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Review

Role of histone modification on transcriptional regulation and HIV-1 gene expression: possible mechanisms of periodontal diseases in AIDS progression

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Abstract: Although approximately 200 distinct cell types – including fibroblasts, neurons, and hematopoietic cells – possess the same DNA sequence, they have diverse functions in humans and exhibit considerably different gene expression patterns. It has become increasingly clear that epigenetic regulation plays an important role in gene expression. There are two major forms of epigenetic regulation: posttranslational modification of DNA-associated histone proteins in chromatin and methylation of DNA. These forms are regulated by distinct but coupled pathways. Notably, histone Lys acetylation by histone acetyltransferase and deacetylation by histone deacetylases play a crucial role in on-off regulation of gene expression. It is now understood that epigenetics plays an important role not only in the regulation of gene expression but also in the pathogenesis of a broad range of diseases such as cancer and microbial infections. We have determined that epigenetic regulation is involved in the establishment and maintenance of HIV-1 latency and in the reactivation of HIV-1 by periodontopathic bacteria. In this review, we focus on the effect of histone modification on transcriptional regulation and the contribution thereof to the regulation of HIV-1 gene expression during the lytic and latent stages of HIV-1 infection. Likewise, we discuss the mechanisms by which periodontal diseases may accelerate AIDS progression in infected individuals as a new systemic disease caused by periodontitis and describe potential therapeutic interventions based on epigenetic mechanisms. (J Oral Sci 53, 1-13, 2011)

Keywords: epigenetic regulation; HIV; AIDS; periodontal diseases; butyric acid; histone.

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Introduction

In the nuclei of eukaryotic cells, long genomic DNA tightly combines with histone and nonhistone proteins to form a dynamic polymer called chromatin. The structural repeating unit of chromatin is the nucleosome, which consists of 145-147 bp of DNA wrapped in 1.65 turns around histone proteins consisting of two copies each of H2A, H2B, H3, and H4. The linker histone, H1, assists in further compaction of the chromatin into higher-order structures. Chromatin is present in two main forms, called "euchromatin" and "heterochromatin" (1). Euchromatin is less condensed and more transcriptionally permissive and is present in promoter and enhancer elements of transcriptionally active genes. In contrast, the highly condensed and silent heterochromatin is found in transcriptionally inactive regions (1, 2). Posttranslational modifications of the N-terminal region of each core histone play an important role in the control of the structural organization of chromatin and its transcriptional status. The histone N-terminal tail region protrudes from the globular center of the nucleosome, where it may interact with other nuclear factors, and is subject to a variety of post-

translational modifications, such as acetylation, methylation, phosphorylation, ubiquitination, and glycosylation (1, 2) (Fig. 1). These modifications occur in response to various stimuli and affect the binding of other nuclear proteins to the histone tails, which subsequently induce structural changes in chromatin or recruit other proteins. The ability of nuclear proteins to specifically associate with certain histone modifications is the basis of the histone code hypothesis (3), which postulates that nuclear proteins function to activate or inhibit transcription or, similarly, to maintain a specific chromatin structure.

Among the histone modifications, acetylation and methylation of histone 3 and histone 4 (H3 and H4) play a central epigenetic role in the organization of chromatin domains and the up- or downregulation of gene expression (1-3). Generally, acetylation of Lys (K) residues in the promoter region of these histones by histone acetyltransferases (HATs) facilitates a switch from repressive heterochromatin to permissive euchromatin, which allows increased accessibility of transcription factors to DNA and activation of gene transcription. Conversely, the deacetylation of histones by histone deacetylases (HDACs) is associated with transcriptional silencing and gene repression (1, 2). Unlike histone Lys acetylation, histone Lys methylation has been found to play both positive and negative roles in transcriptional regulation, depending on the residue (3, 4). This modification is induced by histone methyltransferases (HMTs) and can be reversibly controlled by histone demethylases (HDMs) (5). Methylation of histone proteins affects the binding of histone modification enzymes to the chromatin, which then induces other posttranslational modifications, such as histone phosphorylation and DNA methylation (4, 5).

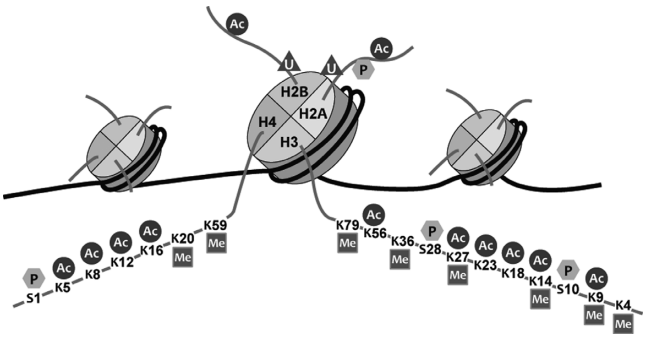

Fig. 1 Schematic diagram of a nucleosome and histone modifications.
Posttranslational modifications of the N-terminal region of each core histone (H2A, H2B, H3, and H4) play a central epigenetic role in the organization of chromatin structure and the up- or downregulation of gene expression. The figure shows the histone tails of H3 and H4, which contain posttranslational modifications, such as acetylation (Ac), methylation (Me), phosphorylation (P), and ubiquitination (U). Epigenetic mechanisms determine the phenotype, without altering primary DNA sequences.

