Feasibility of using Gag proteins to reawaken dormant HIV infection based on bioinformatics analysis

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Abstract: The technique of using drugs to target latent virus reservoirs has been introduced to reawaken dormant viruses such that the immune system can attack them, but further tests have shown this method fails. In this study, the author attempted to analyze whether drugs can be used to reawaken dormant virus reservoirs and proposed the use of viral proteins to activate the sleeping virus. 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. The author hence believes that the latency-reversing drugs are involved in the process of the transcription of cancer genes, and because the virus genome they reawaken contains the same NF-κB binding sites, the drugs indirectly reawaken dormant retrovirus infection. Related studies showed 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 contrast, Gag proteins can more reliably be used to directly reawaken dormant HIV infection, which recruits human uncharged tRNA to serve as the reverse transcription primer.

Keywords:HIV; HTLV; NF-κB; tRNA; Gag

1. 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]. 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 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 [2]. Hence, further investigation is needed to determine the applicability of this method. In this study, the author attempted to mathematically analyze whether latency-reversing drugs can reawaken the sleeping retrovirus.

2. Methods

Because HIV uses the host's NF-κB signaling pathway to activate viral transcription [3], the author designed the following experiment. First, the author prepared several T cells and HIV-1 RNAs. The HIV genome contains at least nine genes, including Gag, Pol and Env [4]. The *IGF1R* gene is located on human chromosome 15, which contains at least 21 exons, such as ENSE00003838363 and

ENSE00001316091 [5]. Using mathematical models can help understand phenomena in biology. In mathematics, the genome can be defined as a set of elements by listing the elements between curly brackets and separated by commas:

$$H = \{\text{Gag}, \text{Pol}, \text{Env}\}, \qquad I = \{\text{ENSE00003838363}, \text{ENSE00001316091}\},$$

where H denotes the set of HIV genomes and I represents the set of *IGF1R* genes. The *IGF1R* gene is one of the known target genes of androgen receptor activation [6]. Hence, the process of transcription can be written in the following form:

$$f(A) = I, \qquad A = \text{Androgen},$$

where the domain of f is the set of genes that RNA polymerase II will transcribe, and A represents the androgen. The CRISPR–Cas9 enzyme [7,8] then copies the enhancer of the *IGF1R* gene into the promoter-proximal region of HIV-1 RNAs. Subsequently, T cells are infected with the modified virus, and the form can then be rewritten as follows:

$$f(A) = I \cup H.$$

Androgens are then injected into the T cells. After the *IGF1R* gene is transcribed by RNA polymerase II [9], HIV will also 'wake up' [10]. Python is one of the most popular programming languages [11] and can be used to write scripts that can check the accuracy of mathematical formulas:

```
1   H = {'Gag','Pol','Env'}                           4   def f(x):
2   I = {'ENSE00003838363','ENSE00001316091'}         5       if x == A:
3   A = "Androgen"                                     6           return I | H
```

The set H represents the HIV genome, and the set I represents the *IGF1R* gene. The string A is then defined to represent androgen, whereas $f(x)$ represents the function of RNA polymerase II, which returns the *IGF1R* gene and HIV genome applied to the string A. The result of $H \leq f(A)$ is then printed to verify whether the virus was activated. As a result, the Python program returns True, which indicates that dormant HIV infection is reawakened.

```
7   print(H <= f(A)) #True
```

Can this possibly mean that androgen reawakens sleeping HIV? The answer is that androgen reawakens both the *IGF1R* gene and HIV genome and not only the retrovirus. In fact, even the Python program returns a result of False.

```
8   print(H == f(A)) #False
```

The collection of elements returned by the method includes the HIV set, which does not mean that the two sets are equal. Mathematically, their relationship can be expressed as follows:

$$H \subseteq f(A), \qquad H \neq f(A).$$

The author will not actually copy the enhancer of the *IGF1R* gene into the virus due to related studies: the promoter-proximal (enhancer) region of the HIV-1 long terminal repeat contains two adjacent NF-κB binding sites that play a central role in mediating inducible HIV-1 gene expression [3,12,13].

