Journal of
Antivirals & Antiretrovirals

Wayengera, J Antivir Antiretrovir 2015, 7:2
DOI: 10.4172/jaa.1000121

Review Article Open Access

Genomic-Regulation of Active Retroviral Elements as a Model for HIV Cure

Misaki Wayengera*

Unit of Genetics & Genomics, Depts of Pathology & Medical Microbiology, Makerere University College of Health Sciences P O Box 7072 Kampala, Uganda

***Corresponding author:** Misaki Wayengera, Unit of Genetics & Genomics, Depts of Pathology & Medical Microbiology, Makerere University College of Health Sciences P O Box 7072 Kampala, Uganda, Tel: +256782450610; E-mail: wmisaki@yahoo.com

Received date: February 26, 2015, **Accepted date:** April 27, 2015, **Published date:** May 04, 2015

Abstract

Human immunodeficiency virus type 1 (HIV-1) is a recently emerged retrovirus that causes acquired immunodeficiency syndrome (AIDS). Whereas the human genome is comprised in large by remnants of retroviral genomes representing evolutionary-foot-prints of ancient retroviral activity, humans and non-human primates (NHP) alike have evolved a constellation of innate mechanisms to either silence or disable those still active genomic retroviral (L1) elements. In light of the global pandemic wrought by human infection with a retroviral member-human immunodeficiency virus (HIV) and the challenges faced by attempts to eradicate integrated latent HIV provirus, we argue here that it is evidenced to postulate that those natural mechanisms for regulating active retroviral elements in man can artificially be harnessed to functionally cure HIV.

Keywords: HIV; AIDS; HIV cure; Models; Genome therapies; L1 retroelements

Introduction

Infection with human immunodeficiency virus type I (HIV-1) is the cause of acquired immunodeficiency syndrome (AIDS) [1]. The first cases of AIDS were reported in the united states (U.S) by the centre for disease control (CDC) Atlanta in 1980 [1,2]. During the past 34 years, about 64 million people world-over have been infected with HIV-1, 30 millions of whom have lost their lives to AIDS-related illnesses [1,2]. These figures make HIV/AIDS one of humanity's worst pandemics. The advent of HIV/AIDS treatment with highly active antiretroviral therapy (HAART) in the 1990s transformed a previously fatal illness into the chronic infection [3-5]. HAART is able to suppress HIV-1 replication without completely eliminating virus. HAART does NOT however confer cure [3-5]. PLWHA on HAART must therefore stay on treatment indefinitely, since ceasation of HAART results into a rebound of viremia. This scenario presents toxicity, resistance and compliance concerns for the individual PLWHA. On a wider context, maintaining the growing number of PLWHA in dare need of HAART (over 5 million now) carries national, regional and global socio-economic cost-implications.

Research towards a preventive HIV-1 has remained unfruitful [6,7]. Following the isolation and genetic characterization of HIV in 1983, many thought a vaccine was near in the offing. Exactly 34 years after, all trialled vaccine prototypes have yielded disappointing results [8]. The first vaccine efforts were directed at inducing antibodies against the gp-120 until it was discovered that HIV-1 had various clades, and all were inherently highly mutable [6-8]. This gave rise to the idea of using multivariate vaccines. Disappointing results accrued from the trial of a prototype multivariate vaccine-VaxGen in 2003, also served to give birth to new postulates including the observation that, besides antibody responses, T cell immune responses were a necessary added requirement to arrest HIV [8,9]. Similar negative results were however reported with an adenovirus vector based T cell vaccine in the STEP and HVTN 505 trials in Sept 2007 [9,10]. The RV 144 trial in Thailand beginning in 2003 was the first-ever vaccine to report positive but moderate effectiveness to protect against primary infection with HIV-1 in 2009 [11]. Results of this trial give the first supporting evidence of any vaccine being effective in lowering the risk of contracting HIV [11]. The initial report shows that the rate of HIV infection among volunteers who received the experimental vaccine was 31% lower than the rate of HIV infection in volunteers who received the placebo [11]. A subsequent immune-correlates study generated the hypotheses that V1V2 antibodies may have contributed to protection against HIV-1 infection, whereas high levels of Env-specific IgA antibodies may have mitigated the effects of protective antibodies. Vaccines that are designed to induce higher levels of V1V2 antibodies and lower levels of Env-specific IgA antibodies than are induced by the RV144 vaccine may have improved efficacy against HIV-1 infection [12]. This design of vaccines has, however, not yet been perfected to-date. Additional evidence that a conventional vaccine may be possible, derives from work with broadly neutralizing antibodies (bNAbs), and fact that certain individuals remain asymptomatic for decades after HIV infection [13-16].

