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Bacterial Short Chain Fatty Acids Push All The Buttons Needed To Reactivate Latent Viruses

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Abstract

The genomes of herpesviruses and HIV become silent during latency through multiple chromatin silencing mechanisms including: histone deacetylation, repressive histone methylation, and DNA methylation. Reactivation of the latent virus requires removal of the chromatin silencing marks and their replacement by activating modifications such as histone acetylation and activating histone methylation. In a complementary mechanism, RNA Polymerase II (RNAP II) elongation is regulated by the positive transcription elongation factor b (P-TEFb)-dependent phosphorylation of Ser2 residues on its C-terminal domain. In resting T-cells latently infected by HIV, expression of P-TEFb is restricted. We found that a group of short chain fatty acids (SCFAs) produced by oral bacteria not only promote histone acetylation but also change the histone methylation dynamics by decreasing repressive histone methylation while increasing activating histone methylation. SCFAs also block DNA methylation and activate P-TEFb to enable elongation of stalled RNA polymerase II. Thus these molecules do not simply act as histone deacetylase (HDAC) inhibitors as previously claimed. Instead, they impact multiple complementary epigenetic regulatory mechanisms to promote highly efficient reactivation of latent viruses.

Following primary acute infection, herpesviruses enter a latent phase without viral gene expression and virion production. The viral genomes are maintained in the nucleus of the host cells as autonomous replicating DNA molecules or episomes. HIV is also able to establish latent infections in resting T-cells after integration of its genome into host chromosome. For both herpesviruses and HIV, latency permits a life-long persistent infection, and the latently infected cells are sources of productive viral replication leading to recurrent infections. Although the two viruses are genetically distinct and have very different replication cycles, during latency the viral genomes are silenced through shared epigenetic mechanisms primarily involving histone deacetylation, repressive histone methylation, and DNA methylation [1–3]. Removal of these silencing marks results in the reactivation of the latent virus.

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Oral bacterial pathogens secrete high concentrations of short chain fatty acids (SCFAs) including butyric acid, propionic acid, isovaleric acid, and isobutyric acid. SCFAs at concentrations found in the saliva of gingivitis patients strongly reactivate latent Kaposi's sarcoma-associated herpesvirus (KSHV) and HIV-1 [4, 5]. In contrast to previous reports that only butyric acid induces reactivation of latent KSHV and HIV-1 [6, 7], we found that all four SCFAs promote viral gene expression in a dose-dependent manner. When used in combination, they also exert an additive effect on viral gene expression, suggesting that the different SCFAs can work together to promote viral replication. SCFAs have been known for their abilities to inhibit class-1/2 histone deacetylases (HDACs) [8–10]. In addition to class 1/2 HDAC inhibitor (HDACi) activity, we found that SCFAs also down regulate expression of the class-3 HDAC SIRT1. As a consequence, exposure of KSHV and HIV-1 latently infected cells to SCFAs results in histone hyperacetylation. Unexpectedly, we found that SCFAs also suppress expression of the histone methyltransferases EZH2 and SUV39H1 to reduce the levels of the repressive histone tri-methylation marks H3K9me3 and H3K27me3 respectively. Moreover, using an unknown mechanism, SCFAs also increase the level of the activating histone tri-methylation mark H3K4me3 [4]. Data from chromatin immunoprecipitation (ChIP) assays confirm that treatment with SCFAs results in increased levels of acetylated histones and the activating histone methylation mark H3K4me3 and decreased levels of repressive histone tri-methylation marks H3K9me3 and H3K27me3 at the promoters of the KSHV key lytic gene RTA (ORF50) and the HIV-1 provirus [4, 5]. Therefore SCFAs do not simply act as HDAC inhibitors as previously assumed but have much broader effects on the epigenetic silencing machinery and can both remove silencing histone modification marks from and add activating histone modification marks to the viral chromatins.

Data from ChIP assays indicate that SIRT1, EZH2, and SUV39H1occupy the promoters of KSHV and HIV-1 during latency. Inhibition or "knock-down" of any one of these enzymes can be sufficient to induce reactivation of latent KSHV and HIV-1 [4, 5, 11–13]. This truly was a paradox as each of these enzymes catalyzes reaction for a different type of histone modification. One explanation for this paradox may be that the different epigenetic regulators assemble into large complexes and regulate each other. Indeed, our data demonstrated that inhibition of any one of these enzymes negatively impacts expression of the other ones [4], which confirms previous suggestions that different epigenetic regulators "cross talk" with each other [14, 15]. Our data showing the co-localization of these epigenetic regulators at the latent viral promoters (and their dissociation from the viral chromatins during reactivation) further supports the hypothesis that they act in concert to silence and activate the viral chromatins by controlling the levels of histone acetylation and the opposing histone methylations.

Compared to histone modifications, DNA methylation seems to play a less important role in viral genome silencing and affects mainly late events of viral gene transactivation [16]. The promoter of KSHV key lytic gene RTA is heavily methylated during latency but is demethylated upon stimulation with sodium butyrate [16], suggesting that SCFAs also play a role in removing CpG methylation during viral reactivation. The promoter of HIV-1 has also been observed to be heavily methylated in certain latently infected cells, however, the DNA methylation status does not affect the early events of HIV-1 transactivation[17]. Interestingly,

SCFAs, but not other HDAC inhibitors such as trichostatin-A (TSA) and suberoylanilide hydroxamic acid (SAHA), reverse DNA methylation-mediated repression in a HDAC inhibition-independent manner [18]. These results highlight the broader activity of SCFAs compared to more traditional HDAC inhibitors.

The SCFAs-induced chromatin modifications are necessary for the recruitment of transcription factors and the RNAP II complex to the viral promoters. However, effective transcription of viral genes depends not only on initiation, but also on the elongation competence of the recruited RNA polymerase II complex, which is regulated by P-TEFb-dependent phosphorylation of Ser2 residues on its C-terminal domain and phosphorylation of the negative elongation factor (NELF) [19]. In primary resting T-cells, RNAP II elongation and HIV-1 Tat activity are highly restricted due to very low levels of P-TEFb expression. In a completely unexpected result, we found that SCFAs are also able to induce P-TEFb in HIV-1 latently infected primary T-cells through induction of cyclin T1 (CycT1) expression and CDK9 phosphorylation[5]. In contrast to a recent study demonstrating that HDAC3 plays a role in suppression of P-TEFb through reduced acetylation of CDK9 and CycT1[20], our data suggest that SCFAs activation of P-TEFb mainly depends on phosphorylation of protein kinase C (PKC) rather than on HDAC inhibition. Consistent with this is our observation that the HDAC inhibitor SAHA only modestly activates P-TEFb [5].

In summary, SCFAs not only induce multiple epigenetic changes to reactivate latent virus but are also able to activate P-TEFb to enable elongation of the RNA polymerase II for effective transcription of viral genes. SCFAs are present in high levels in the oral cavity of patients with severe periodontal disease, in the gut, and in the genital tract in women with bacterial vaginosis. The broad transcriptional activities of these bacterial metabolic by-products lead to reactivation of latent viruses and may help to explain why bacterial pathogens can exacerbate the course of disease in HIV patients.

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