Several studies claim that AZD5582 can reawaken sleeping HIV and SIV, but the effectiveness rate was found to be only 42% [14,15,16]. 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 [17].

Latency-reversing drugs are involved in the process of transcription of cancer genes, and the virus genome reawakened by these drugs contains NF-κB binding sites; thus, the drugs do not directly reawaken dormant retrovirus infection. This method may not be easily understood by some readers; thus, the author provides another example:

To ensure that babies are not carried away by the wrong parents in a hospital (nucleus) with three newborn babies, Adam (HIV genome), Bob (*IGF1R* gene), and Claire (cancer gene), babies and their parents are given wristbands with their corresponding names (enhancer region). Adam is a naughty boy who secretly made a copy of Claire's wristband (NF-κB binding sites) and placed it on his hand. Because nurses (RNA polymerase II) cannot recognize the appearance of babies, babies can only be identified by their wristbands. When Claire's parents (NF-κB) wanted to take their child away from the hospital, the parents handed over their wristband to the nurses and asked them to find their child. Because both Adam and Claire had Claire's name written on their wristbands, both babies were taken away by the parents.

Based on this example, it feels like the previous studies were attempting to use Claire's wristband (NF-κB binding sites) to find Adam (HIV genome), which is clearly inappropriate. More importantly, the mutation rate of HIV-1 is extremely high [18]; if Claire's name on the wristband mutates to Clara or Clark (another cancer gene), the drugs that target Claire will have no effect on the mutated virus.

In addition to AZD5582, many studies claim that latency-reversing drugs, including ciapavir [19], bryostatin-1 [20], disulfiram [21], ingenol-B [22], and prostratin [23], can be used to reawaken sleeping HIV. These latency-reversing drugs have also been used for the treatment of cancer: disulfiram inhibits prostate cancer cell growth [24], bryostatin-1 exhibits potent antitumor activity in vitro and in vivo in human tumor xenografts [25], semisynthetic ingenol compounds show potent antitumor activity on all cancer cell lines evaluated [26], and prostratin exerts a potential anticancer effect through SIK3 inhibition [27].

One type of latency-reversing drug approach will not work in different patients infected with different types of mutated viruses unless multiple drugs are used at the same time. However, the mutation rate of HIV-1 is extremely high, which means that scientists have to constantly develop new drugs for new viruses. More importantly, the virus uses the NF-κB pathway to enhance its expression, which does not mean that the virus must have an NF-κB primer binding site. If the primer binding site is mutated into the enhancer of other genes unrelated to NF-κB, latency-reversing drugs will have no effect on patients. The author thus believes that instead of using Claire, Clara and Clark's wristbands to find Adam indirectly, it would be better to use Adam's wristbands to find Adam directly. In other words, the use of viral proteins can more reliably reawaken dormant retroviruses.

Viral RNA is specifically packaged into virions, not *IGF1R* or cancer RNA; thus, the virus can accurately identify viral RNA. Therefore, viral proteins carry information that can identify viral RNA, just as the androgen receptor activates the *IGF1R* gene. It is possible that a certain viral protein has a similar function to NF-κB or androgen receptor, which can be used to identify viral RNA directly.

It is well known that HIV recruits human uncharged tRNA to serve as the reverse transcription primer [28], and tRNA serves as the physical link between the mRNA and the amino acid sequences of proteins [29]. The author hence believes that uncharged tRNA serves as the physical link between the promoter and the protein receptors, which are recruited by RNA polymerase II. To determine

which viral proteins match the primer binding site, a Python program was written to match all proteins with their own gene sequences and display them graphically.

3. Results

Latent HIV can synthesize a 5'-3' RNA chain by transcribing the existing 3'-5' complementary DNA strand after cellular infection [30,31]. 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).