Although it has recently become clear that epigenetic regulation is a central regulator system of gene expression, emerging evidence implicates epigenetic dysregulation in the pathogenesis of a broad range of diseases and in disruptions of normal cell function. Indeed, aberrant DNA methylation status and posttranslational modifications of histones have been found in many human diseases, including cancer, diabetes, autoimmune diseases, and microbial infections (6). In addition, the repressive methylation states of H3 Lys9 (H3-K9) and H3 Lys27 (H3-K27) have been detected in the promoter regions of aberrantly silenced tumor suppressor genes in cancer cells, together with increased DNA methylation and reduced histone acetylation (7). Moreover, unusual expression of histone modification enzymes, including HATs, HDACs, and HMTs, has been observed in cells of patients with cancer and autoimmune diseases (8, 9). In viral infection, epigenetic reprogramming of the viral or host-cell genome is important in the induction of pathological changes by the virus. Many studies have shown that viruses can cause DNA methylation of the host-cell genome by interacting with the host epigenetic machinery, thus controlling the silencing of cellular genes (10-12). DNA methylation of viral genomes is also known to play a major role in virus-associated human cancer and virus silencing (10-12). In addition to DNA methylation, recent studies have revealed a new mechanism that regulates viral replication and latency, which are involved in histone modifications caused by chromatin remodeling factors, such as HATs and HDACs (12-16). In virus replication involving histone modification, the regulation of human immunodeficiency virus-1 (HIV-1) gene expression during lytic and latent stages of HIV-1 infection has been intensively studied. We have previously demonstrated that a product of periodontopathic bacteria, butyric acid, might markedly induce reactivation of latent HIV-1 through its action as an HDAC inhibitor.

This review describes the current understanding of the relationship between the molecular mechanisms of histone modification and gene expression and explains how this relationship may pertain to the establishment of HIV-1 latency and its reactivation. In addition, we present a hypothetical model of the role of periodontal diseases as a risk factor for AIDS progression in individuals infected with HIV-1.

Histone acetylation/deacetylation: a central switch between "open" and "closed" chromatin

The dynamic equilibrium between histone acetylation catalyzed by HATs and histone deacetylation catalyzed by HDACs plays a crucial role in the on-off regulation of gene expression (1). In most species, histone H3 is primarily acetylated at multiple Lys sites, including H3-K9, -K14, -K18, -K23, -K27, and -K56. Acetylation of histone H4 has been observed at H4-K5, -K8, -K12, and -K16. Acetylation of the positively charged N-terminal tails of core histones by HATs disrupts the electrostatic interactions of histones with the negatively charged phosphate backbone of DNA, which loosens the chromatin structure. These reactions thus promote chromatin opening and enable DNA-binding transcriptional factor to access the chromatin, thereby enhancing gene expression at specific regions (1, 2).

Several mammalian proteins have been identified as nuclear HATs, including cyclic AMP-responsive enhancer binding protein (CREB)-binding protein (CBP), p300, p300/CBP-associated factor (PCAF), GCN5, and Tip60 (17,18) (Fig. 2). CBP and p300 are ubiquitously expressed, highly conserved transcriptional coactivators that exhibit significant HAT activity against core histones. Interaction of CBP/p300 with acetylated Lys residues promotes additional acetylation of histones. CBP and p300 are also involved in the acetylation of nonhistone proteins, including many transcription factors (such as nuclear factor κB [NF-κB], p53, nuclear hormone receptors, CREB, and c-Fos), which subsequently increase the binding activity of transcriptional factors to the DNA. In addition, acetylation of CBP/p300 itself has been demonstrated to enhance its HAT activity and affects the expression of a wide variety of genes. PCAF and GCN5 also exhibit significant HAT activity against core histones (18). Interestingly, CBP/p300, PCAF, and GCN5 bind the HIV-1 transactivator Tat protein during HIV-1 infection, thereby inducing chromatin remodelling of proviral genes (16).

In contrast, a closed chromatin structure is promoted by deacetylation of Lys residues by HDACs, which are the

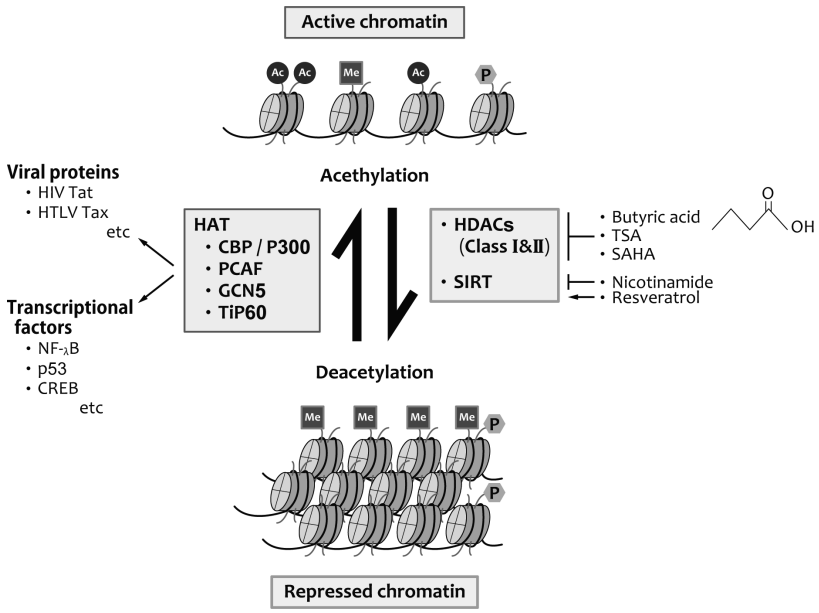

Fig. 2 Histone acetylation/deacetylation is mediated by HATs and HDACs. The dynamic equilibrium between histone Lys acetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylation catalyzed by histone deacetylases (HDACs) is crucial for on-off regulation of gene expression. Acetylation of the N-terminal tails of core histones by HATs (eg, CBP/p300, PCAF, GCN5, and Tip60) leads to open, transcriptionally active chromatin. HATs can also promote acetylation of nonhistone proteins, including many transcription factors and the viral transactivators. Acetyl groups are removed by HDACs, the counterpart of HATs, which promote a closed, repressed chromatin structure. SIRT is a class III HDAC, which promotes histone deacetylation by NAD-dependent mechanisms.

