consequently, even latent viruses containing functional NF-κB sites remain unresponsive to drugs that activate NF-κB. Thus, it is evident that the activation of NF-κB alone does not suffice to trigger latent HIV, contradicting the central hypothesis of this study.

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Several studies claim that AZD5582 can reawaken sleeping HIV and SIV, but the effectiveness rate was found to be only 42%. [31–33] Most importantly, the novel small-molecule IAP inhibitor AZD5582 has been used for the treatment of cancer and reportedly causes cIAP1 degradation and thus induces apoptosis in the MDA-MB-231 breast cancer cell line at subnanomolar concentrations in vitro. [34]

In addition to AZD5582, many studies claim that LRAs, including ciapavir [35], bryostatin-1 [36], disulfiram [37], ingenol-B [38], and prostratin [39], can be used to reawaken sleeping HIV. These LRAs have also been used for the treatment of cancer: disulfiram inhibits prostate cancer cell growth [40], bryostatin-1 exhibits potent antitumor activity in vitro and in vivo in human tumor xenografts [41], semisynthetic ingenol compounds show potent antitumor activity on all cancer cell lines evaluated [42], and prostratin exerts a potential anticancer effect through SIK3 inhibition [43].

The LRAs associated with cancer are directed towards the NF-κB binding sites. This indicates that these LRAs are specifically designed to affect or interact with the NF-κB signaling pathway, which is relevant to cancer. The LRAs target the NF-κB binding sites instead of the HIV-1 primer binding site. As a result, LRAs do not directly reawaken dormant retrovirus infections. Therefore, the author suggests that rather than using LRAs to indirectly activate NF-κB binding sites, it might be more effective to employ other agents, such as viral proteins, to directly activate the HIV-1 primer binding site.

Viral RNA is specifically packaged into virions, not NF-κB or cancer RNA. This specificity allows the virus to accurately identify its own genetic material. Consequently, viral proteins carry information that enables the recognition of viral RNA, much like how LRAs activate NF-κB binding sites. It is conceivable that a specific viral protein may possess a function analogous to NF-κB, which could be harnessed to directly identify the HIV-1 primer binding site.

It is well known that HIV-1 recruits human uncharged tRNA (Lys) to serve as the reverse transcription primer [44], and tRNA also functions as the physical link between the mRNA and the amino acid sequences of proteins [45]. Therefore, the author posits that uncharged tRNA serves as the physical link between the promoter and the protein receptors, which are recruited by RNA polymerase II. To identify the specific viral protein that matches the primer binding site, a Python program was developed to match all proteins with their respective gene sequences and visualize them graphically.

Results

Latent HIV can synthesize a 5'-3' RNA chain by transcribing the existing 3'-5' complementary DNA strand after cellular infection [46,47]. The author uses the x-axis to represent the protein and the y-axis to represent the primer binding site. Negative numbers indicate that the protein or tRNA may have rotated 180 degrees (which did not happen) or been bound in the 3'-to-5' direction (if both values are negative).

The author also expanded the analysis to include other retroviruses, including Deltaretroviruses (HTLV and STLV) and Lentiviruses (HIV, SIV, and FIV). The gene data was sourced from the GenBank database at the NCBI. The author used the following sequences for the analysis: NC_001436 (HTLV-1) [48], NC_001488 (HTLV-2) [49], NC_000858 (STLV-1) [50], NC_001815 (STLV-2) [51], NC_001802 (HIV-1) [52], NC_001722 (HIV-2) [53], NC_001549 (SIV) [54], and NC_001482 (FIV) [55].

Having 2 amino acid sequences of the matching points leads to many possibilities, and it is thus impossible to confirm which protein matches the primer binding site. When there are 4 amino acid sequences, no matching target can be found. However, when there are 3 amino acid sequences, there is exactly one perfect matching region. Different types of retroviruses are represented by different patterns and colors, and their sequences around the primer binding site are matched with their own proteins, as shown in Figure 1.

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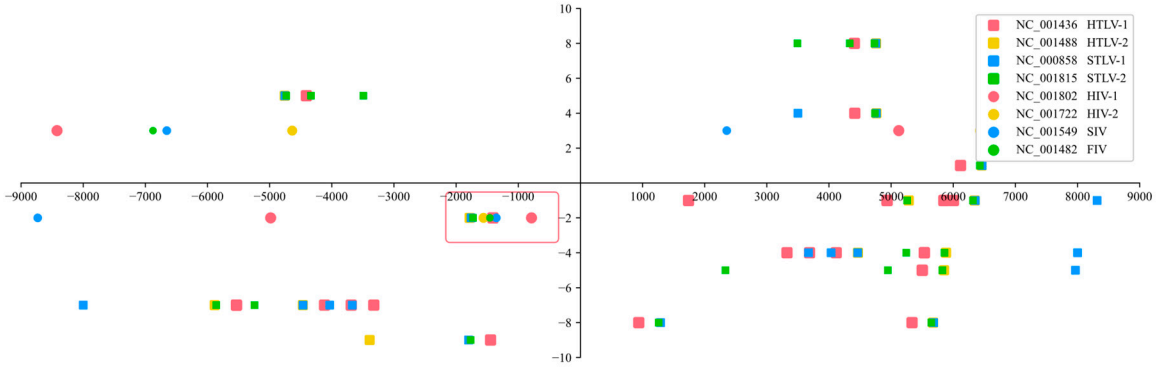

Figure 1. Coordinates of matched points.

