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Therapeutics targeting protein acetylation perturb latency of human viruses

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Abstract

Persistent viral infections are widespread and represent significant public health burdens. Some viruses endure in a latent state by co-opting the host epigenetic machinery to manipulate viral gene expression. Small molecules targeting epigenetic pathways are now in the clinic for certain cancers and are considered as potential treatment strategies to reverse latency in HIV-infected individuals. In this review, we discuss how drugs interfering with one epigenetic pathway, protein acetylation, perturb latency of three families of pathogenic human viruses—retroviruses, herpesviruses, and papillomaviruses.

Viral epigenetics and the tussle between repression and activation in the nucleus

Certain viruses replicate in the cell nucleus and have evolved elaborate strategies with which to utilize the nuclear environment for their own genetic fitness. Generally, a viral infection results in one of two outcomes—i.) a productive infection, in which the virus completes its replication cycle and progeny virions are assembled, or ii.) a latent infection, in which viral genetic material is present, but little to no viral proteins are produced. The decision between these two fates is multifactorial, and is influenced by the differentiation state of the host cell, availability of select host/viral factors, and viral gene circuitry. While a productive infection often evokes a fierce immune response, a latent infection allows the virus to chronically persist for long durations without alarming host immune cells.

Upon transiting to the nucleus, viral genomes often are assembled into nucleosomes and chromatinized[(1–5)]. The nucleosome is the organizational unit of eukaryotic chromatin in which ~146bp of DNA are tightly wrapped around an octameric core of 4 distinct acidic histone proteins (H2A, H2B, H3, and H4). The nucleosome represents a ~6 fold reduction in the physical space required to store DNA, and inherently poses a significant barrier to DNA-derived processes such as transcription[(6)]. Importantly, viral latency is a reversible

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phenomenon of transcriptional silencing achieved in part by appropriating host epigenetic processes. Such epigenetic processes include posttranslational modifications to histones, exchange of histone variants, and active assembly, disassembly, and remodeling of chromatin by molecular machines. This regulation of chromatin structure and DNA accessibility contributes to the decision between latent and productive states of viral infection. Viral DNA is often condensed into nucleosomes during latency, while during productive infection, viral chromatin is labile and bound by factors that maintain a permissive chromatin state.

Manipulation of viral latency, particularly via epigenetic-based approaches, is an emergent therapeutic avenue to combat latent viral infections in humans. Current antiviral treatments are largely limited to drugs that specifically target viral enzymes only present during a productive infection. Latent virus is refractory to these treatment strategies, yet in many cases is sufficient to reseed productive infection within the host once treatment is stopped. Latency thus represents an attractive target for viral eradication in chronically infected patients. There are at least three approaches to tackle viral latency – i.) permanent suppression, in which latent virus is irreversibly inactivated, for example by a drug or an *in vivo* gene-editing method, ii.) forced reactivation ("shock and kill")[7], in which latent virus is specifically reactivated by one drug in the presence of antivirals that target replicative virus, theoretically allowing reduction or clearance of latently infected cells by the immune system, and iii.) immune modulation, in which an infected patient's immune system is specifically primed to clear latently infected cells.

At this time, epigenetic drugs targeting protein acetylation, a posttranslational modification best studied with regard to histones, are the most clinically advanced[8]. Here, we focus on protein acetylation and how this pathway may be therapeutically exploited for the treatment of three distinct families of human pathogenic viruses—retroviruses, herpesviruses, and papillomaviruses (Table 1). While these viruses share the capacity to co-opt the host nucleus and establish latent infections, they confront the nuclear environment in unique ways. Retroviruses such as HIV are RNA viruses that undergo reverse transcription and integrate resultant proviral DNA into host chromatin. Herpesviruses are large DNA viruses that shuttle their complex genomes to the nucleus where they associate with host chromatin as a circular episome, or "mini-chromosome." Similar to herpesviruses, papillomaviruses are also maintained as episomes although may integrate into host chromatin under certain conditions. Small molecules targeting catalysis and recognition of protein acetylation are being explored for their effects on latency of these viruses and have distinct effects on viral transcription. Here, we review acetylation-targeted therapeutics and available preclinical and clinical data investigating the application of these drugs in latent viral infections.

Drugs targeting the protein acetylation network

Protein acetylation is one of the best-studied epigenetic mechanisms of transcriptional control[9]. While first discovered to modify histones[10], acetylation is now known to regulate many epigenetic factors, including important transcriptional regulators such as NF-κB[11], P-TEFb[12], and RNA polymerase II itself[13]. Acetylation impacts protein function by altering stability, localization, and affinity for other proteins and nucleic acids. This is

achieved via at least three mechanisms– i.) neutralization of the positively charged unmodified lysine residue, ii.) generation of novel protein:protein interfaces, and iii.) suppression of other posttranslational modifications that can occur on lysine residues (i.e., methylation, ubiquitination, etc.). These mechanisms are not mutually exclusive, and likely work in concert to enact functional outcomes associated with acetylation. For example, transcriptional activation correlating with histone acetylation may be explained by both electrostatic antagonism of histone:DNA contacts and site-specific recognition of histone acetyl-lysine residues by a bromodomain-containing effector protein, as discussed below.