counterpart of HATs that catalyze the hydrolytic removal of acetyl groups (1,19) (Fig. 2). Histone deacetylation diminishes the accessibility of the transcriptional factors to nucleosomal DNA, thereby leading to repression of gene expression (2). Mammalian HDACs can be divided into three classes (19). Class I HDACs (HDAC1, 2, 3, and 8) are localized in the nucleus. Class II HDACs (HDAC4, 5, 6, 7, 9, and 10) shuttle between the nucleus and the cytoplasm. It is well known that drugs, such as trichostatin A and butyric acid, which inhibit HDAC activity, can prevent hypoacetylation of histones, leading to chromatin remodeling and induction of gene expression (2, 19). The third class of HDACs comprises the human sirtuin (SIRT) enzymes, which are related to the yeast transcriptional repressor protein Sir2 (20, 21). The genes of the SIRT family (SIRT1-7) are highly conserved and encode nicotinamide adenine dinucleotide $(NAD)^+$-dependent protein deacetylases, which are known to function in transcriptional silencing processes through the deacetylation of histones H3 and H4. SIRT1 deacetylase activity is inhibited by nicotinamide and activated by resveratrol. In HIV-1 infection, SIRT1 promotes deacetylation of the HIV-1 Tat protein (22).

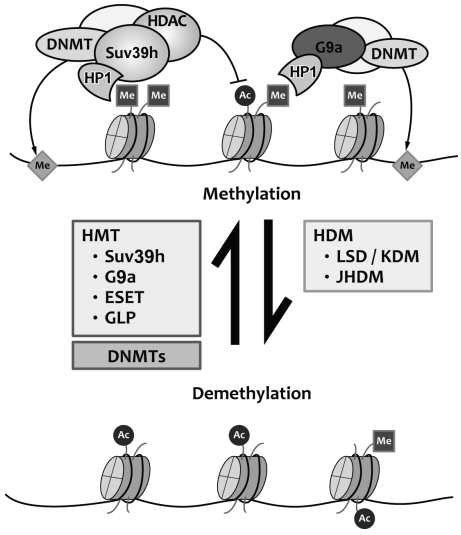

Fig. 3 Regulation of histone methylation and demethylation.
Methylation of histone residues can either activate or repress gene expression, depending on the residue. This modification is induced by histone methyltransferases (HMTs), such as Suv39h, G9a, ESET/ SETDB, and GLP, which are characterized by a conserved SET domain and removed by HDMs. Suv39h and G9a are responsible for methylation of H3-K9 and subsequent recruitment of heterochromatin protein HP1 and HDACs. The HMTs, HP1, and HDACs are all capable of binding and recruiting DNMTs, which then facilitate DNA methylation.

Role of histone methylation in transcriptional regulation

In 1999, it was discovered that various Lys residues in nucleosomal histones are mono-, di-, or trimethylated on their ε-amino groups and that these methyl marks affect chromatin structure and transcription (4, 5). In most species, histone H3 is primarily methylated at Lys residues, including H3 at -K4, -K9, -K14, -K27, -K36, and -K79. Histone H4 methylation has been observed at H4 at -K20 and -K59. In contrast to acetylation, methylation of histone residues can either activate or repress gene expression, depending on the residue (4,5). For example, methylation of H3 at K4 (H3-K4me), H3 at K36 (H3-K36me), and H3 at K79 (H3-K79me) is generally associated with transcriptionally active chromatin, while methylation of H3 at K9 (H3-K9me), H4 at K20 (H4-K20me), and H4 at K27 (H3-K27me) has been associated with transcriptionally silent regions (4, 5).

Methylation of histone H3-K9 is strongly associated with formation of crossed chromatin and gene silencing (4,23-25). Four mammalian H3-K9 methyltransferases have been identified in mammalian cells (4, 5, 26) (Fig. 3), and these methyltransferases each contain a catalytically active SET (Suv39, Enhancer of Zeste, Trithorax) domain, which is responsible for Lys methyltransferase activity. These include Suv39h1 and the closely related Suv39h2, G9a and the closely related GLP/EuHMTase1, and ESET/ SETDB. Suv39h1 contains both SET and chromo domains and selectively methylates histone H3-K9. G9a is a key enzyme responsible for H3-K9 dimethylation (H3-K9me2) in mammals, as disruption of the G9a gene results in a drastic decrease in H3-K9 methylation mainly in euchromatic regions (26, 27). Methylation of H3-K9 recruits heterochromatin protein 1 (HP1) proteins – a family of heterochromatic adaptor molecules involved in both gene silencing and nucleosomal chromatin structure – and HDACs to form heterochromatin. Then, complexes of HMTs, HP1, and HDACs are able to recruit DNA methyltransferases (DNMTs), which facilitate methylation of DNA (4, 28).

Methylation of histones at Lys residues is believed to be a reversible process (Fig. 3). In 2004, Shi et al. first discovered that an HDM, namely, lysine-specific histone demethylase 1 (LSD1), is a FAD-dependent amine oxidase that can demethylate H3-K4me and H3-K4me2 (29). Two years after the discovery of LSD1, jumonji C domain-

containing histone demethylation enzymes were identified as HDMs that demethylate H3-K9 and -K36 (30). HDMs are emerging as important factors in developmental processes and have been linked to human diseases, such as neurogenic disorders and cancer (4, 5).

