Research Highlights

Highlights from the latest articles in epigenomics

New inhibitors of HDAC to purge latent HIV-1 reservoir

Evaluation of: Choi BS, Lee HS, Oh YT *et al.*: Novel histone deacetylase inhibitors CG05 and CG06 effectively reactivate latently infected HIV-1. *AIDS* 24(4), 609–611 (2010).

Although the highly active antiretroviral therapy (HAART) is extremely effective at decreasing the HIV-1 load below the detection limit, HIV-1 establishes latent infection in a reservoir of resting memory CD4⁺ T cells. Latent HIV-1 evades host immune responses and antiretroviral therapy, and represents the main obstacle in the eradication of the virus from infected patients. Numerous transcriptional and post-transcriptional mechanisms contribute to the development of HIV-1 latency. A major transcriptional block is the lack or sequestering of activating transcription factors in resting CD4⁺ T cells. The site of virus integration can also have a profound effect upon HIV transcriptional expression. Once integrated, HIV is subjected to the same epigenetic regulatory mechanisms as cellular genes. Certain chromatin-modifying enzymes have been shown to maintain HIV latency. These include histone deacetylases (HDACs), which create a more condensed, repressive chromatin environment and remove important docking signals for activating transcription factors [1]. The histone methyltransferases have also been reported to regulate HIV latency in cell-line models [2]. In addition to histone modifications, methylation of integrated proviral genomes has been shown to control resistance of latent HIV-1 to reactivation signals, and thus determine the stability of the HIV-1 latency [3].

A future strategy for the eradication of the latent reservoir is based on the idea of reactivating the latent virus concomitantly

with HAART [4]. Infected cells could undergo apoptosis after virus expression, and HAART would prevent the infection of new cells. Antiviral immune responses might also aid in the clearance of infected cells and prevent the spread of viral infection. Reactivation of latent virus in primary memory T cells requires the activation of Lck and NFAT, but not NF- κ B [5]. Translocation of NFAT into the nucleus is accompanied by the recruitment of chromatin remodeling factors such as SWI/SNF, and histone acetyl transferases such as CBP and p300, followed by changes in the local chromatin structure [6], and histone acetyl transferases, such as CBP and p300. Complementary reports demonstrated that the class I HDACs 1, 2 and 3 are expressed in the nuclei of resting CD4⁺ T cells and associate with the HIV promoter in cell-line models of latency. Accordingly, selective chemical inhibition of HDACs 1, 2 and 3 can induce *ex vivo* viral outgrowth from the resting CD4⁺ T cells of HIV-infected individuals better than the nonselective HDAC inhibitor, valproic acid. By contrast, inhibitors selective for class II HDACs 4, 5, 7 and 9 do not induce outgrowth of latent HIV [1]. It has now been reported that novel HDAC inhibitors, CG05 and CG06 effectively reactivate latent HIV-1 [7]. In the ACH2 cell line, both inhibitors show a dose-dependent acetylation of histone H3 at Lys9 and Lys27, a higher reactivation activity of HIV-1 than suberoylanilide hydroxamic acid and a lower toxicity compared with PXD101. Both inhibitors will undoubtedly be included in clinical trials of HIV-1 reactivation from the resting CD4⁺ T cells of HIV-infected individuals. Recent reports showing that some other HDAC inhibitors, such as ITF2357 [8], MCT-1 and MCT-3 [9], and MRK-1 [1] also efficiently reactivate HIV from latently infected cells illustrate intense research in this field. Future investigation of histone

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modifications, including H2A, H2B and H4 will help elucidate the detailed mechanism of both CG05 and CG06 action so that these inhibitors might be used in the therapy of latent HIV infection.

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Reactivation of HIV-1 latent reservoir by an inhibitor of H3K9me2 methyl transferase G9a

Evaluation of: Imai K, Togami H, Okamoto T: Involvement of histone H3 Lysine 9 (H3K9) methyl transferase G9a in the maintenance of HIV-1 latency and its reactivation by BIX01294. *J. Biol. Chem.* 285(22), 16538–16545 (2010).

The current protocols of HAART are efficient in decreasing the HIV-1 load below the limit of detection and blocking the progress of infected patients towards the fulminant AIDS. Despite the potency of HAART, however, HIV-1 establishes latent reservoir in resting memory CD4⁺ T cells, which escape host immune responses and antiretroviral therapy. HIV-1 latency is thus the main obstacle to the eradication of the virus from infected individuals. Transcriptional shutdown and multi-step formation of restrictive chromatin at long terminal repeats (LTR) are two interconnected events leading to the latent state of the HIV-1 provirus. HIV-1

LTR-driven transcription is silenced in the absence of cellular transcription initiation factors such as NF- κ B and NFAT [1]. Furthermore, it requires recruitment of the histone deacetylase type 1 (HDAC-1) [2], histone methyltransferase Suv39H1 [3], and heterochromatin protein HP1 to the chromatin around the HIV-1 LTR. It was also shown that CpG methylation of the HIV-1 5' LTR is an additional epigenetic restriction mechanism, which controls resistance of latent HIV-1 to reactivation signals and thus determines the stability of the HIV-1 latency [4].

Antilateness strategies aimed at virus eradication would purge the latent reservoir by inducing transcriptional activation of latent virus concomitantly with HAART [5]. Infected cells could undergo apoptosis after virus expression, and HAART would prevent infection of new cells. Antiviral immune responses might also aid in the clearance of infected cells, and prevent the spread of viral infection.

Recent reports show that Lck and NFAT, but not NF- κ B, are required for optimal latent virus reactivation in primary memory T cells [1]. Transcriptional reactivation is accompanied by changes in the local chromatin structure and by the recruitment of chromatin remodeling factors such as SWI/SNF [6] and histone acetyl transferases such as CBP and p300.

It has now been reported that dimethylation of H3K9 (H3K9me2) by histone methyltransferase G9a is responsible for the maintenance of HIV-1 silencing [7]. Depending on its position, the methylation of histones plays either positive or negative role in transcriptional regulation. Thus, methylation at K4 and R17 of histone H3 is generally associated with active genes, whereas trimethylation of H3 at K9 (H3K9me3) and 27 (H3K27me) has been associated with inactive genes [8]. It was also noted that H3K9me3 is a hallmark of a stable and poorly reversible transcriptional repression in comparison

with H3K27me [4,8]. Although the effect of Suv39H1 on H3K9 trimethylation and silencing of HIV-1 provirus is known [3], the role of histone methylation by G9a in the maintenance of HIV-1 latency has not yet been elucidated. Since the extent of histone methylation by G9a is much greater than that of Suv39H1, it is not surprising that a specific inhibitor of G9a, BIX01294, can reactivate expression of HIV-1 from latently infected cells such as ACH-2 and OM10.1 [7].

Since G9a is known to associate with DNMT and that G9a-knockout cells revealed significantly reduced DNA methylation [9], it is possible that G9a indirectly induces DNA methylation. G9a inhibitors thus potentiate HIV-1 reactivation from latency. Elucidation of the epigenetic silencing mechanism of the HIV-1 provirus in latently infected cells is crucial for our understanding the pathophysiological process of HIV-1 infection and, particularly, for the further development of novel antiretroviral therapies.

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