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Histone deacetylase inhibitors and HIV latency

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Abstract

Purpose of review—Interest has re-emerged in approaches to eradicate HIV infection. A series of modifications of nucleosomal histones within chromatin are a key mechanism of HIV gene regulation that alters the recruitment of transcription factors to viral DNA. The balance of these histone modifications in the vicinity of the HIV LTR plays an important role in the maintenance of proviral quiescence in rare latently infected cells, and presents a target for therapies aimed at purging this reservoir of persistent HIV infection.

Recent findings—Altering the balance of acetylase and deacetylase activity within CD4+ lymphocytes using histone deacetylase (HDAC) inhibitors, or other epigenetic drugs, has recently emerged as a promising approach to purge the reservoir of persistent infection. Multiple molecular mechanisms appear to underlie the establishment and maintenance of persistent, latent HIV infection, most frequently in the resting central memory CD4+ T cell. HDAC inhibitors perturb this balance, induce expression of integrated provirus, and may allow attack of this primary form of persistent HIV infection.

Summary—While HDAC inhibitors are a promising approach, a better understanding of relevant mechanisms of latency in vivo, and better tools to translate this knowledge into therapies are needed.

Keywords

HIV; latency; resting CD4+ T cells; histone deacetylase inhibitor

Introduction

The viral long terminal repeat (LTR) promoter of the human immunodeficiency virus type 1 (HIV-1) is a key battlefield on which the host–virus interaction plays out, as HIV co-opts numerous cellular factors to control the rate of viral transcript production. In addition to cellular factors that bind LTR DNA sequences, acting as classical *cis*regulators, the viral Tat *trans*-activator binds to TAR, the leader mRNA sequence that serves as a unique target in the regulation of LTR transcription. In most settings, HIV rapidly appropriates the resources of the activated CD4+ T cell to transform it into a factory for virus production.

However, the down-regulation of expression of the genome of HIV-1 plays a pivotal role in the rare latent stage of the life cycle of this pathogenic retrovirus. In addition to factors that

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bind DNA (or in the case of the LTR, TAR RNA) and regulate transcription complex assembly and processivity, transcription is regulated by alterations of nucleosomes within chromatin. *In vitro* studies have shown that higher order structure imposed by nucleosomes can reduce the accessibility of specific activators, general transcription factors, and RNA polymerase to both enhancer and promoter regions, inhibiting transcription initiation (1).

There has been a growing understanding of inhibition of eukaryotic transcription at the molecular level. Diverse mechanisms of action have been proposed for transcriptional repressors, and some transcription factors were shown to affect chromatin structure [2]. Chromatin structure plays an important role in regulating gene expression. Multiple signaling pathways result in enzymatic covalent modifications (acetylation, phosphorylation, methylation) of specific amino acids in histone tail domains. The “histone code” hypothesis holds that combinations of distinct modifications occurring at particular sites on the histone tail direct which proteins are capable of interacting with histone-DNA complexes and determine gene activity [3].

Already more than 50 enzymes are known that selectively modify the histone tail thus providing the means to make a combinatorial histone code. One canonical example is that of the histone acetylases, acting to allow the transcriptional machinery access to the DNA template, compete with histone deacetylases that blunt transcription by reducing accessibility of DNA templates. [4]. In parallel, enzyme families that modify histones act on host transcription factors as well, inducing regulatory modifications which alter their functional properties, cellular localization, or cellular half-lives. As the understanding of the complex networks that specifically regulate individual host genes increases, so too will the opportunities to modulate HIV gene expression within the host cell. However, the clinical application of this understanding will be challenging, as undue alteration of key controls of host gene expression, and therefore intolerable effects on host cell function, must be avoided.

Once integrated into the host cell genome, the HIV LTR requires remodeling to allow expression, and histone acetylation to respond to NF-κB activation [5]. Local chromatin effects have long been thought to contribute to the durable suppression of HIV proviral expression, and latently infected cells recovered from a T lymphocyte cell line infected in vitro were recently found to contain HIV integrated in or close to alphoid repeat elements in heterochromatin [6].