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 Fig. 1.
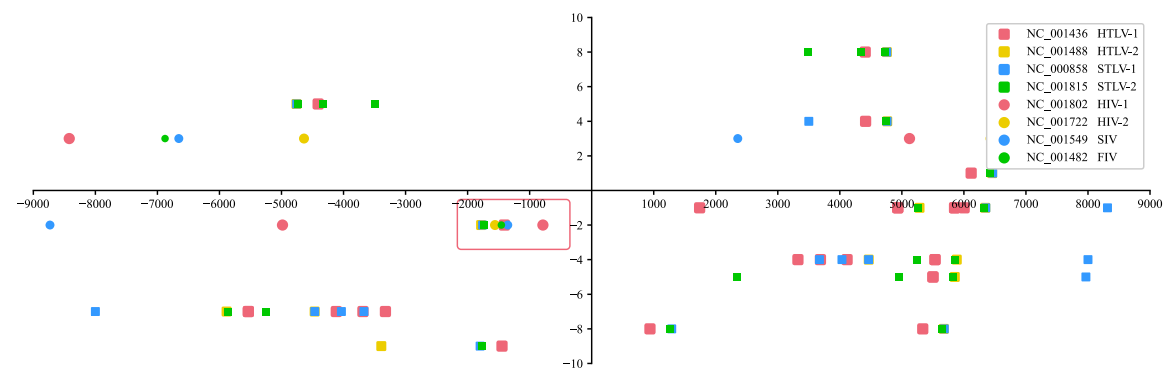
Figure 1. Coordinates of matched points.

As shown in Fig. 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 Deltaretrovirus (HTLV and STLV) or only Lentivirus (HIV, SIV and FIV), 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 [32,33,34,35,36,37,38,39], as shown in Fig. 2.

Figure 2. Deltaretrovirus and Lentivirus.

As shown in Fig. 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 Gag proteins can match the primer binding site.

Related studies showed that the genomic Gag/Gag-Pol complex recruits the LysRS/tRNA complex [40], the selective packaging of the tRNA primer requires HIV-1 Gag and Gag-Pol [41], and an interaction between LysRS and Gag is observed in vitro [42]. Since HIV-1 initiates the process of reverse transcription by using tRNA(Lys) to bind to the genomic RNA at the primer binding site [43], it has been proven that the gag protein matches the primer binding site.

In HIV-1, Gag/LysRS interaction depends on Gag sequences within the C-terminal domain (CTD) of CA around amino acids 283-363 [44] and motif 1 of LysRS around amino acids 208-259 [42]. 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.

4. Discussion

Because the primer binding sites of different viruses are extremely stable, other amino acid sequences of Gag proteins may also match these sites, but there is no sufficient information to confirm this hypothesis at present. The current information demonstrates that drugs fail to reawaken dormant HIV infection, and it is more reliable to use viral proteins to directly reawaken dormant retroviruses. Compared with the use of latency-reversing drugs to activate NF-κB primer binding sites, the use of Gag proteins can directly reawaken retrovirus without considering the mutation of the NF-κB primer binding site at adjacent locations, and the pattern exists in both Deltaretrovirus and Lentivirus, which means that the treatment can be used for most patients without the need to take multiple drugs at the same time. More importantly, the CRISPR–Cas9 enzyme can be used to modify the amino acid sequences of viral proteins to avoid uncharged tRNAs it recruits to match the NF-κB primer binding site, which results in avoiding the activation of cancer genes and reducing unknown risks to patients. It is also possible to design a new NF-κB, which has amino acid sequences of Gag proteins, to reawaken dormant HIV infection.

5. Conclusions

Latency-reversing drugs are involved in the transcription of cancer genes, and the virus genomes that they reawaken contain the same NF-κB primer binding sites; thus, the drugs are not directly

reawaken dormant HIV infection. 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 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 showed 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 contrast, Gag proteins can more reliably be used to directly reawaken dormant HIV infection, which recruits human uncharged tRNA to serve as the reverse transcription primer.

List of abbreviations

NF-κB: Nuclear factor kappa light chain enhancer of activated B cells
IGF1R: Insulin-like growth factor 1 receptor
CRISPRs: Clustered regularly interspaced short palindromic repeats
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
CTD: C-terminal domain
NTD: N-terminal domain
Gag: Group-specific antigen
CA: Capsid

Ethics approval and consent to participate
Not applicable.
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.
Competing interests
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