Protein acetylation is catalyzed enzymatically by lysine/histone acetyltransferases (KATs, also known at HATs)[14, 15]. There are currently ~25 known human KATs, and these enzymes are mostly known as transcriptional activators. They fall in different classes based on structural and functional homology: GNAT (i.e., GCN5, PCAF), MYST (i.e., TIP60, MOF, MOZ), p300 (p300, CBP), transcription factor-related (i.e., TAF1/TBP), and nuclear receptor-related (i.e., CLOCK). KATs contain a conserved glutamate residue that serves as a base to increase the nucleophilicity of the lysine ε-amino group allowing addition to the acetyl-Coenzyme A (CoA) carbonyl, upon which an unstable tetrahydral intermediate forms. Collapse of the tetrahedral intermediate releases reaction products CoA and acetyl-lysine. MYST family KATs employ a slightly different reaction mechanism using transfer of acetyl-CoA to a cysteine residue in the active site as an intermediate step of catalysis.

KATs are only now emerging as important and feasible drug targets[16], with p300/CBP at the forefront. Lys-CoA was the first synthetic inhibitor for the p300/CBP KAT[17] operating via a unique mechanism in which the drugs interferes with a long catalytic loop (L1) of the KAT domain that normally positions the substrate and acetyl-CoA in a basic pocket to favor spontaneous acetylation (Figure 1A)[18]. C646 is a cell-permeable pyrazolone-furan p300/CBP inhibitior that is currently being investigated mostly for cancers and inflammation[19]. Other KAT inhibitors (KATi) include isothiazolones as PCAF inhibitors[20], α-methylene-γ-butyrolactones as GCN5 inhibitors[21], and 6-Alkylsalicylates as TIP60 inhibitors[22]. Remarkably, several natural products including curcumin[23] (turmeric), garcinol[24] (*Garcinia indica* fruit rind), and anacardic acid[25] (cashew nuts) inhibit KATs with considerable potency. Curcumin targets p300/CBP specifically via a yet unknown mechanism while garcinol and anacardic acid are broader KAT inhibitors that may function by mimicking acetyl-CoA. Recent evidence shows that salicylic acid, a well-known anti-inflammatory compound, is a potent inhibitor of the p300/CBP KAT and can delay onset and clinical development of Alzheimer's disease in mice by inhibiting acetylation of the pathogenic tau protein[26]. Notably, KAT activators have been described, such as CTB (N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-benzamide)[25], which alters the p300 structure to increase enzymatic activity through an unknown mechanism.

The enzymes opposing KAT activity are lysine deacetylases (KDACs, also known as HDACs) and are well-studied drug targets[27]. KDACs are comprised of four distinct classes based upon structural homology where class I, II, and IV KDACs utilize a divalent zinc ion coordinated in the active site to catalyze deacetylation upon nucleophilic attack of water on the acetyl-lysine residue. Class III KDACs or sirtuins operate via a distinct mechanism involving nicotinamide adenenine dinucleotide (NAD^+) in lieu of an active site metal. Class

I KDACs localize mainly to the nucleus, where they largely repress cellular transcription, while Class II and IV KDACs generally shuttle between the nucleus and the cytoplasm. Sirtuins are found in the nucleus, cytoplasm and in mitochondria.

KDAC inhibitors (KDACi) primarily target class I and II KDACs, and some (i.e., vorinostat, romidepsin, panobinostat) are clinically approved for the treatment of certain types of cancer, such as T cell lymphomas and multiple myeloma. To date, there are over 500 ongoing/recruiting clinical trials involving KDACi for different indications. Although chemically diverse, these compounds share a basic structure of a variable cap that aids in recognition of the KDAC surface, an aliphatic spacer, and the active moiety functional group. KDACi mostly chelate the divalent zinc cation in the deacetylase active site to achieve reversible inhibition (Figure 1B). KDACi fall roughly into four structural classes—i.) short-chain aliphatic acids, such as valproic acid and sodium butyrate ii.) hydroxamates, such as trichostatin A (TSA) and vorinostat iii.) benzamides, such as entinostat and iv.) cyclic tetrapeptides and depsipeptides, such as trapoxin A and romidepsin. Class III KDACs, particularly SIRT1, are attractive drug targets given their connection with life- and health-span extension[28]. EX-527 is a specific SIRT1 inhibitor and functions by engaging the enzyme during an NAD^+-dependent intermediate step in catalysis[29]. In contrast, the natural phenol resveratrol as well as several small-molecule drugs activate SIRT1 activity through allosteric mechanisms[30].