Compounds that alter protein acetylation were recognized to induce expression of HIV more than 20 years ago [7]. A few years thereafter, the effects of sodium butyrate were described in a cellular model of proviral latency, and were hypothesized to be primarily linked to the induction of histone acetylation, overcoming an unknown block to HIV expression [8].

Evidence for a role of HDAC in the maintenance of HIV latency

In laboratory models of chronic HIV infection, histone acetylation and increased accessibility of chromatin about the LTR has been associated with transcriptional activation [9-11]. Therefore, deacetylation of histones could establish a chromatin organization that could maintain or establish quiescence of integrated viral DNA. The first example of a host regulatory network that exerted this effect of the HIV LTR was the demonstration of the cooperative recruitment of HDAC1 to the HIV LTR by the host transcription factors YY1 and LSF [12].

YY1 is transcription factor widely distributed in human cells that recognizes a host of viral and cellular promoters, and can activate or repress transcription. Coull et al. demonstrated that YY1 binds the HIV-1 LTR, and represses LTR expression and viral production. YY1

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recognizes this sequence only via interaction with LSF, through which YY1 exerted its repressive effects on viral expression via the recruitment of HDAC1.

Williams [13] later reported that the p50 homodimer of NF-κB also recruits HDAC1 to the LTR. Imai [14] suggested that HDAC1 could also be recruited to the LTR by AP-4. Shortly thereafter a fourth complex containing c-Myc and Sp1 was shown to be capable of recruiting HDAC1 to the LTR [15]. Finally, Tyagi and Karn [16] have identified CBF-1 as a *fifth* factor capable of recruiting HDAC1 to the LTR. Additionally, HDAC2 has recently been found to occupy the LTR in studies performed in a human microglial cell line [17]. This finding echoes the ability of multiple coactivator complexes to recruit histone acetylases (HATs) to promoters, including the HIV LTR [18]. One might speculate that this strikingly redundant use of a mechanism of transcriptional regulation might suggest that these mechanisms were selected by evolutionary pressure to evade the immune system. This is possible but, in our view, unlikely. HIV possesses many effective mechanisms to thwart the immune response, and proviral latency only becomes relevant in the setting of antiviral therapy. It seems more likely that as the transcription factor complexes and mechanisms involved are host-derived, that the evolution of host genetic control has led to the use of multifunctional transcription complexes that can act to recruit repressors or activators in different settings. As the virus is forced to parasitize the host's transcriptional machinery, its use of host activators is inevitably linked to counter-regulatory mechanisms. The virus has evolved the Tat activator to cpo-opt the host's gene expression machinery, but in rare circumstances the viral transactivator is unable to overcome host repressive mechanisms.

More recently our laboratory has demonstrated that the class I HDACs 1, 2, and 3 may be particularly important to maintaining HIV latency. These HDACs associate with the HIV promoter in cell line models of latency, are expressed in the nuclei of resting CD4+ T cells, and selective chemical inhibition of HDACs 1, 2, and 3 can efficiently induce *ex vivo* viral outgrowth from the resting CD4+ T cells of HIV infected individuals. In contrast, inhibitors selective for certain members of the class II HDACs, including HDACs 4, 5, 7, and 9, do not induce outgrowth of latent HIV [19,20].

Targeting HDAC as a strategies to purge latent provirus

Although there appear to be multiple molecular mechanisms that contribute to HIV latency and persistent infection [21], due to the therapeutic tools currently at hand, we will focus our discussion on the attempts in the near term to bring epigenetic therapies to bear on HIV latency. The existence of multiple mechanisms that recruit HDACs to the proviral promoter may be of high therapeutic significance. HDACs are recruited to the highly conserved initiator region of the HIV promoter by several distinct complexes as discussed above, utilizing factors ubiquitous in cell types infected by HIV-1 and required for basal and activated expression. Potent inhibitors specific for HDACs relevant to HIV LTR regulation may be effective therapeutics to disrupt latent HIV infection, and might avoid toxicities that could accompany global HDAC inhibition. Further, HDAC inhibition does not increase the ability of HIV to spread, as it does not increase the susceptibility of peripheral blood mononuclear cells to HIV infection [22]. It has recently been noted that high-level HDAC inhibition ex vivo may impair the ability of CD8 cells to produce factors inhibitory for HIV expression [23]. If HDAC inhibitors are to be used as part of a therapeutic strategy to purge proviral infection, this effect might somewhat increase the importance that such approaches be coupled to very potent ART. However, it might also be noted that if the goal is to induce viral expression to allow purging of infected cells, the down-regulation of CD8-associated antiviral factor might be desirable, in that it may allow more effective purging in combination with potent ART. Such observations highlight the complexity of targeting