Overall, designs of previously successful vaccines (as is the case of antimicrobial agents) can be evidenced to possess existing effective natural models of the same. However, the classical vaccination approaches that have been successful in the control of various viral diseases by priming the adaptive immunity to recognize the viral envelope proteins have failed in the case of HIV-1. Some have stated that an HIV vaccine may not be possible without significant theoretical advances [6-10,17]. There are a number of factors that cause development of an HIV vaccine to differ from the development of other classic vaccines [6-10,17,18]:

- Classic vaccines mimic natural immunity against reinfection generally seen in individuals recovered from infection; there are almost no recovered AIDS patients.
- Most vaccines protect against disease, not against infection; HIV infection may remain latent for long periods before causing AIDS.

- Most effective vaccines are whole-killed or live-attenuated organisms; killed HIV-1 does not retain antigenicity and the use of a live retrovirus vaccine raises safety issues.
- Most vaccines protect against infections that are infrequently encountered; HIV may be encountered daily by individuals at high risk.
- Most vaccines protect against infections through mucosal surfaces of the respiratory or gastrointestinal tract; the great majority of HIV infection is through the genital tract.

On a similar context, bacteria endowed with the restriction modification (RM) system are able to restrict tropism by bacteriophages (hencefore, simply denoted phages). This by production of restriction enzymes (REases). REases recognize palindromic nucleotide base-pair sequences within the xenogenic phage-DNA before its integrated in the bacteria and repetitively cleave therein until the phage DNA is disabled or inactivated. RM systems therefore represent an innate-defense of bacteria against tropism by phages [19,20]. Our group has previously proposed that the mechanism of action of this bacteria innate-defense against phages, represents a model for developing genetic and or genomic vaccines against human infesting. We [19,20], over 10 years ago, conceived the idea that the natural antiviral innate defense system whereby bacteria inactivate bacteriophages through target mutagenesis, offered a model for an antiviral gene therapy against HIV. By operating a highly specialized nucleic acid-enzymatic system, the Restriction Modification (R-M) system, bacteria are able to primarily restrict tropism by xenogenic phage-DNAs. This, through the production of restriction enzymes (REases) that recognize palindromic sequences within the infecting phage DNA and repetitively cleave therein till the phage is rendered defective. In order to increase the efficacy of this system on the small phage-genomes, bacteria have evolved REases that only recognize short (5-12) palindromic sequences. This adaptation carries with it a major disadvantage in that it renders the bacteria genome equally susceptible to REase cleavage. The bacteria genome is however protected by epigenetic methylation of homologous target sequences within itself. Lack of a specialized host-genome protective mechanism such as that exhibited by bacteria through the 'methylation component of the R-M system' hindered the in-vivo application of our earlier approaches using bacteria derived proviral-HIV-targeting REases as gene therapeutics [21].

Our group previously advanced its preclinical prototypes from bacteria-derived REases, to the artificial REases generated by linking a FoKI DNA cleavage domain, to an array of DNA binding protein-motif cordinated by a zinc ion (Zinc Finger Nucleases, ZFN) [22]. Because of the longer stretch of DNA recognized by ZFN relative to bacteria-derived REases that conveys better safety in-vivo, ZFN have become a powerful tool for host-genome editing [22]. This has resurrected the idea that human infecting viruses can effectively be targeted in-vivo with little or no toxicity to the host genome. Indeed, several groups have recently successfully used ZFN-related natural (homing endonucleases) and artificial enzymes (ZFN, TALEN, CRISPER/Cas) [discussed later] derived from bacteria precursors to either silence or 'excise" HIV-1 among cells.