Epigenetic studies of microbial infections

The importance of epigenetic regulation in microbial infections is becoming increasingly appreciated. DNA methylation of viral genomes plays a major role in virus-associated human cancer and virus silencing. Indeed, DNA hypermethylation of human T-cell leukemia virus type 1 (HTLV-1), which carries the 5-long terminal repeat (LTR) sequence, was observed in individuals with adult T-cell leukemia (31). Virus DNA methylation increases from early to late stages of tumorigenesis (32). In contrast, viruses and bacteria may cause DNA methylation of the host-cell genome, thus promoting the repression or silencing of host cellular genes, and may thus be involved in the development of human diseases such as cancer. In one example of this, a good correlation was observed between *Helicobacter pylori*-induced aberrant DNA methylation in chronic gastritis and gastric cancer, although the molecular mechanisms for this correlation are unclear (33). *H. pylori* infection induced promoter DNA methylation of the E-cadherin gene, an adhesion molecule involved in tumor invasion and metastasis (34). Epstein-Barr virus (EBV) infection also stimulates E-cadherin gene methylation (35). In addition, *Campylobacter rectus* is involved in periodontal diseases and increases the risk of preterm births that could induce hypermethylation in the promoter of the insulin-like growth factor 2 gene in the placenta of infected mice (36).

Recent studies have revealed a new mechanism that regulates microbial progression involving histone modifications. Interestingly, Wurtele et al. reported that deacetylation of histone H3-K56 is required for cell viability in several clinically important pathogenic fungi (37). They found that reduced levels of H3-K56 acetylation by Hst3p – which is a member of the family of NAD^+-dependent HDACs known as SIRT – sensitizes *Candida albicans* to antifungal agents. Importantly, reduction of H3-K56 acetylation by Hst3p inhibition or nicotinamide reduces the virulence of *C. albicans* in mice. During virus replication, the genome of herpes simplex virus type 1 (HSV-1) rapidly associates with histone and nonhistone chromosomal proteins after infecting the host cells. HSV-1 is subject to chromatin-based regulation of transcription and replication (13, 14). The replication of human cytomegalovirus (HCMV) is also controlled during modification of histone proteins (38). Recent reports indicate that a switch between the lytic and latent stages of HSV-1 and HCMV infection is determined by the chromatin state of the viruses (14, 39). Moreover, it was demonstrated that hepatitis B virus (HBV) replication is associated with specific epigenetic marks, such as the acetylation or diacetylation of H3 and H4 (40). Interestingly, interactions between HBV encode the regulatory HBx protein and HATs, such as CBP and p300, which are associated with gene expression; those with HDAC1 have been associated with gene repression (41, 42). In the next section, we focus on regulation of the expression of the HIV-1 gene, which is one of the best described genes associated with transcriptional control at the chromatin level.

Latency is the main obstacle in eradicating HIV-1 from patients

HIV is a cytopathic retrovirus and the primary etiological agent of acquired immunodeficiency syndrome (AIDS) and related disorders. Twenty-eight years after the discovery of HIV-1 in 1983, we are still unable to eliminate the virus from infected patients. According to the latest report of the Joint United Nations Program on HIV/AIDS, about 33 million people globally are living with AIDS. Although there is no effective AIDS vaccine, the introduction of highly active antiretroviral therapy (HAART) in 1996 greatly extended the survival of infected patients. Current HAART protocols consist of combinations of antiretroviral agents that efficiently decrease the HIV-1 load to below the detection limit, thereby reducing mortality due to HIV-1 infection. However, despite the potency of HAART, latent HIV-1 infection is established in reservoirs of resting $CD4^+$ T cells, which escape host immune responses and antiretroviral therapy (43). In fact, for many patients with HIV, latently infected cells persist over long a period of time and harbor integrated proviruses capable of reseeding virus production after cessation of therapy (16, 43). Siliciano and colleagues estimated the mean half-life of latently infected cells at 44.2 months (44). Because current HAART does not attack latent viral infection, it has been estimated that it would require over 70 years of uninterrupted HAART to eliminate the pool of latently infected cells, based on previous estimates of a latent reservoir size of approximately 10^6 cells (44, 45). Therefore, the ability of HIV-1 to establish a latent infection is crucial to the pathogenesis of AIDS, and elucidation of the molecular mechanisms involved in the maintenance and breakdown of HIV latency is required for a comprehensive understanding of the pathophysiology of HIV-1 infection and the development of novel therapies.

Molecular mechanism of HIV-1 transcription from LTR

Of the various stages of the viral life cycle, HIV-1 gene expression is the principal determinant of the viral replication rate leading to disease progression of AIDS. After viral infection, HIV-1 proviral DNA is synthesized and incorporated into nucleosomes, and the transcriptional activity of the provirus from its LTR is under the control of the regional nucleosomal structure (16, 45). It has been suggested that epigenetic modification of the nucleosome structure (called Nuc-1) near the viral mRNA start site plays a regulatory role in inducing 5'LTR-driven transcription and viral expression (45, 46). The compaction of HIV-1 proviral DNA and its permissiveness for viral transcription are directly dependent on histone posttranslational modification, such as acetylation and methylation, which are distinct reactions that serve to recruit various regulatory protein complexes towards HIV-1 LTR and eventually up- or downregulate HIV-1 gene expression and viral replication (46).