As shown in Figure 1, inside the red box, the coordinates of 8 different viruses appear at the same time and are extremely close, which means that they represent the same protein. Other locations contained either only Deltaretroviruses or only Lentiviruses, and the spacing between the different color coordinates was too large, indicating that they were not even the same protein and were therefore excluded. If the virus amino acid sequences of the protein mutated, its primer binding site remained the same, which means that it was not the matching target.

In the GenBank database, the primer binding site of the HTLV-2 (NC_001488) genome is approximately nt 766 to 783, and that of the HIV-1 (NC_001802) genome is approximately nt 182 to 199. Their primer binding sites start with TGG and end with GGGA, and after aligning the sequences, their matching points can be found in the same position, as shown in Figure 2.

NC_001436 HTLV-1	400	CACAGTTGGGGGCTCGTCCGGGATTCGAGC	429
	416	↳ GCTCGGGGG	407
	317	Q K L L Q A R G H T N S P	319
	1383	CAAAAATTACTACAGGCCCGAGGGCACACTAATAGCCCT	1421
NC_001488 HTLV-2	760	AACAATTGGGGGCTCGTCCGGGATTTGAAT	789
	776	↳ GCTCGGGGG	767
	323	Q K I L Q A R G H T N S P	325
	1758	CAAAAAATCTTACAAGCCCGCGGACACACTAACAGCCCC	1796
NC_000858 STLV-1	752	CACAGGTGGGGGCTCGTCCGGGATACGAGC	781
	768	↳ GCTCGGGGG	759
	317	Q K L L Q A R G H T N S P	319
	1735	CAGAAACTACTACAGGCCCGAGGACACACTAATAGCCCT	1773
NC_001815 STLV-2	709	AACAAGTGGGGGCTCGTCCGGGATACCTAC	738
	725	↳ GCTCGGGGG	716
	322	Q K L L Q A R G H T N S P	324
	1704	CAAAAATTGCTGCAGGCCCGGGGCCATACTAATAGCCCC	1742

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Figure 2. Deltaretrovirus and Lentivirus.

As shown in Figure 2, the sequences on the second line represent the 3'-5' complementary DNA strand that gag proteins match with, and the arrow indicates the 3'-5' direction. The Gag proteins of the viruses match the same primer binding site, even though the viruses are highly different.

To determine whether this finding is a coincidence, the author analyzed the probability. Because viruses of the same type, Deltaretrovirus or Lentivirus, have the same primer binding site, one virus can be considered a mutation from another. The author used the HTLV-1 and HIV-1 genomes as templates and used Pairwise Sequence Alignment (EMBOSS Needle) to compare the genetic similarity of different viruses. The similarity of the NC_001488 (HTLV-2), NC_000858 (STLV-1) and NC_001815 (STLV-2) genomes to the NC_001436 (HTLV-1) genome was 59.2%, 89.3% and 61.0%, respectively. The similarity of the NC_001722 (HIV-2), NC_001549 (SIV) and NC_001482 (FIV) genomes to the NC_001802 (HIV-1) genome was 51.1%, 54.1% and 49.1%, respectively. The similarity of six viruses S can be written as

$$S = (0.592, 0.893, 0.61, 0.511, 0.541, 0.491)\,.$$

The average probabilities of the amino acid sequences A (GCT, GCC, GCA, GCG), R (CGT, CGC, CGA, CGG, AGA, AGG), G (GGT, GGC, GGA, GGG), S (TCT, TCC, TCA, TCG, AGT, AGC) and P (CCT, CCC, CCA, CCG) remaining unchanged after a mutation are 3/63, 5/63, 3/63, 5/63, and 3/63, respectively. Thus, the average probabilities of the amino acid sequences ARG and SPR remaining unchanged after a mutation are 11/189 and 13/189, respectively. Therefore, the average probability that 3 amino acid sequences of six viruses remain unchanged after a mutation can be represented by M as follows:

$$M = \left(\frac{11}{189}, \frac{11}{189}, \frac{11}{189}, \frac{13}{189}, \frac{13}{189}, \frac{13}{189}\right).$$

Assuming that each gene sequence has the same probability of mutation, the number of amino acid sequence mutations increases with increases in the diversity of the viruses. The probability that 3 amino acid sequences of different viruses match the same primer binding site is

$$P = \prod_{i=1}^{n} M_i^{3(1-S_i)} \approx 3.67636 \times 10^{-9}\,.$$

The result shows that the probability is approximately 3.67636×10^{-9}, which is extremely small; thus, it can be determined that it is not a coincidence.