It is a well-known fact that the human genome is comprised in large part of the remnants of ancient retroviral infections. These remnants do not, however, cause disease as they in one way or other effectively have been rendered functionally defective [23-32]. Therefore, the only prerequisite for an effective prophylaxis (or genetic cure among those already latently infected) against HIV-1 infection is that the incoming (infecting) or latent provirus be mutated sufficiently to prevent production of progeny virus and continued pathogenesis. Indeed, the mechanisms for silencing active L1 genomic retro-element by the three prime repair exonuclease 1 (TREX1) [31] and excision repair cross complementing 1 (ERCC) [32] appear to mimic these very steps.

About half of our DNA bears homology to known classes of repeats, which may be classified as non-long terminal repeats (non-LTR, 17.88%), long terminal repeats (LTR, 9.24%), DNA repeats (3.42%), simple repeats (0.91%), other repeats (0.83%), and satellite repeats (0.43%). These repetitive elements may further be subdivided into two principal types, either **(a)** tandem (each repeat unit is adjacent to others) or **(b)** interspersed [23]. While tandem repeats are formed in situ by replication or recombination events, those interspersed repeat sequences are derived from transposable elements. Transposable elements may be of either DNA or RNA origin, a delineation largely based on the mechanism of their spread. DNA transposons use a "cut-and-paste" mechanism, while RNA transposons exploit a "copy-and-paste" mode of moving in genomes. RNA transposons (retrotransposons, retroposons, or retroelements) use an RNA intermediate (lariate) to reproduce and or spread. This behavior makes them resemble retroviruses in large, and evolutionary biologists have used this hinch to uncover the fact that the human genome comprise in large remnants of retroviral genomes. While most of these remnants represent evolutionary-foot-prints of ancient retroviral activity, the only active mobile DNAs in modern-day humans are the autonomous L1 retrotransposon and non-protein-coding (nonautonomous) sequences its machinery mobilizes [23,24].

The life cycle of L1 entails three steps that, in the eyes of the seasoned virologists can be noted to comprise two-intermediate steps of retroviral life cycle at the point of genome integration and exit. The first step is transcription of an L1 genomic-DNA locus into RNA, which is mediated by RNA polymerase II from an internal L1 or antisense L1 promoter. Transferred to the nucleus. The second step is termed target-primed reverse transcription (TPRT). In the course of TPRT, ORF2p cleaves the target DNA (often at a 59-TTTTAA-39 consensus sequence) and uses the 39 hydroxyl group to prime the reverse transcription reaction [23].

Innate Regulation of Active L1 Retroviral Elements

Left unchecked, it is clear that active L1 retro-elements within the genome carry with them the potential for denovo mutagenesis. Whereas some of these de-novo mutations may have beneficial adaptive effects, others are known to be carcinogenic [23]. It therefore becomes relevant that the mutagenic effects of active L1 retroviral elements on the host are minimized or regulated. L1 is regulated by distinct pathways in different cell contexts.

Silencing by hyper-methylation and de-acetylation

- In the male germline, L1 is inhibited via an elaborate system involving Piwi-interacting RNAs (piRNAs) that ultimately methylates genomic L1 sequences. This depends on methylation regulator DNMT3L [23] and PIWIL4 (also known as MIWI2) [25], as well as PIWI proteins involved in piRNA production.
- In embryonic stem cells, inherited L1 methylation is maintained by DNA methyltransferases DNMT1, DNMT3A, and DNMT3B [26].

- In embryonal carcinoma cell lines, newly retrotransposed L1 sequences are silenced by histone alterations, including deacetylation of H4 and dimethylation of H3K9 [27].

Therefore, silencing of proviral HIV-1 integral within latently infected cells may theoretically be achieved through hypermethylation and de-acetylation.

Silencing by other innate-mechanisms

In addition to the epigenetic silencing mentioned above, other innate-defence proteins have been implicated in L1 repression in various somatic tissues. These include:

- Methyl CpG binding protein 2 (MECP2): MeCP2 protein-whose difficiency results into the Rett Syndrome, is essential for normal nerve function by turning off (by repression or silencing) several other genes. This prevents these genes from making proteins when they are not needed). The human proteins MECP2 (this protein), MBD1, MBD2, MBD3 and MBD4 comprise a family of nuclear proteins related by the presence in each of a methyl-CpG binding domain (MBD). Each of these proteins, with the exception of MBD3, is capable of binding specifically to methylated DNA. MECP2, MBD1 and MBD2 can also repress transcription from methylated gene promoters [28].
- Lymphoid-specific helicase (HELLS). Lymphoid-specific helicase is an enzyme that in humans is encoded by the HELLS gene. Helicases function in processes involving DNA strand separation, including replication, repair, recombination, and transcription. HELLS is thought to be involved with cellular proliferation and may play a role in leukemogenesis [29-33].
- Retinoblastoma protein-containing complex (RB1): Retinoblastoma protein (abbreviated pRb, RB or RB1) is a tumor suppressor protein that is dysfunctional in several major cancers. A major function of pRb is to prevent excessive cell growth by inhibiting cell cycle progression until a cell is ready to divide. It is also a recruiter of several chromatin remodelling enzymes such as methylases and acetylases [32-37].
- Three prime repair exonuclease 1 (TREX1): Three prime repair exonuclease 1 is an enzyme that in humans is encoded by the TREX1 gene.This gene encodes the major 3'->5' DNA exonuclease in human cells. The protein is a non-processive exonuclease that may serve a proofreading function for a human DNA polymerase. It is also a component of the SET complex, and acts to rapidly degrade 3' ends of nicked DNA during granzyme A-mediated cell death. Mutations in this gene result in Aicardi-Goutieres sydrome, chilblain lupus, RVCL (retinal vasculopathy with cerebral leukodystrophy), and Cree encephalitis [31].
- Excision repair cross complementing 1 (ERCC): Excision repair cross-complementing (ERCC) is a set of proteins which are involved in DNA repair.In humans, ERCC proteins are transcribed from the ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, and ERCC8 genes. ERCC 6 and 8 are associated with Cockayne syndrome [32,38].
- Apolipoprotein B mRNA editing enzyme, catalytic proteins APOBEC3A, APOBEC3B, and APOBEC1 [32,39].

Much as some of these innate mechanisms function by mediating hypermethylation and de-acetylation; an alternative is for proviral HIV-1 resident in latently infected cells, to be targeted for either hypermutation or excision. Eriksson S, et al., [40] recently observed that some of the resting memory $CD4^+$ T cells already contain defective viral genomes, which do not express viral genes in response to latency-reversing agents. Prior to this, Sanchez G et al. [41] had demonstrated an accumulation of defective viral genomes in peripheral blood monocyte cells (PBMCs) of HIV-1 infected individuals in the absence of latency-reversing agents. Keiffer TL et al., [42] also demonstrated G-to-A hypermutation in protease and reverse transcriptase (RT) regions of HIV-1 residing in resting $CD4^+$ T cells *in vivo*. These defective viral genomes were recently found to be functionally inactivated, since the same could not be reactivated by latency reversing agents [35].

Harnessing L1 Retro-Element Innate-Regulators for HIV Cure Research

In as much as the above innate-regulators seem to effectively prevent L1 expression [3], their translational significance is yet to be explored in the field of HIV and other retrovirus therapeutic research. In light of the global pandemic wrought by human infection with the retroviral member-human immunodeficiency virus type one (HIV-1) and the challenges faced by attempts to eradicate integrated latent HIV provirus, it is evidenced to postulate that those natural mechanisms for regulating active retroviral elements in man can artificially be harnessed to functionally cure HIV. The advent of HIV/AIDS treatment with highly active antiretroviral therapy (HAART) in the 1990s transformed a previously fatal illness into a chronic infection [3-5]. Specifically, HAART enables a reduction of plasma HIV-1 RNA levels in treated patients to below the detection limit of clinical assays (1-50 copies of HIV-1 RNA/ml) [3-5]. HAART-induced suppression of viremia falsely led to the initial hope that HIV may be eradicated within 2-3 years of treatment [3]. This has, however, not been achieved as a latent form of HIV-1 infection persists *in vivo* [4,5]. Consequently, HIV/AIDS treatment with HAART demands a life-long commitment of which the long term impact on the patient remains a question. Notably, a small fraction of resting memory $CD4^+$ T cells carry non-replicating, integrated viral genomes that cannot be targeted and eliminated by HAART. While these cells do not produce virus particles during the resting state, they can give rise to replication-competent virus following cellular activation [5]. More still, in as much as these latently infected resting memory $CD4^+$ T cells are rare (1 in a million); they are stable even in patients on prolonged HAART [5,43-50]. Interruption of HAART leads to a rebound in viremia [51,52], typically from an archival variant [53]. The reservoir of latent HIV is widely recognized as the major barrier to HIV-1 eradication by HAART [54].