Protein acetylation is controlled non-enzymatically via the bromodomain, an important "reader" module of ~110 amino acids that binds acetyl-lysine residues[31]. 61 distinct bromodomains are found encoded in 46 proteins within the human proteome, with 1–6 bromodomains found in a single protein. All are nuclear factors and comprise modifying enzymes, nucleosome remodelers, and transcription factors. The bromodomain:acetyl-lysine interaction has relatively low affinity *in vitro*[32], yet the local chromatin environment and the complex in which the bromodomain-containing protein assembles may enhance bromodomain binding *in vivo*. The structure of the bromodomain:acetyl-lysine interaction is well characterized and serves as a valuable tool for drug development. The bromodomain adopts a distinctive structure comprised of four anti-parallel α-helices that generate a hydrophobic cleft in which the acetyl-lysine is bound. A key hydrogen bond is made between the acetyl-lysine and a conserved asparagine that is found in nearly all bromodomains. This asparagine is a critical target for inhibitors of the bromodomain:acetyl-lysine interaction (Figure 1C), most notably the benzodiazepine derivatives JQ1[33] and I-BET[34], both inhibitors of the double bromodomain and extraterminal domain (BET) subfamily of proteins. Because JQ1 is active against certain cancers based on its ability to suppress expression of the oncogenic transcription factor c-Myc[35], several BET inhibitors (BETi) are currently in early clinical trials.

Human viruses, latency, and acetylation-targeted therapeutics

Retroviruses (Human immunodeficiency virus (HIV))

Retroviruses like HIV encode small, diploid, single-stranded, positive-sense RNA genomes that are reverse transcribed and integrated into host chromatin. HIV infects primarily $CD4^+$ T cells and macrophages to establish a life-long infection that, if untreated, leads to the

development of acquired immunodeficiency syndrome (AIDS). It is estimated that 35 million people are living with HIV/AIDS, and the majority of infections are in developing countries. With the introduction of effective antiretroviral therapy, infected individuals rarely develop AIDS and have nearly normal life spans. However, treatment is not curative and requires life-long adherence due to a small reservoir of latently infected cells in which viral transcription is durably suppressed after integration into host chromatin[36]. HIV latency occurs mainly in resting memory T cells, but also in macrophages, microglial cells, gut-associated lymphoid tissues, and hematopoietic progenitor cells. Establishment of HIV latency likely occurs early in infection[37] and is influenced by the integration site[38], availability of certain host factors[39], and stochastic transcriptional changes coupled to the strong positive feedback loop driven by the viral transactivator of transcription (Tat)[40].

HIV transcription is orchestrated by a single promoter located within the viral 5′ long terminal repeat (LTR) that drives the expression of a multiply spliced mRNA that fuels viral replication[41]. The HIV promoter behaves similarly to that of a signal-inducible human gene in that it is highly responsive to extracellular stimuli and readily initiates RNA synthesis in host chromatin, yet does not proceed past 30–100 nt of synthesis. This phenomenon is known as proximal promoter pausing of the host RNA polymerase II complex and also occurs at a large fraction of human genes, particularly those that encode important cell type-specific regulators of fate and plasticity[42]. Negative transcription elongation factors, such as NELF and DSIF, sterically hinder polymerase passage past the proximal pause site (Figure 2A). Productive HIV transcription relies on the recruitment of the positive transcription elongation factor b (P-TEFb) and associated “super elongation” factors by HIV Tat to TAR, an RNA element present in the 5′ extremity of initiating viral transcripts. Thus, HIV transcription represents a positive feedback loop in which minimal processive transcription can result in synthesis of Tat that feeds back to fuel robust transcription elongation.

The latent integrated HIV provirus is readily chromatinized to adopt several well-defined nucleosomes independently of the integration site, including a nucleosome positioned immediately downstream of the HIV transcription start site (nuc-1)[43]. Factor acetylation has long been recognized as a critical regulator of the HIV locus, as early studies showed that KDACi reactivated viral transcription in latently infected T cell lines by disrupting nuc-1 positioning[44]. These studies have been extended to different KDACi spanning all structural classes in different models of latency, including Jurkat latency (J-Lat) cell lines and primary cells[45]. These findings indicate that during HIV latency, nuc-1 undergoes constitutive deacetylation by KDACs to maintain transcriptional silencing. Indeed, class I KDACs are recruited to the HIV promoter (i.e., via c-Myc, Sp1[46], and YY1[47]) and TSA treatment induces nuc-1 histone H4 hyperacetylation[47, 48]. Importantly, Tat cooperates with KATs (i.e., p300/CBP, PCAF[49]) to further mediate local acetylation of the HIV locus. The importance of KATs in Tat-mediated transcription is underscored by the potent transcriptional inhibition mediated by KATi curcumin[50]. In addition to histones, acetylation of *trans*-acting factors regulates HIV transcription, with targets including Tat[51], P-TEFb[12], and subunits of NF-kB[11]. Latency reversal with KDACi is robust in cell line models and has been tested clinically[52], but appears most promising in patient cells when combined with T cell activators such as protein kinase C (PKC) agonists[53]. Although many

targets of acetylation act at the HIV promoter directly, indirect effects of KDACi on cellular genes involved in regulation of HIV transcription remain to be rigorously explored. Importantly, it is estimated that KDACi treatment manipulates expression of 2–10% of total genes[54], and what the effect of these host transcriptomic changes are on viral latency reactivation is underappreciated.