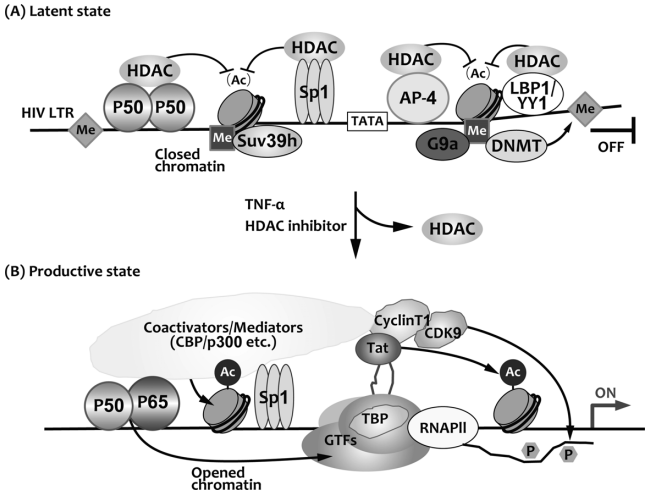

Fig. 4 Model of HIV-1 proviral latency and its reactivation via histone modifications.
(A) Latent state: Closed chromatin structure at the latent HIV-1 LTR. Negative transcription factors (eg, AP-4, YY-1/ leader-binding protein-1 (LBP-1), NF-κB p50 homodimer, and Sp1-myc complex) have been shown to mediate HDAC recruitment to LTR and, consequently, inhibit transcription from the viral promoter. The HTMs, such as Suv39h1 and G9a, are responsible for chromatin-mediated HIV-1 transcriptional latency through methylation of H3-K9. (B) Productive state: Upon cell stimulation, such as by HDAC inhibitors (eg, butyric acid and trichostatin A) and TNF-α, transcriptional activators, including NF-κB, are recruited along with coactivators (eg, CBP/p300 and PCAF) exhibiting histone acetyltransferase activity. Local histones are acetylated, and the abovementioned negative regulators are dismissed together with HDAC proteins, thus initiating transcription. Interaction of Tat and p-TEFb (Cyclin T1/CDK9) promotes phosphorylation of the C-terminal domain (CTD) of RNA polymerase II (RNAP II), thereby leading to transcriptional elongation of the HIV-1 gene.

HIV-1 transcription is driven by promoter activity in the 5'LTR of the integrated provirus and is regulated by cellular factors (eg, NF-κB, CREB, Sp1, and AP-1) that bind to multiple *cis*-regulatory elements located in LTR as well as the virally encoded transactivator Tat protein (16, 47) (Fig. 4). The early phase, or initiation, of transcription is mainly activated by cellular transcription factors, and only a few transcripts elongate throughout the viral genome, resulting in the transcription of Tat. In the absence of Tat, transcription of HIV mRNAs can be initiated, but these cannot be efficiently elongated to produce full-length viral RNA genome. Tat interacts specifically with cyclin T1 (CycT1), a regulatory partner of cyclin-dependent kinase 9 (CDK9), in the positive transcription elongation factor (P-TEFb) complex and, together with CycT1, binds cooperatively to the transactivation responsive element (TAR), where a bulged RNA loop structure located at the 5'-end of nascent viral RNA transcripts is used to recruit P-TEFb and promote the transcriptional elongation of HIV-1(48, 49). The assembly of the Tat-TAR-P-TEFb complex at the HIV-1 promoter activates CDK9 kinase, which further autophosphorylates P-TEFb and hyperphosphorylates the C-terminal domain of RNA polymerase II, leading to the formation of more-processive elongation complexes that synthesize full-length HIV viral mRNA (49). Activation of HIV-1 gene expression by Tat and cytokines, such as tumor necrosis factor-α (TNF-α), is accompanied by histone acetylation, leading to loss or rearrangement of chromatin structure at Nuc-1 (16).

Histone deacetylation and methylation are involved in maintaining HIV-1 latency

In contrast to productively infected cells, latently infected cells harbor the proviral HIV-1 genome, which is integrated into the silent chromatin, thereby ensuring the persistence of transcriptionally inactive proviruses. It has become clear that HDACs are critical regulators of HIV-1 latency. Accumulating evidence indicates that the presence of HDAC1 leads to the repression of HIV-1 LTR, whereas inhibition of HDAC1 results in HIV-1 gene expression (50-55). To date, negative transcription factors such as Yin-Yang-1 (YY-1) (50), NF-κB, p50 homodimer (51), and Sp1-myc complex (52) have been shown to mediate HDAC1 recruitment to LTR and consequently inhibit transcription

from the viral promoter (Fig. 4). We also reported that activator protein-4 (AP-4) acts as a transcriptional repressor by recruiting HDAC and masking the TATA-box-binding protein (TBP) to HIV-1 TATA box molecules and is involved in the maintenance of viral latency (53). The consensus AP-4 site, CAGCTG, is located -21 to -16 nucleotides immediately downstream of the TATA box in the HIV-1 LTR, and the binding site appears to be conserved in most HIV-1 isolates. When AP-4 is knocked out by siRNA, HIV-1 replication was greatly augmented in cells transfected with a full-length HIV-1 clone. Others have observed that HDAC inhibitors (HDACis), such as butyric acid, suberoylanilide hydroxamic acid (SAHA), and valproic acid, might lead to histone acetylation and induce HIV transcription in latently infected cells. (46, 54, 55)

In addition, it has recently been observed that histone H3-K9 methyltransferases such as Suv39h1 and G9a are responsible for chromatin-mediated HIV-1 transcriptional latency through methylation of H3-K9 (28, 56, 57). Marban et al. reported that the corepressor COUP-TF-interacting protein 2 (CTIP2) recruits Suv39h1 to promote local histone H3 lysine 9 methylation at the HIV-1 LTR (56). We revealed that G9a significantly inhibited both basal and TNF-α- or Tat-induced HIV-1 gene expression (57). Interestingly, a specific inhibitor of G9a, BIX01294, was able to reactivate expression of HIV-1 from latently infected cells via downregulation of H3-K9me2. Moreover, synergistic activation of HIV-1 replication by BIX01294 and SAHA or the DNMT inhibitor 5-aza-2'-deoxycytidine has been observed (57). Although these studies indicate that epigenetic silencing is deeply involved in the maintenance of HIV-1 transcriptional latency, it is not known how HIV latency switches to full HIV replication.