Existing strategies to eradicate latent proviral HIV-1

Strategies aimed at depleting the latent reservoir have been devised, and many are now in advanced preclinical testing or early clinical trials [55]. Depleting the latent reservior may typically be achieved in two ways, either by **(i)** reactivating latent virus and thereby accelerating its clearance, or **(ii)** targeting latently infected resting memory CD4+ve T cells for destruction. Among the approaches for the reactivation of latent HIV-1 is T cell activating cytokines [56-61], T cell receptor and T cell receptor signaling pathway agonists [62-64], histone deacetylase inhibitors [65-67], DNA methylase inhibitors [68,69], and compounds like 5-hydroxynaphthalene-1,4-dione and disulfiram [70]. Some of these approaches like the histone-deacetylase inhibitors and DNA methlyase inhibitors awaken latent virus through countering the natural L1 silencing by hypermethylation and de-acetylation pathways discussed above [23-38]. It nonetheless remains

unclear what their effectiveness towards the ultimate goal of HIV cure will be. Groups elsewhere have proposed targeted-destruction of latently infected T cells as a strategy to deplete the latent reservoir. Even if this became clinically possible to some degree of specificity, however, it is unclear what effects the widespread T-cell destruction would have on the ability of the infected individual to reconstitute an effective immune response. Several groups have recently moved to research strategies that are similar to or analogous to the second mechanism for silencing L1 by other innate-mechanisms above i.e hypermutation or excision. For example, use of proviral DNA cleaving enzymes like the CRE-recombinase [71], homing endonucleases (HE) [72], zinc finger nucleases (ZFN) [22,73], CRISPER/Cas and RNA-directed gene editing [74] seems to mimic natural activity of the the three prime repair exonuclease 1 (TREX1) [31], and excision repair cross complementing 1 (ERCC) [32]. Nevertheless, majority of the mechanisms for L1 regulation are yet to be explored.

Gaps and insights into the future of applying L1 regulators for HIV-1 cure research

In as much as some of the mechanisms of action by the active-L1 genomic regulators discussed above have been explored towards HIV cure research, many more remain unexplored. With the exception of the manner of action by CRE-recombiniase [71], homing endonucleases (HE) [72], and zinc finger nucleases (ZFN), TALEN, CRISPER/Cas or RNA-directed genome editing with the intent to mutate and disable proviral DNA[22,73-75] that may mimic the three prime repair exonuclease 1(TREX1) [31] and excision repair cross-complement (ERCC)[32]; most existing anti-latency agents-aiming to awaken latent HIV for HAART targeting, indeed act in opposition to the natural L1 genomic regulators. This may be justified by the fact that the goal of current HIV cure science has been to exorcise latent HIV by awakening provirus to render it susceptible to HAART. Nonetheless, it appears that this goal is misdirected, and our focus should be on repressing, not awakening, HIV. Specifically, without eliminating or disabling the proviral HIV template used for the synthesis of new HIV virions which is integrated within the genomes of latently infected cells, the cycle of progeny HIV viron production cannot be stopped by a mere combination of latency reversing-agents and HAART alone, no matter how many times the latter combination is co-applied in absence of target reservior control, or destruction (see potential dangers associated with this underlined above). Thats because, integrated virus cannot miraculously jump-out or disappear from its genomic confines since the same only acts as a template during viral and at no time, ever leaves. The current strategies that awaken and or stimulate latent provirus to replicate, only serve to stimulate and or enhance its utility as a template for new virions. Thats why we are calling for a moratorium to accelerate research to harness those models for proviral HIV DNA silencing or disabling towards cure research (Table 1). Moreover, that a constellation of mechanisms rather than just a single one have been evolved by the human genome to contain the L1 elements, underlines the fact that a combination of approaches rather than a single 'magic bullet' may be required to disable or silence and functionally cure HIV-1. For example, whereas humans have at the genome level evolved a constellation of antiretroviral genes with potential to target HIV [76-79], their presence is insufficient for controlling smart retroviruses like HIV-1 which have evolved counter-evasive genes against the same. Antiretroviral genes are part of the innate defense system and in retroviral literature-are denoted restriction factors (RFs). Some of the well-characterized RFs today, include APOBEC, TRIMS, Tetherin/BST-2, SAMDH-1 and MHC-I. Although the current repertoire of RFs in human was possibly historically acquired under the evolutionary pressure of other lentiviruses other than HIV, HIV has itself also evolved various mechanisms to counter the effects of these antiviral genes on its subsequent host invasion and tropism. For instance, using the nef genes, HIV is known to down-regulate the class I MCH molecules on infected cells, thereby masking its presence and thereby avoiding immune surveillance and destruction [75-77]. The viral infectivity factor (vif) gene, on the hand, is used by HIV to down-regulate APOBEC3, and thereby avoid intracellular destruction [77,78]. Using the vpr gene (vpu/x is SIV and HIV-2), HIV-1 similarly counters the effects of SAMDH-1 [79]. Thus, smarter-ways to overcome these HIV's evasive mechanisms against RFs may open a new way to HIV-cure research and development.