The functional outcomes of acetyl-lysine recognition by bromodomain-containing proteins are complex at the HIV promoter. Acetylated Tat was originally shown to generate an interaction surface for the PCAF bromodomain[55], and initial attempts to target this interaction therapeutically to inhibit Tat transactivation yielded two compounds[56]. In contrast, BETi reactivate HIV from latency across different experimental systems, including primary cells[57–60]. The BET protein BRD4 and Tat are competitive for limiting levels of P-TEFb[61], which in part explains how displacement of BET proteins from chromatin favors Tat-mediated transactivation[58]. BETi nonetheless reverse latency in models devoid of Tat[59], suggesting that other mechanisms contribute to the activity of these compounds in HIV latency reversal. Plausible hypotheses include increasing levels of active P-TEFb required for HIV transcription, BET proteins exerting direct repressive functions at the HIV promoter, and indirect transcriptional effects[62]. Similar to KDACi, BETi synergize with low dose PKC agonists in primary cells[53]. Importantly, KDACi and BETi do not synergize in HIV latency reactivation[57], suggesting that these compounds work through similar pathways.

Herpesviruses (Herpes simplex virus (HSV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV))

Herpesviruses are extremely pervasive viruses that maintain their large double-stranded DNA genomes as circular episomes topologically associated with host chromatin. Under a productive infection, herpesvirus DNA is scarcely associated with nucleosomes and is transcribed in a highly coordinated and sequential manner; groups of genes are expressed at immediate early (IE), early (E), and late (L) stages of infection. IE genes are transcribed and translated in the absence of *de novo* viral protein synthesis and maintain a chromatin environment permissive to transcription by destroying nuclear compartments important for subsequent virion formation (i.e., PML nuclear bodies), reducing nucleosome occupancy, and/or altering the activity of histone-modifying enzymes. E genes function to replicate viral DNA, while L genes are expressed only after DNA replication has begun. Productive or lytic infection is brief as infected cells are mainly destroyed due to cytopathic effects of the virus. The herpesvirus family has thus evolved intricate mechanisms to persist in a latent form in certain cell types.

Herpesviral latency is complex, and characterized by the variable gain of a restricted gene expression program that allows for production of proteins and noncoding RNAs to enforce latency and alter the host. Strikingly, herpesviruses regulate their chromatin in a locus-specific manner with select regions open and active during latency. We focus here on an α-herpesvirus, HSV, and two γ-herpesviruses, EBV and KSHV. HSV establishes latency in the nervous system, allowing long-term persistence and access to epithelial tissues where

productive infection occurs[63]. The γ-herpesviruses are tumor viruses whose latency programs are causally associated with certain cancers[64, 65].

HSV

While HSV productively infects oral (HSV-1) or genital (HSV-1, −2) epithelial cells causing cold sores or genital lesions, it is also neuroinvasive and establishes latency in ganglionic neurons innervating the site of primary exposure. HSV-1 is quite common both in developed and developing countries, with >80% of the population being infected by the age of 50. HSV-2 is less common, with ~30% of the population being infected by age 50. There are several models how HSV latency is established in neurons[63]: i.) VP16, a viral tegument protein required for IE expression, is lost during retrograde transport to the neuronal nucleus ii.) host factors required for VP16-mediated IE expression (i.e., Oct-1 and HCF1) are missing or limited in neurons and iii.) VP16 is not properly posttranslationally modified in neurons. Importantly, latent HSV retains expression of latency-associated transcripts (LATs) thought to cause RNA-mediated heterochromatinization of the HSV genome, antisense inhibition of IE transcription, and inhibition of neuronal apoptosis. Stress to the host (i.e., fever, UV light, physical/emotional stress, nerve damage) can reactivate latent HSV in neurons, leading to viral particle assembly, anterograde transport to the epidermis where productive, symptomatic infection and transmission can occur.

The latent HSV genome that persists in neurons is largely heterochromatinized and displays a regular pattern of host-derived nucleosomes bearing modifications indicative of repressed transcription (i.e., H3K9me3 and H3K27me3)[66, 67]. Accordingly, acetylation levels on the latent HSV genome are low, although the neuronal-specific LAT promoter bears hyperacetylated histones[68]. Acetylation of HSV-associated histones at lytic genes is a hallmark of productive infection[69]. Curcumin is a potent inhibitor of lytic HSV[70], but also blocks latency reactivation in ganglionic cultures stimulated with nerve growth factor[71]. The effects of curcumin on HSV latency are independent of p300/CBP inhibition[70], and genetic knockdown of KATs does not decrease characteristic histone acetylation marks (i.e., H3K9ac and H3K14ac) at IE promoters during induction from latency[72]. This suggests non-histone targets or indirect effects explain the effects of KAT inhibition on HSV expression. Interestingly, activation of the p300/CBP KAT activity by CTB promotes latency reactivation by inducing IE expression and inhibiting LAT expression[71], supporting the model that KATs are positive regulators of HSV IE gene expression.

