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Mathematical analysis: feasibility of using viral proteins to reawaken dormant retrovirus infection

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Abstract

The technique of using drugs to target latent virus reservoirs has been introduced to reawaken dormant viruses so that the immune system can attack them. However, further tests have shown this method to fail in laboratory tests. In this work, the author tries to mathematically 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 sequences ARG of gag proteins of HTLV1, HTLV2, STLV1 and STLV2 match with their primer binding site GGGGGCTCG in the 3'-to-5' direction, and the amino acid sequences SPR of gag proteins of HIV1, HIV2, SIV and FIV match with their primer binding site GGCGCCCGA in the 3'-to-5' direction. The gag proteins are promising for reawakening dormant retrovirus infection. The author hence believes that the latency-reversing drugs were involved in the process of transcription of cancer genes, and the virus genome they reawaken were just happened to contain the same NF-κB binding sites, so the drugs were indirectly reawakened dormant retrovirus infection. On the other hand, it is more reliable to use viral proteins to directly reawaken retrovirus, just as androgen receptor activates the *IGF1R* gene.

Keywords:

Retrovirus, NF-κB, HIV, HTLV

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a disease caused by human immunodeficiency virus (HIV). The virus attacks immune system cells in the body to use their machinery to make copies of itself. However, some HIV-infected immune cells enter a state in which they do not produce new virus, called the resting or latent state. These form a latent HIV reservoir, in which HIV can hide for years, avoiding 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 so that they can be identified and targeted by HIV therapy. However, scientists at Johns Hopkins reported that compounds they hoped would 'wake up' dormant reservoirs of HIV inside the immune system. T cells have failed to do so in laboratory tests on white blood cells taken directly from patients infected with HIV [2]. Hence, more investigation is needed to determine the applicability of this method. In this article, the author tries to mathematically analyze whether latency-reversing drugs can reawaken the sleeping retrovirus.

2. Methods

Since HIV uses the host NF-κB signaling pathway to activate viral transcription [3], the author designed an experiment as follows. First, the author prepared several T cells and HIV-1 double-stranded DNA, which

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were converted by reverse transcription. 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]. In mathematics, a set is a collection of elements, so the genome can be defined as a set of elements, by listing its elements between curly brackets, separated by commas:

$$H = \{\text{gag}, \text{pol}, \text{env}\} \qquad I = \{\text{ENSE00003838363}, \text{ENSE00001316091}\}$$

Where H denotes the set of HIV genome, and I represent the set of the *IGF1R* gene. The *IGF1R* gene is one of the known target genes of androgen receptor activation [6]. In mathematics, a function from a set X to a set Y is an assignment of an element of Y to each element of X. Hence, the process of transcription can be written in the following form:

$$f(x) = y \qquad f(A) = I \qquad A = \{\text{androgen}, \text{androgen receptor}\}$$

Where f is the function of RNA polymerase II, and A denotes the collection of androgen and its receptor. Next, the CRISPR-Cas9 enzyme [7,8] is used to copy the enhancer of the *IGF1R* gene into the promoter-proximal region of HIV double-stranded DNA. Then, T cells are infected with the modified virus, so the form can be rewritten as follows:

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

Finally, androgen and its receptor are 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 to check the accuracy of mathematical formulas:

```
H = {'gag','pol','env'}
I = {'ENSE00003838363','ENSE00001316091'}
A = {'androgen','androgen receptor'}
```

```
def f(x):
    if x == A:
        return I | H
```

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

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

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

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

The collection of elements returned by the method includes the HIV set, which doesn't mean that the two sets are equal. In mathematics, 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 because there are 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 they also claim that the effectiveness rate is only 42% [14,15,16]. Most importantly, the novel small-molecule IAP inhibitor AZD5582 was used for the treatment of cancer. It was reported to cause cIAP1 degradation, inducing apoptosis in the MDA-MB-231 breast cancer cell line at subnanomolar concentrations in vitro [17].

The latency-reversing drugs were involved in the process of transcription of cancer genes, and the virus genome they reawaken happened to contain the NF-κB binding sites, so the drugs were not directly reawakened dormant retrovirus infection.

This method may be difficult to understand, so the author gives another example.

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

From this example, it feels like those studies are trying to use Claire's wristbands (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.

Besides AZD5582, many studies claim that latency-reversing drugs can be used to reawaken sleeping HIV, including ciapavir [19], bryostatin-1 [20], disulfiram [21], ingenol-B [22], and prostratin [23]. These latency-reversing drugs were also used for the treatment of cancer: disulfiram has inhibited prostate cancer cell growth [24], bryostatin-1 has demonstrated potent antitumor activity in vitro and in vivo in human tumor xenografts [25], semisynthetic ingenol compounds demonstrated potent antitumor activity on all cancer cell

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lines evaluated [26], and prostratin has 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, activating genes related to cancer may pose unknown risks. The author hence believes that instead of using Claire, Clara and Clark's wristbands to find Adam indirectly, it is better to use Adam's wristbands to find Adam directly. In other words, it is more reliable to use viral proteins to reawaken retroviruses.

Viral RNA is specifically packaged into virions, not *IGF1R* or cancer RNA, so the virus can accurately identify viral RNA. Therefore, the viral proteins carry information that can identify the viral RNA, just as androgen receptor activates the *IGF1R* gene.

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]. Therefore, the author proposed a model of protein reawakening includes that uncharged tRNA serves as the physical link between the promoter and the protein receptors, which are recruited by RNA polymerase II. An example is illustrated in Fig. 1 [30,31,32].