Relationship between periodontal diseases and HIV/AIDS

Chronic immune activation associated with coinfection by non-HIV pathogens may be a critical factor in the severity and progression of AIDS and is known to increase the risk of HIV transmission in infected individuals. It has been established that viral replication is induced by certain stimuli elicited from outside HIV-1 infected cells, including proinflammatory cytokines and even coinfection with other pathogens such as mycobacterium and herpes viruses (58, 59). In fact, AIDS development is more rapid in individuals with dual infections (58, 59). Therefore, understanding the role of immune activation associated with non-HIV coinfection in HIV replication is important in the development of novel therapeutic strategies to control AIDS progression during opportunistic infection.

Periodontal diseases, caused by subgingival infection with oral bacteria, are found worldwide and are among the most prevalent microbial diseases in humans. Severe periodontitis can result in loosening of teeth, occasional pain, impaired mastication, and eventual tooth loss (60). *Porphyromonas gingivalis* (*P. gingivalis*) is a common Gram-negative, nonmotile anaerobe that appears to play an important role in periodontal destruction in adult periodontitis (60). This bacterium produces an elaborate variety of virulence factors, including lipopolysaccharides (LPS), fimbriae, and proteases.

A positive association between periodontal disease and HIV-1 infection has been reported. More *P. gingivalis* bacilli were found in HIV-1-positive individuals than in an otherwise healthy control group (61, 62). In addition, there was a significant correlation between periodontitis stage and both HIV-1 proviral DNA load in gingival crevicular fluid and HIV-1 RNA viral load in plasma and saliva (63, 64). Moreover, expression of HIV-1 receptors/coreceptors is increased by chronic periodontitis (65). It was recently demonstrated that *P. gingivalis* upregulates CCR5 in oral keratinocytes (66) and thus facilitates subsequent infection of HIV into permissive cells, such as macrophages, in a CCR5-dependent manner (67). These results suggest that periodontal disease affects HIV-1 activation and AIDS progression.

Porphyromonas gingivalis promotes HIV-1 reactivation via chromatin remodeling

We previously reported that a short-chain fatty acid (SCFA) species of extracellularly secreted metabolites of *P. gingivalis*, most likely butyric acid, could be involved in periodontal disease (68, 69), and high concentrations of butyric acid have been observed in periodontal pockets (70). Because butyric acid inhibits the enzymatic activity of HDACs by competing with HDAC substrate at the active site pocket containing its catalytic center (71), we hypothesized that *P. gingivalis* activates the expression of HIV-1 genes from latently infected cells. We observed that *P. gingivalis* reactivates HIV-1 replication from latently infected cells while concomitantly increasing Lys acetylation of histone H3 and H4 (72) (Fig. 5). This activity could be ascribable bacterial culture supernatant rather than other bacterial components, such as fimbriae, LPS, or proteases. Although *P. gingivalis* produces several SCFAs, only butyric acid accelerates transcription of HIV-1 from latently infected cells. The bacterial culture supernatant of *P. gingivalis* bacilli and butyric acid could induce histone acetylation. Chromatin immunoprecipitation assays revealed that a corepressor complex containing HDAC1 and AP-4 was dissociated from the HIV-1 LTR promoter upon stimulation with supernatants from *P. gingivalis*

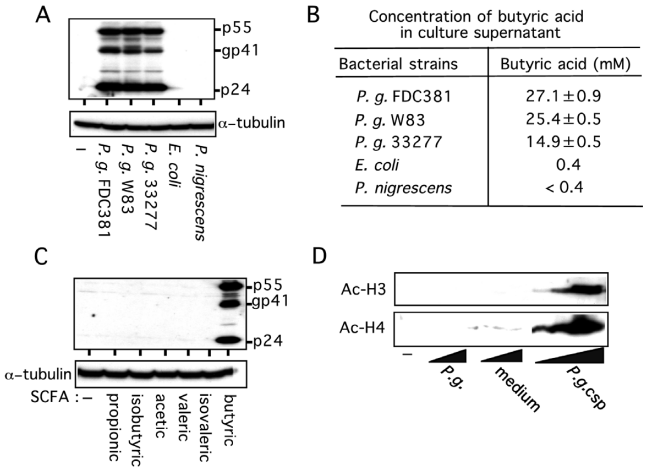

Fig. 5 *P. gingivalis* facilitates HIV-1 reactivation through chromatin remodeling.
(A) Stimulation of latent HIV-1 replication by culture supernatants of various bacterial strains. T cells infected with latent HIV-1 were incubated with culture supernatants of various bacteria, and the cell lysate was analyzed to detect virus proteins by immunoblotting with sera collected from individuals with AIDS. Three representative strains of *P. gingivalis* (*P. g.*) reactivated HIV-1 replication. The lack of such activity in the culture supernatants of *Prevotella nigrescens* and *Escherichia coli* might be due to their inability to produce butyric acid. (B) Butyric acid concentration in the culture supernatants of various bacteria was measured by gas chromatography. *Porphyromonas* strains produced the highest concentrations of butyric acid. (C) Effects of various short-chain fatty acids (SCFAs) on HIV-1 replication. HIV-1 induction was solely attributable to butyric acid among the SCFAs secreted by *P. gingivalis*. (D) Hyperacetylation of histones by *P. gingivalis*. Latent HIV-1-infected cells were incubated with CSP and analyzed for acetylated histone proteins. CSP, culture supernatant of *P. gingivalis* FDC381. (modified from Ref. 72)