KDACi, including short-chain aliphatic acids and hydroxymates, consistently reactivate HSV from latency both in cell-based[73] and animal models of latency[74]. KDAC-mediated surveillance of viral chromatin maintains latency through KDAC1/KDAC2-containing CoREST/REST repressive complexes associated with HSV chromatin[75]. KDACi are thought to inhibit CoREST/REST complexes at IE genes, which is supported by the potency of entinostat, a benzamide KDAC inhibitor that shows specificity for KDAC1 at low doses, in murine trigeminal ganglia[71]. Interestingly, the viral IE gene product ICP0 serves as an autonomous KDAC inhibitor by disrupting the interaction between CoREST and KDAC1/KDAC2[75]. Overall, these findings support a model wherein a dynamic equilibrium of acetylation at HSV chromatin locally governs the decision between lytic and latent

infection[76]. KDACi induce the formation of progeny virions from neurons, but it remains to be tested whether forced reactivation in the presence of antiherpesviral agents can lead to clearance of viral reservoirs.

EBV

EBV causes mononucleosis and in its latent form is associated with different types of cancer, such as Burkitt's and Hodgkins lymphomas[65]. EBV productively infects endothelial cells in the oropharynx during oral contact, yet the virus eventually reaches the B cell compartment where it can establish a latent infection that persists lifelong. EBV is widespread, with >90% of US adults having detectable antibodies indicative of current or previous EBV infection. EBV latency is complex and consists of four types (0–III) that may reflect distinct differentiation states of infected host cells. EBV latency types are ranked in order of increasing gene expression: latency type 0 (resting B cells) displays no detectable gene expression, latency type I (Burkitt's lymphoma, proliferating B cells) expresses EBV nuclear antigen 1 (EBNA1) and viral microRNAs, latency type II (Hodgkin's lymphoma, carcinomas) expresses EBNA1, microRNAs, and latency membrane proteins (LMPs), and latency type III (immortalized lymphoblastoid cell lines) expresses six EBNA proteins and three LMPs. Molecular mechanisms of type III latency are best studied, likely due to the tractable experimental system of immortalized lymphoblastoid cell lines. During primary infection, EBNA2 accumulates and, through interaction with host transcription factor PU.1, activates the Cp promoter that in turn is responsible for activation of type III latency. Type III latency (also known as "Cp on latency") leads to the production of a ~120kb polycistronic RNA that is differentially spliced to encode for EBV latency proteins that drive immortalization of B cells *in vitro*. It is important to note that latency type III can relax to more restrictive patterns of gene expression of type II and I, although this phenomenon is not well understood. Lytic induction requires activation of the Zta promoter that drives expression of the IE gene product Zta (also known as BZLF1), a transcription factor required for expression of lytic genes (Figure 2B).

The EBV episome is well studied with respect to epigenetic regulatory mechanisms, including DNA methylation, histone posttranslational modifications, chromatin assembly/disassembly, and long-range chromosomal interactions with itself and the human genome[77, 78]. Histone acetylation marks, including those at histone H3K9 and H3K27, are present at the Cp region under type III latency, at the Zta promoter during lytic induction, and more generally at LMP and microRNA promoters in lymphoblastoid cell lines[79, 80]. This indicates that histone acetylation is involved in the regulation of EBV latency type decision and lytic induction in a locus-specific manner. The KAT inhibitor curcumin negatively regulates EBV reactivation and EBV-mediated B cell immortalization, underscoring the functional importance of cellular KATs in activating lytic infection. Conversely, structurally diverse KDACi, including valporic acid, vorinostat, and panobinostat, induce lytic EBV gene expression in different models[81–83]. Certain cell lines carrying EBV, however, are refractory to induction of lytic infection by KDACi, even though global histone hyperacetylation occurs in these models[84]. In responsive cell lines, it is assumed that hyperacetylation of the Zta promoter and consequent transcriptional activation causes lytic gene expression. In resistant cell lines, the model is that indirect, inhibitory

transcriptional effects of the drugs dominate. When combined with drugs targeting replicative virus, such as ganciclovir[85], KDACi become toxic to B cells undergoing lytic EBV infection. This is a first indication that forced reactivation may reduce latent EBV reservoirs and block cancer development.

Various bromodomain:acetyl-lysine interactions are functionally important for the EBV life cycle. Firstly, the bromodomain of the CBP KAT is necessary for Zta-facilitated histone acetylation and lytic induction[86]. Secondly, the BET protein BRD4 interacts with EBNA1 and is critical for its full transactivation capacity[87]. Accordingly, the BET inhibitor JQ1 reduces Cp transcription via loss of P-TEFb[88] and reduces activity of virally induced "super-enhancers," or areas of active transcription with high densities of the transcriptional coactivator complex mediator and BRD4 that uniquely tie together the fate of the EBV genome and the cell state of its lymphoblastoid host[89].

KSHV

KSHV is causally linked to several malignancies in immunocompromised individuals, including Kaposi's sarcoma and primary effusion lymphoma (PEL)[64]. It is estimated that 10% of the population in North America is infected with KSHV, and this number is considerably higher in developing countries. KSHV displays tropism for lymphocytes, fibroblasts, epithelial, and endothelial cells. Similar to other herpesviruses, KSHV latency is characterized by a restricted pattern of gene expression, although several proteins (i.e., LANA, vFLIP, and vCyclin) and noncoding RNAs are expressed and function in latency maintenance, immune modulation, and cell transformation. Most infected cells in KSHV-associated tumors display a latent phenotype, suggesting that latency is required for oncogenesis, although lytic KSHV is linked to cancer development as well[90]. The switch between lytic and latent states is governed by the viral IE gene product RTA (also known as ORF50), which activates lytic gene expression through interactions with several host transcription factors (i.e., AP1, C/EBPα) and has E3 ubiquitin ligase activity.