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cellular factors as part of antiviral therapy, and the need for very detailed studies of potential therapeutic approaches.

A pilot clinical experiment to test the ability of the weak, global HDAC inhibitor valproic acid (VPA) to deplete resting CD4+ T cell infection was reported several years ago [24]. In order for such clinical experiments to replicate the results of *in vitro* observations, several events must occur. First, resting cells induced to express HIV must be cleared, either by direct viral cytopathic effect, immune clearance, or terminal expansion and cell death induced as a consequence of viral replication. While VPA itself does not appear to induce cell activation or proliferation, cellular programs induced by viral replication could do so. Secondly, the decline in reservoir size must be greater than the range of observational error of the experimental system. Finally, virus emerging from resting cells must not spread to other susceptible cells, as even a rare infectious event that results in a new quiescent resting cell infection might be enough to maintain the size of this small, persistent cellular reservoir.

In this first clinical experiment, four HIV-infected volunteers with documentation of plasma HIV RNA consistently <50 copies/ml for 24 to 75 months entered the study.

Prior to the initiation of VPA treatment, antiviral therapy was intensified by the addition of enfuvirtide (T-20), to safeguard against the spread of HIV to uninfected cells during the period of VPA therapy. Therefore, any decline in the number of infected resting CD4 cells in this pilot trial could be attributed to VPA, T-20, or both.

To determine whether treatment with VPA affected the number of infected resting CD4+ cells, the frequency of cells capable of producing HIV-1 (infectious unit per billion cells, IUPB) was enumerated by limiting-dilution culture assays of resting CD4+ T cells after activation. Leukopheresis to determine IUPB was performed two times prior to, and after the administration of T-20 and VPA. Pooled IUPB from resting cell outgrowth assays prior to T-20 and VPA declined 29%, >84%, 68%, and 73%. As no virus was recovered in outgrowth assays of patient 2 at week 18, maximal IUPB and minimal decline is modeled on an estimate of virus recovery in one macroculture. Therefore the finding of a decline in resting cell infection of more than 50% after only 4 months of therapy suggested a new and potent effect attributed to VPA and/or T-20, greater than the variance of the resting cell outgrowth assay in 3 of the 4 patients.

However, subsequent studies have not confirmed this initial result. Little effect of VPA was seen in two observational studies in patients taking VPA with ART for other indications, and in one prospective trial in which VPA was given without intensified ART [25-27]. In an attempt to clarify these discordant findings, the stability of resting CD4+ T cell infection and residual viremia was measured in patients who added VPA with or without raltegravir (RAL), or T-20 with or without VPA, to standard ART [28].

In three patients on standard ART a depletion of resting cell infection was observed after 16 weeks of VPA, but this effect waned over up to 96 weeks of further VPA. In two patients T-20 added to stable ART had no effect on resting cell infection.

Simultaneous intensification with T-20 and addition of VPA had no effect on resting cell infection frequency in one of these pre-treated patients, and resulted in a 46% decline in a second. No significant depletion of resting cell infection (> 50%) was seen in six volunteers after the addition of RAL and VPA. In 4 of the 6 patients this lack of effect might be attributed to intermittent viremia, low VPA levels, or intermittent study therapy adherence. Therefore, the prospective addition of VPA and RAL, VPA and T-20, or T-20 failed to progressively reduce the frequency of resting cell infection, or ablate intermittent and low-level viremia. While VPA may have a modest and limited effect on the frequency of resting

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cell infection in some patients, even these preliminary studies in small populations suggest that new approaches such as more potent HDAC inhibition, alone or in combination with intensified ART or other agents that may disrupt proviral latency must be pursued.