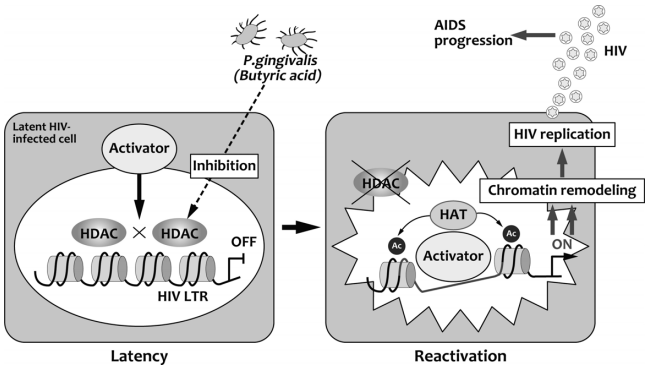

Fig. 6 Schematic representation of latent HIV-1 reactivation by *P. gingivalis*.
Porphyromonas gingivalis produces high concentrations of butyric acid, which acts as a potent inhibitor of HDACs and induces histone acetylation at the HIV-1 LTR, leading to reactivation of HIV-1 in latently infected cells.

concomitant with the association of acetylated histone and RNA polymerase II (72).

Our results suggest that *P. gingivalis* infection is able to markedly induce reactivation of latent HIV-1 proviruses via chromatin modification. This is the first evidence of a molecular link between a bacterial metabolite and AIDS progression at the transcriptional level. Our findings highlight the role of biochemical modification of nucleosomal histone proteins – by acetylation or deacetylation – in the transcriptional activity of integrated HIV-1 proviruses and indicate that histone acetylation is induced by *P. gingivalis* infection and thus is actively involved in the breakdown of viral latency (Fig. 6). Although our *in vitro* experimental data strongly support a relationship between periodontopathic bacteria and HIV-1 activation, which might be present *in vivo*, additional basic and clinical studies are required to clarify the role of periodontitis as a risk factor for HIV-1 activation and AIDS progression in infected individuals.

Concluding Remarks and Future Perspectives

Possible mechanisms of periodontal diseases in AIDS progression

During the past 10 years, evidence has accumulated regarding the role of chronic periodontitis as a risk factor for several systemic diseases, including preterm birth, heart disease, diabetes, and pulmonary disease (60). These associations implicate *P. gingivalis* infection as a possible cause of a number of systemic diseases. Our study demonstrated a cause-and-effect relationship between *P. gingivalis* infection and transcriptional induction of latent HIV-1. Butyric acid produced by *P. gingivalis* could promote gene expression of latent HIV-1, thus making *P. gingivalis* infection a risk factor for AIDS progression due to its ability to upregulate HIV-1 replication systemically or locally, ie, in the oral cavity. In fact, butyric acid is regarded as the most potent inhibitor of HDAC (71), and it markedly augments HIV-1 replication in latently infected primary mononuclear cells from HIV-1-infected individuals (73). Interestingly, elevated concentrations of butyric acid (range, 4.7 to 13.8 mM) were detected in affected dental plaque, and the mean ± standard deviation butyric acid

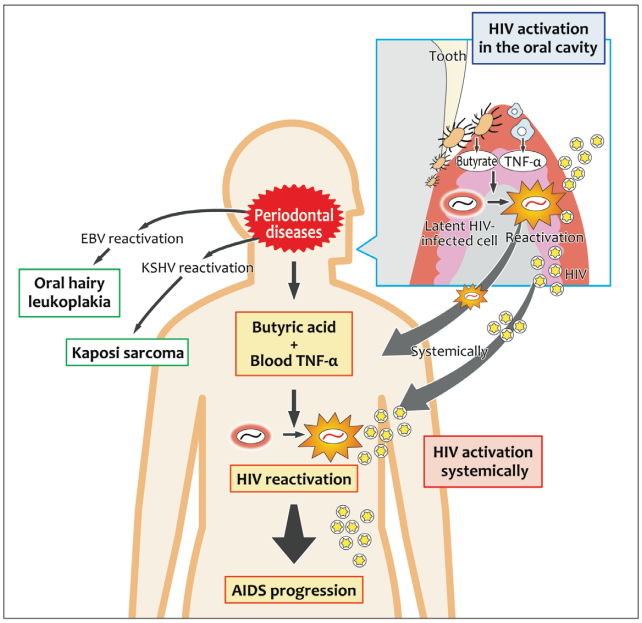

Fig. 7 Possible roles of periodontal diseases in AIDS progression.
In individuals with AIDS, HIV-infected lymphocytes and monocytes are abundant at oral sites, including periodontal pockets, gingival tissues, and salivary glands. Direct interaction of these cells with periodontopathic bacteria and/or indirect interaction with soluble factors (eg, butyric acid and TNF-α) could induce local HIV-1 replication in the oral cavity. It is possible that cells in which viral transcription has been reactivated by a stimulus spread throughout the body via the blood. In addition, TNF-α concentrations are known to be elevated in individuals with periodontal disease, which suggests that periodontal disease is a trigger for local and systemic breakdown of latent infection and may be a risk factor for AIDS progression. Moreover, we have observed reactivation of EBV and Kaposi sarcoma-associated herpes virus (KSHV/HHV8) by periodontopathic bacteria. Thus, periodontal disease may be involved in the onset of AIDS-related pathological changes in oral hairy leukoplakia and Kaposi sarcoma.