Epigenetic regulation of KSHV latency is mechanistically similar to EBV, in that locus-specific chromatin modifications contribute to the latent phenotype. During latency, histone hypoacetylation is observed at the RTA promoter[91], while nucleosomes encompassing the LANA binding sites required for episome replication are hyperacetylated[92]. Very little work has been done on the effects of KATi on KSHV latency, although these enzymes are functionally important in the KSHV life cycle, and several viral factors directly associate with KATs (i.e, RTA with p300/CBP[93]). Different KDACi, including valproic acid, TSA, and sodium butyrate, reverse KSHV latency[91, 94], suggesting that class I and II KDACs constitutively act to maintain KSHV chromatin in a latent state. Modulation of class III KDAC activity also disturbs KSHV latency. Nictotinamide and sirtinol reactivate KSHV latency, accompanied by increases in histone acetylation at the RTA promoter[95]. Interestingly, SIRT1 interacts with RTA and binds its promoter. The SIRT1 activator, resveratrol, inhibits KSHV reactivation, concordant with a model of continuous surveillance of KSHV chromatin by class III KDACs. Importantly, KSHV LANA is acetylated in infected cells[96] and may play an unappreciated role in the response of latent KSHV to KDACi.

KSHV LANA directly interacts with the BET ET domain[97], and this interaction may be functional in altering host cell cycling and in tethering the replicative viral episome to chromatin during mitosis. The role of BET proteins in KSHV transcription remains relatively unexplored although it is known that JQ1 does not stimulate KSHV lytic induction[98]. However, JQ1 effectively kills PEL cells by targeting c-Myc[98], and may therefore be valuable in treating KSHV-associated cancers.

Papillomaviruses (Human papillomavirus (HPV))

HPVs encompass of group of >200 different DNA viruses that infect squamous epithelial cells to produce a variety of lesions, including genital/anal warts and certain cancers[99]. HPV is considered the most common sexually transmitted disease, with ~79 million people infected in the United States. The viral genome is ~8kb in size and is maintained normally as an episome, although integration of the HPV genome does occur and is a major risk factor for carcinogenesis. The HPV life cycle is intimately coupled to the differentiation state of its host cell in the epithelial striatum; latent virus persists at a low copy number in mitotically slowed cells in the basal layer, while productive infection is initiated as the latently infected cell asymmetrically divides and migrates to the upper epithelium. Different HPV genotypes are classified as high-risk or low-risk according to their capacity to malignantly transform cells. Oncogenesis can arise from high-risk HPV infection (i.e., HPV16, −18) mainly due to increased expression of viral E6 and E7 oncoproteins, which deregulate p53 and Rb, respectively, to induce cell transformation. The HPV E2 protein, a sequence-specific regulatory factor important for maintenance of the replicative episome and viral transcription, tightly limits expression of E6 and E7. Loss of E2 function, notably through an integration event disrupting the E2 gene, leads to E6 and E7 expression and virally induced carcinogenesis.

Epigenetic alterations to viral chromatin during high-risk HPV infection and cervical carcinogenesis have been studied, with methylation of viral DNA being the best characterized[100, 101]. Nucleosomes readily form over HPV DNA at the early and late promoter regions both *in vitro* and in cell lines harboring extrachromosomal[102] and integrated HPV genomes[103, 104]. Strikingly, the nucleosomes of the early and late promoters bear acetylated histones in undifferentiated cells, suggesting that HPV chromatin is in an open state throughout its replication cycle[105]. These histones become further acetylated during differentiation[105]. Interestingly, curcumin inhibits E6 and E7 expression in HeLa cells, suggesting that histone hyperacetylation may be important for active transcription of these viral factors[106]. However, HPV proteins also manipulate acetylation of nonhistone factors. E6 inhibits p300-mediated acetylation of the tumor suppressor p53[107], and E7 upregulates SIRT1 to induce constitutive deacetylation of p53[108], both outcomes implicated in p53 deregulation and oncogenesis. KDACi such as valproic acid and sodium butyrate may restore p53 function by inducing its hyperacetylation and consequently inhibiting its degradation. Cervical cancer cells are sensitive to KDACi, likely through upregulation of the p21 tumor suppressor. Although in various cell lines and model systems KDACi induce expression and copy number of HPV, in primary tumors E6 and E7 expression was unchanged during valproic acid treatment[109]. Collectively, these findings

indicate that KDACi do not target HPV chromatin directly, but rather disrupt the ability of the virus to control acetylation pathways in the infected cell.