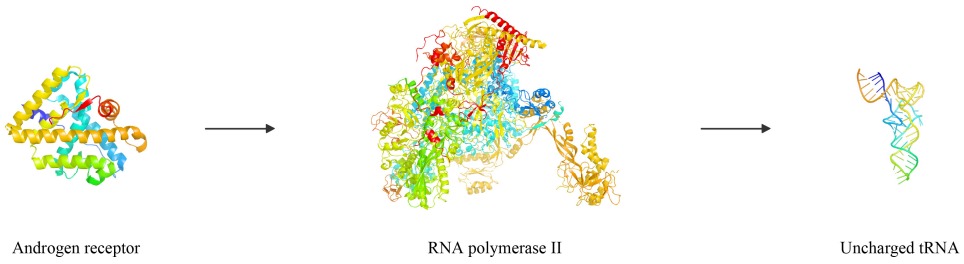

Figure 1. Protein reawakens

The author hence believes that a certain viral protein has a similar function to NF-κB or androgen receptor, which can be used to identify the viral RNA directly. To determine which viral protein matched the primer binding site, a Python program was written to match all the proteins with their own gene sequences and display them graphically.

3. Results

DNA is always synthesized in the 5'-to-3' direction, but reverse transcriptase synthesizes negative-strand DNA in the 3'-to-5' direction [33,34]. The viral proteins recruited by RNA polymerase II may also be rotated 180 degrees, which creates 4 ways for uncharged tRNA to match the primer binding site. 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 rotated 180 degrees or was synthesized in the 3'-to-5' direction when both values were negative.

Having 2 amino acid sequences of the matching points leads to many possibilities, such that it is impossible

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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 with different patterns and colors, and their sequences around the primer binding site are matched with their own proteins, as shown in Fig. 2.

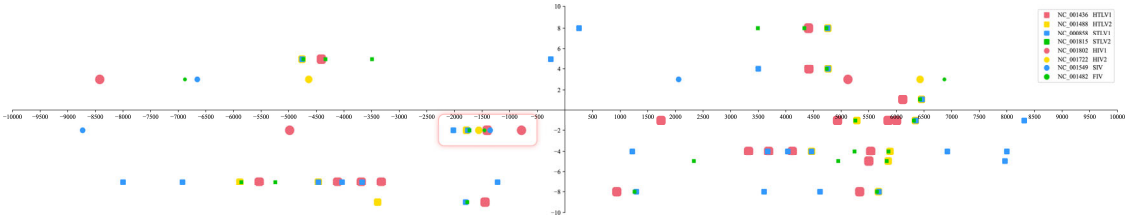

Figure 2. Coordinates of matched points

Fig. 2 shows that inside the red box, the coordinates of 8 different viruses appear at the same time, and they are extremely close, which means they represent the same protein. In other locations, there was either only Deltaretrovirus or only Lentivirus, 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 HTLV2 NC_001488 genome is approximately nt 766 to 783, and that of the HIV1 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 [35,36,37,38,39,40,41,42], as shown in Fig. 3.

NC_001436 HTLV1	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 HTLV2	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 STLV1	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 STLV2	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 3. Deltaretrovirus and Lentivirus

It can be determined that their gag proteins match with the same primer binding site, even though the viruses are highly different.

To determine whether it is a coincidence, the author analyzes its probability. Since 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 HTLV1 and HIV1 genome as templates and used Pairwise Sequence Alignment to compare the genetic similarity of viruses. The similarity of HTLV2 NC_001488, STLV1 NC_000858 and STLV2 NC_001815 to HTLV1 NC_001436 genomes are 59.2%, 89.3% and 61.0%, respectively. The similarity of HIV2 NC_001722, SIV NC_001549 and FIV NC_001482 to HIV1 NC_001802 genomes are 51.1%, 54.1% and 49.1%, respectively. The similarity of viruses S can be written as:

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

The average probabilities of 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, 3/63, respectively. So, the average probabilities of 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

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mutations increases with the diversity of 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, so it can be determined that gag proteins can match the primer binding site.

4. Discussion

Since the primer binding sites of different viruses are extremely stable, other proteins may also match in adjacent locations, but there is no sufficient information to determine this now. What can certainly be concluded is that thus far, drugs fail to reawaken dormant HIV infection, and it is more reliable to use viral proteins to reawaken retrovirus, just as androgen receptor activates the IGF1R gene.

5. Conclusions

The latency-reversing drugs were involved in the process of transcription of cancer genes, and the virus genome they reawaken were just happened to contain the same NF-κB binding sites, so the drugs were not directly reawakened dormant retrovirus infection. The amino acid sequences ARG of gag proteins of HTLV1, HTLV2, STLV1 and STLV2 match with their primer binding site GGGGGCTCG in the 3'-to-5' direction, and the amino acid sequences SPR of gag proteins of HIV1, HIV2, SIV and FIV match with their primer binding site GGCGCCCGA in the 3'-to-5' direction. The gag proteins are promising for reawakening dormant retrovirus infection.

List of abbreviations

NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
IGF1R: Insulin-like growth factor 1 receptor
CRISPR: Clustered regularly interspaced short palindromic repeats
IAP: Inhibitors 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

Ethics approval and consent to participate

Not applicable.

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Consent to publish

The author gives the 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

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

Competing interests

There are no conflicts of interest.

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Contributions

S.C. wrote the manuscript.

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