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Related studies have shown that the genomic Gag/Gag-Pol complex recruits the LysRS/tRNA complex [56]. The selective packaging of the tRNA primer requires HIV-1 Gag and Gag-Pol [57], and an interaction between LysRS and Gag is observed in vitro [58]. Relevant studies have demonstrated that Gag proteins match the HIV primer binding site and possess the potential to directly activate dormant retroviruses.

In HIV-1, Gag/LysRS interaction depends on Gag sequences within the C-terminal domain (CTD) of CA around amino acids 283-363 [59] and motif 1 of LysRS around amino acids 208-259 [58]. It should be noted that the amino acid sequence SPR of the Gag protein is located at amino acids 148-150 within the N-terminal domain (NTD) of CA, specifically at the NTD-NTD interface 1.

Discussion

Because the primer binding sites of different viruses are extremely stable, other amino acid sequences of viral proteins may also match these sites, but there is no sufficient information to confirm this hypothesis at present. In addition, the mutation rate of HIV-1 is extremely high. While analyzing those sequences around the HIV-1 long terminal repeat, the author didn't find any similarity in the adjacent NF-κB binding sites of different viruses. This suggests that the NF-κB binding sites will mutate as the virus mutates. Consequently, scientists must constantly develop new LRAs for new viruses. More importantly, the virus utilizes the NF-κB pathway to enhance its expression, but this doesn't necessarily imply that the virus must always possess an NF-κB binding site. If the NF-κB binding site mutates to become an enhancer of other genes unrelated to NF-κB, LRAs will have no effect on patients.

When comparing the use of LRAs to activate NF-κB binding sites, the utilization of Gag proteins has the advantage of directly reawakening the retrovirus, irrespective of the mutation of the NF-κB binding site at adjacent locations. This pattern is consistent in both Deltaretrovirus and Lentivirus, implying that this treatment could potentially be applied to most patients without requiring the simultaneous administration of multiple LRAs. Moreover, a noteworthy aspect is that the CRISPR-Cas9 enzyme can be employed to modify the amino acid sequences of viral proteins. This modification can prevent the recruitment of uncharged tRNAs to match the NF-κB binding site, thus avoiding the activation of cancer-related genes and mitigating unforeseen risks to patients.

Conclusion

The enhancer region of the HIV-1 LTR contains two adjacent NF-κB binding sites that play a central role in mediating inducible HIV-1 gene expression. The LRAs target the NF-κB binding sites instead of the HIV-1 primer binding site. As a result, LRAs do not directly reawaken dormant retrovirus infections. The results show that the amino acid sequence ARG of Gag proteins of HTLV-1, HTLV-2, STLV-1 and STLV-2 match their primer binding site GGGGGCTCG in the 3'-to-5' direction and that the amino acid sequence SPR of Gag proteins of HIV-1, HIV-2, SIV and FIV match their primer binding site GGCGCCCGA in the 3'-to-5' direction. Related studies have shown that the genomic Gag/Gag-Pol complex recruits the LysRS/tRNA complex. The selective packaging of the tRNA primer requires HIV-1 Gag and Gag-Pol, and an interaction between LysRS and Gag is observed in vitro. In HIV-1, Gag/LysRS interaction depends on Gag sequences within the CTD of CA around amino acids 283-363 and motif 1 of LysRS around amino acids 208-259. It should be noted that the amino acid sequence SPR of the Gag protein is located at amino acids 148-150 within the NTD of CA, specifically at the NTD-NTD interface 1. Although this research is purely bioinformatics analysis, the relevant studies have demonstrated that Gag proteins match the HIV-1 primer binding site and possess the potential to directly activate dormant retroviruses.

List of abbreviations

LRAs: Latency-reversing agents
CD4: Cluster of differentiation 4
NF-κB: Nuclear factor kappa light chain enhancer of activated B cells

CRISPR: Clustered regularly interspaced short palindromic repeat
IAP: Inhibitor of apoptosis protein
cIAP1: Cellular inhibitor of apoptosis protein 1
SIK3: Salt-inducible kinase 3
HTLV: Human T-lymphotropic virus
STLV: Simian T-lymphotropic virus
HIV: Human immunodeficiency virus
SIV: Simian immunodeficiency virus
FIV: Feline immunodeficiency virus
LysRS: Lysyl-tRNA synthetase
PBS: Primer binding site
LTR: Long terminal repeat
CTD: C-terminal domain
NTD: N-terminal domain
Gag: Group-specific antigen
CA: Capsid

Ethics approval and consent to participate: Not applicable.

Consent to publish: The author gives consent for the publication of identifiable details, which can include photographs and details within the text to be published in the above Journal and Article.

Availability of data and materials: The datasets were produced by Python3, and the tool is available at https://github.com/rheast/genome. Pairwise Sequence Alignment (EMBOSS Needle) was used to identify regions of similarity between two biological sequences with the tool available at https://www.ebi.ac.uk/Tools/psa/. Nucleotides were downloaded from the NCBI database at https://www.ncbi.nlm.nih.gov/nuccore/. The sample nucleotides correspond to the accession numbers NC_001436, NC_001488, NC_000858, NC_001815, NC_001802, NC_001722, NC_001549 and NC_001482.

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