Several groups have shown that BRD4 physically interacts with E2, an interaction functionally implicated in both episome maintenance and its transcriptional regulation[110–112]. BRD4 tethers E2 to mitotic chromatin ensuring proper segregation and replication of the viral genome. The interaction between E2 and BRD4 is mutually exclusive with the active BRD4:P-TEFb complex[113], as both E2 and P-TEFb engage the extreme C-terminus of BRD4. Disruption of active P-TEFb by E2 may account for E2-mediated viral oncogene repression, although other host factors are likely involved[114]. Interaction with BRD4 is also required for full transactivation capacity of E2, likely through E2-independent P-TEFb recruitment. JQ1 dissociates BRD4:E2 from chromatin, disrupts viral episome maintenance, and causes a transient increase in viral genome amplification (Figure 2C)[115–117]. In addition, JQ1 inhibits HPV transcription presumably via P-TEFb[118]. These findings suggest that JQ1 may have therapeutic effects against HPV by i.) transiently stimulating HPV genome amplification and possibly immune detection and ii.) generally suppressing HPV transcription. Further studies are needed to validate these hypotheses.

Conclusions, outlook, and perspective

It is clear that latency programs of distinct viruses utilize protein acetylation as a means to regulate chromatin structure, transcription, and viral fate. Acetylation-targeted therapeutics perturb these latency programs in different ways with several commonalities among the discussed viruses—i.) all encode viral-specific transactivators that reverse latency (Tat, VP16, Zta, RTA, E2) via a mechanism that includes recruitment of KATs, therefore KATi generally enforce latency and inhibit lytic induction, ii.) latent viral chromatin appears to undergo constitutive deacetylation via KDACs, and therefore KDACi generally reactivate viruses from latency, and iii.) BET proteins play distinct roles in viral latency and can impact both viral transcription (i.e., HIV, EBV, and HPV) and replication (HPV).

Given the prevalence of chronic latent infections, particularly by herpesviruses, the effects of epigenetic-based therapies on these viruses should be considered in the clinic, either with therapeutic intention or with awareness of potential adverse drug reactions. Ideally, the potency of these agents to regulate viral transcription programs might be therapeutically harnessed to durably suppress (KATi) or reactivate (KDACi) latent virus. While a “shock and kill” approach has advanced to clinical stages in chronic HIV infection with KDACi[52], the concept of durable viral suppression is currently only emerging[119] and further studies are required to validate KATi in this therapeutic strategy. Development of BETi to treat various cancers is rapidly advancing, but their therapeutic use in viral latency or detailed knowledge about side effects on latent viruses is still in very early stages.

Notably, many epigenetic pathways apart from protein acetylation are currently being explored as therapeutic targets mainly in cancer treatment. These include inhibitors of DNA methyltransferases approved for clinical use and modestly active in reactivating latency of HIV and HSV, with more consistent data for EBV. Protein methyltransferase inhibitors, although not currently in the clinic, may also prove useful in the reactivation of latent

viruses, given strong links between several methyltranferases (i.e., EZH2) and latency of the discussed viruses. Conversely, demethylase inhibitors, such as inhibitors of the lysine-specific demethylase (LSD1) and Jumonji proteins, may support durable viral suppression strategies.

Collectively, these epigenetic-based therapies– alone or in combination– represent an exciting new class of drugs that should be fully explored to reduce, clear, or permanently enforce latency of human viruses in infected patients.

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Keywords

Acetylation
A posttranslational modification in which the acetyl group from acetyl-CoA is transferred enzymatically to the amino group in the ε-position of a lysine side chain.

Lysine acetyltransferases (KATs, also known as HATs)
Enzymes that catalyze the transfer of the acetyl group from acetyl-CoA to lysine residues to form ε-N-acetyl-lysine.

Lysine deacetylases (KDACs, also known as HDACs)
Enzymes that catalyze removal of acetyl groups from acetyl-lysine residues. These enzymes can be ion dependent (class I, II, IV KDACs) or NAD^+-dependent (class III KDACs, or sirtuins).

Bromodomain
A nuclear protein domain of ~110 amino acids that binds and recognizes acetyl-lysine residues.

Latency
A reversible state of non-productive viral infection in which viral genetic material is present, but little to no viral proteins are produced.

Retrovirus
A family of RNA viruses that reverse transcribe and integrate their genomes into host chromatin (i.e., HIV).

Herpesvirus
A family of large DNA viruses that are maintained as multicopy, extrachromosomal episomes (i.e., HSV, EBV, and KSHV).

Papillomaviruses

A family of DNA viruses that persist in infected cells as either a multicopy episome or as an integrant under certain conditions (i.e., HPV).