S. No	L1 genomic-regulators	Existing anti-latency strategy acting here[¥]	Potential HIV cure approach
1	Piwi-interacting RNAs (piRNAs) methylation of L1 sequences	siRNA, DNA methylase inhibitors ,	HIV-1 targeted piRNA
	L1 methylation is maintainance by DNMT1, DNMT3A, and DNMT3B.	DNA methylase inhibitors,	HIV-1 targeted DNMT1, DNMT3A, and DNMT3B
	Methyl CpG binding protein 2 (MECP2)	DNA methylase inhibitors,	HIV-1 targeted MECP2
2	L1 silencing by histone alterations, including deacetylation of H4 and dimethylation of H3K9.	histone deacetylase inhibitors	Deacetylation of H4 and dimethylation of H3K9
3	Lymphoid-specific helicase (HELLS).	-	HIV-1 targeted HELLS
4	Retinoblastoma protein-containing complex (RB1)	-	HIV-1 targeted RB1
5	Three prime repair exonuclease 1 (TREX1) and excision repair cross complementing 1 (ERCC).	CRE-recombinase, homing endonucleases (HEs), and zinc finger nucleases (ZFN)	HIV-1 targeted TREX1 and ERCC
			HIV directed CRE, HEs, ZFN, TALENs & CRISPER/Cas

6	Apolipoprotein B mRNA editing enzyme, catalytic proteins APOBEC3A, APOBEC3B, and APOBEC1.	-	HIV-1 directed APOBEC3A, APOBEC3B, and APOBEC1
			Anti/Counter-HIV-1 evasion of RFs by NEF, VPU, VPR
¥Note that except for the action of CRE-recombinase, homing endonucleases (HE), TALENS and CRISPER/Cas9 and zinc finger nucleases (ZFN); most existing anti-latency agents-aiming to awaken latent HIV for HAART targeting, indeed act in opposition to the natural L1 genomic regulators. Wherever dashes are, there were no existing anti-latency models based on these pathways found.			

Table 1: Active L1 genomic-regulators and their corresponding anti-latency strategies.

Conclusion

In conclusion, given the challenges faced in eradicating integrated latent HIV provirus, we argue here that there is enough evidence to drive the postulate that those natural mechanisms for regulating active retroviral elements in man could collectively be artificially harnessed to functionally cure HIV.

Conflict of Interest

MW's laboratory's major research focus is on 'target-mutagenesis' of human infecting viral DNAs, as a gene-therapeutic and or genomic vaccine model. MW has received research funding towards target mutagenesis of proviral HIV-1 DNA by ZFN and CRISPER/Cas from Glasko-Smith Kline through a competitive grants award process, the Trust in Science-Africa Initiative. MW is a CSO at Restrizymes Biotherapeutics (U) LTD, a company interested in R & D of restriziction enzyme (i.e ZFN/CRISPER/Cas/HE/TALEN) therapeutics.MW declares no financial conflicts of interest.

Acknowledgement

I thank members of my research group, for support on the on-going experimental work. Funding: MW is funded by GlaskoSmithKline (GSK) Trust in Science Africa grant # COL100034581.

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