concentration in the periodontal pockets of patients with periodontal disease was 2.6 ± 0.4 mM in (70, 74). Furthermore, patients with periodontal disease have elevated blood concentrations of TNF-α, which are reported to be involved in systemic disorders such as diabetes and preterm delivery (60). Taken together, these findings indicate that local and systemic synergistic effects of periodontal disease may be involved in AIDS progression (Fig. 7). We hypothesize that immunosuppression due to HIV infection leads to the following chain of pathological events: (1) periodontal disease progression, (2) rising concentrations of butyric acid and TNF-α due to the increase in periodontal bacteria associated with worsening periodontal disease, (3) activation of latent HIV infection by the synergistic effects of local periodontal butyric acid and systemic TNF-α, and (4) AIDS progression.

Treatment of infections associated with AIDS, such as herpes and tuberculosis, slows AIDS progression, and the prevention and treatment of infections is thus the core of AIDS treatment. There are no data to indicate whether prevention and treatment of periodontal disease have a similar effect on AIDS progression, but it is expected that the causal relationship between periodontal disease and AIDS will be clarified in future epidemiological studies.

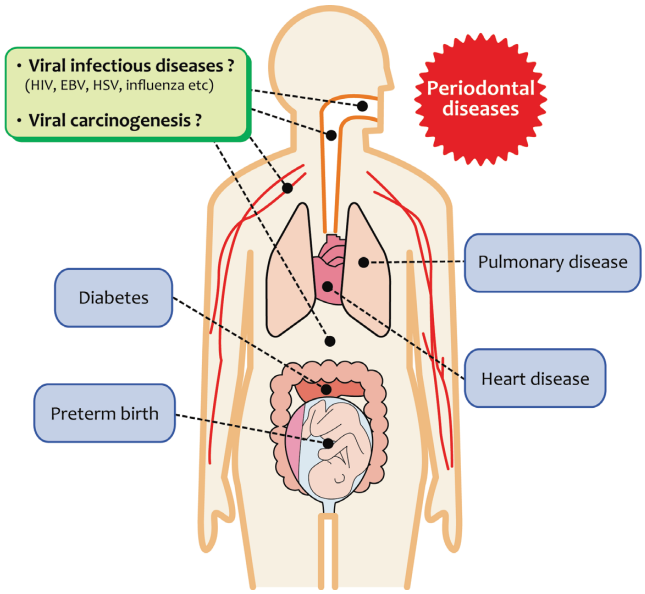

Fig. 8 Periodontal diseases and systemic diseases.
Mounting evidence supports the hypothesis that periodontal diseases are a risk factor for several systemic diseases, including diabetes, preterm birth, heart disease, and pulmonary disease. Our observations suggest that periodontal diseases and other oral diseases also affect viral infections. It is expected that future interdisciplinary studies on the cooperation between medical and other fields will investigate whether the concept of "periodontal medicine" is applicable to viral infections.

Epigenetic disorders and prospects for epigenetic therapy

Because epigenetic alterations, including posttranslational modifications of histone and DNA methylation, are readily revertible by epigenetic drugs such as HDACis

and DNA demethylating compounds, epigenetic therapy has emerged as effective chemotherapy for several intractable diseases, including cancer, rheumatoid arthritis, and viral infectious diseases. Examples of such agents include SAHA, an HDACi that has been approved by the Food and Drug Administration (FDA) for the treatment of cutaneous T cell lymphoma (75,76), and DNMT inhibitors such as 5-azacytidine and 5-aza-2'-deoxycytidine, which have received FDA approval for treatment of myelodysplastic syndrome and leukemia (77,78). In an animal model of arthritis, a significant reduction in arthritis score was observed after administration of SAHA (79). Moreover, a recent study proposed that a strong synergy exists between 5-aza-2'-deoxycytidine and butyrate in the prevention of murine lung cancer (80). Our observations suggest that AP-4 and G9a are responsible for transcriptional quiescence of latent HIV-1 provirus (53, 57), which provides a molecular basis for the reported efficacy of combination therapy using conventional anti-HIV drugs and an HDACi or HMT inhibitor in accelerating HIV-1 clearance from infected individuals. Interestingly, in a clinical study, Lehrman et al. recently reported that combination therapy with intensified HAART and the HDACi valproic acid successfully accelerated HIV-1 clearance from resting $CD4^+$ T cells in individuals infected with HIV-1 (81). Thus, it is plausible that epigenetic drugs will be of clinical benefit in preventing the clinical development of AIDS.

Although epigenetic therapy is useful in treating some epigenetic disorders, adverse effects have been reported for many epigenetic drugs. In addition, specific inhibitors that target individual epigenetic codes or modifiers remain to be developed. Further studies are needed to clarify the role of epigenetic regulation in the etiology of disease and to aid in identifying new diagnostic tools and developing novel epigenetic drugs.

Disruption of the balance of epigenetic networks is known to result in a number of intractable diseases. Based on our study of microbe-host interactions via epigenetic regulation, we have shown that periodontal diseases may affect the development of HIV-1 and other viral infections (Fig. 8). It is expected that enhancing our understanding of the pathogenesis of viral infections from the perspective of epigenetic regulation will lead to new treatments and superior methods of prevention.

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