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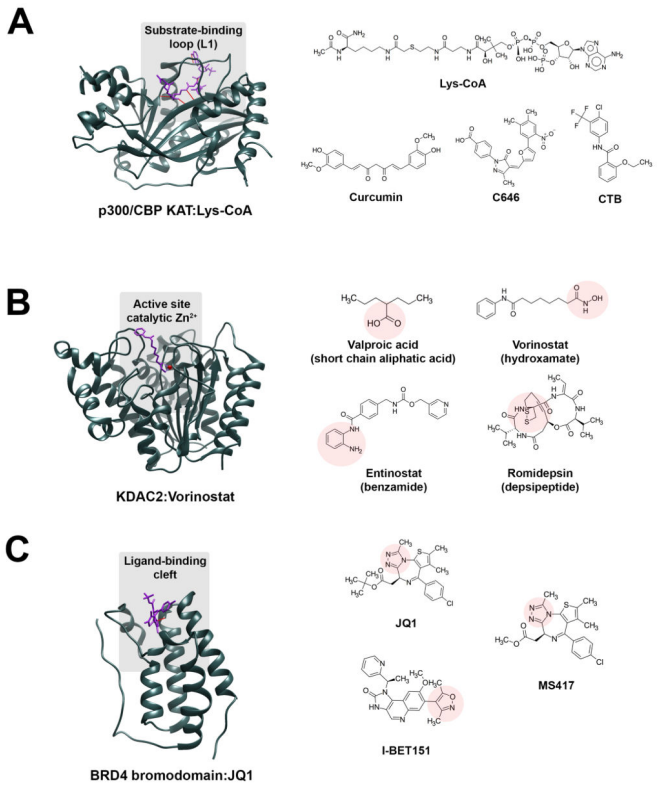

Figure 1.

Drugs targeting catalysis and recognition of protein acetylation – A. Structure of p300 KAT domain (dark grey) in complex with bidentate Lys-CoA inhibitor (purple) with hydrogen bonds (red) formed between the L1 catalytic loop and Lys-CoA shown (*left,* PDB: 3BIY[18]). Chemical structures of select KATi (*right).* B. Structure of KDAC2 KDAC domain (dark grey) in complex with vorinostat (purple) with divalent zinc chelation shown (*left*, PDB: 4LXZ[120]). Chemical structures of select KDACi, with active moieties shadowed in red (*right).* C. Structure of first BRD4 bromodomain (dark grey) in complex with JQ1 (purple) with the hydrogen bond (red) formed between the bromodomain asparagine and JQ1 shown (*left*, PDB: 3MXF[33]). Chemical structures of select BETi with active moieties shadowed in red (*right*).

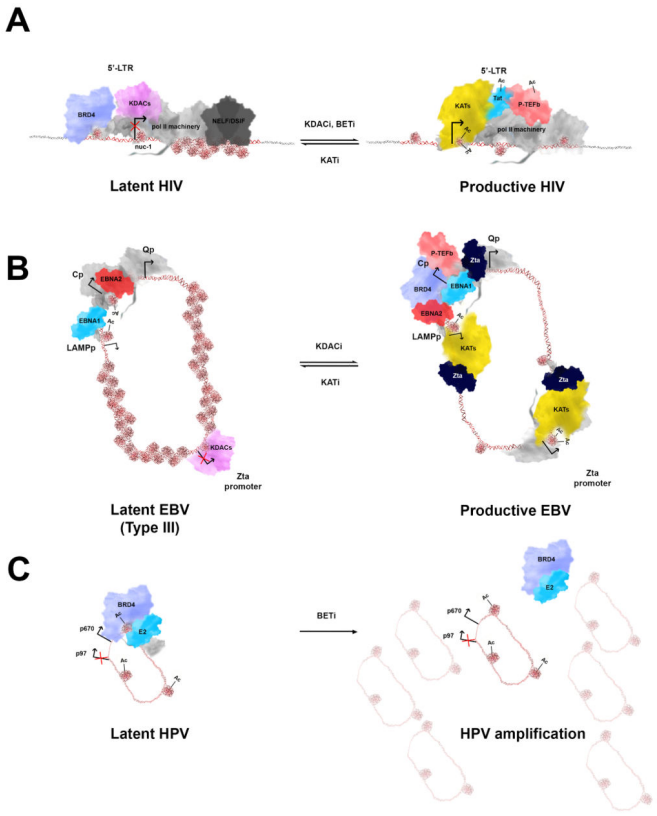

Figure 2.

Effects of acetylation-targeted therapeutics on latency of HIV (A), EBV (B), and HPV (C). A. Latent HIV is disrupted by KDACi via nuc-1 hyperacetylation and acetylation of *trans*-acting factors such as Tat and P-TEFb. BETi reverses latency through relief of potentially repressive BET complexes that inhibit transcription via as yet undefined mechanisms. Conversely, HIV transcription is inhibited by KATi via hypoacetylation of nuc-1 and *trans*-acting factors. B. Latent EBV is reversed by KDACi via hyperacetylation of EBV chromatin, particularly the Zta locus. KATi inhibit EBV transcription by reducing acetylation marks on EBV chromatin. C. BETi induces amplification of HPV chromatin by removing BRD4:E2 tethered HPV episomes from human chromatin.

Table 1

Key facts of virus families in discussion.

Family	Retroviridae	Herpesviridae	Papillomaviridae
Members	HIV	HSV, EBV, KSHV	HPV
Global prevalence	0.8%	90% (HSV), 95% (EBV), 10–50% (KSHV)	12%
Genome size (kb)	9.8	150–210	8
# gene products	9	70–90	8
Genome type	Integrated	Episomal	Episomal, integrated
Latency cell types	Resting CD4+ T cell, macrophages, microglia, GALT, HSC	Neurons (HSV), B lymphocytes (EBV & KSHV)	Basal epithelial cells
Location	Active genes	PML bodies	DNA repair foci