Beyond Valproic Acid

Vorinostat (VOR) or suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor with nanomolar potency selective for the crucial Class I HDACs, can induce the expression of latent HIV from the resting CD4+ T cells of HIV-infected, ART-treated, aviremic patients at a concentration of protein-unbound drug that is achieved with clinical dosing [29]. This observation has been confirmed in other experimental systems [30-31]. Recently, reports have emerged that other potent HDAC inhibitors that are in development for use in oncology can effectively induce the expression of integrated, proviral HIV in several model systems [32,33].

A clinical experiment is in development to directly test the hypothesis that a clinically tolerable dose of VOR can induce expression of HIV within resting CD4+ T cells in vivo. The proposed study will investigate the safety, tolerability and spectrum of side effects of a limited exposure to VOR in combination with ART, but due to the potential for long-term adverse events following prolonged exposure to VOR, the FDA has only approved a short-term evaluation of the activity of VOR. If successful, an assessment of the effect of VOR on the frequency of recovery of HIV from the resting CD4+ cells of patients on ART could be performed, but given the mutagenic potential of VOR such a study might be ethically challenging. However, even if the risks of longterm VOR administration do not allow its use in attempts to eradicate HIV infection, proof-of-concept that HDAC inhibitors can perturb persistent HIV infection (heretofore lacking) will allow further investment and research towards similar approaches to eradicate HIV infection.

Recently, studies have emerged suggesting that combinatorial strategies will be required to effectively and comprehensively purge the pool of replication-competent, integrated, persistent HIV [34-38]. Concepts presented include combining HDAC inhibition with the inhibition of histone and/or DNA methylation, or with various cellular signaling or activating pathways.

Perez [38] studied a novel protein kinase agonist, bryostatin, studied in the 1990’s as a cancer chemotherapeutic, and found that this compound reactivates latent HIV-1. Bryostatin was also synergistic when tested in combination with HDAC inhibitors, and of relevance was found to down-regulate the expression of the HIV-1 coreceptors CD4 and CXCR4 and prevented de novo HIV-1 infection in susceptible cells. While such concepts may be mechanistically valid in experimental system, given the clinical challenges of testing a single HDAC inhibitor such as VOR, combination approaches will be challenging to test in clinical experiments. Given the success of current antiretroviral therapy, the relative good health and significant life expectancy of patients with stably suppressed viremia, clinical approaches to eradication must be very safe and tolerable. Combinatorial strategies will require careful testing in the developing animal models of antiretroviral therapy and HIV infection. Nevertheless, such a multimodality approach that includes potent suppression of HIV replication, therapies that reach all compartments of residual HIV replication, and depletion of any reservoirs of persistent, quiescent proviral infection must be found if the HIV pandemic is to be effectively controlled.

Conclusion

Eradication of infection in HIV-infected individuals is not yet possible, but serious efforts to develop tools for and approaches to this goal are finally beginning. Therapies that target

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latent HIV infection, based on an expanding understanding of the factors that allow an active LTR to revert to a silent state, maintain established latency, or emerge from latency, are beginning to be tested in pre-clinical and clinical studies.

The parallel obstacle of residual viral replication, persisting despite clinically effective HAART, must also be overcome to achieve eradication of HIV infection. It seems likely that a definition of the reservoirs of persistent HIV will lead to techniques to eliminate persistent viremia. Advances in HIV vaccine development may also be employed to enlist the body's own defenses in recognition and clearance of infected cells.

When the known obstacles are overcome, we may discover additional cellular reservoirs of infection and mechanisms of persistence that require alternate or combinatorial therapeutic approaches. But the progress made so far in deciphering HIV pathogenesis and developing treatment strategies gives hope that eradication of established HIV infection is not an impossible goal.

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unclear. Perez highlighted the bifunctional effects of bryostatin that may impair the infection of bystander cells.

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