



Hallmarks of Retroelement Expression in T-Cells Treated With HDAC Inhibitors

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A wide spectrum of drugs have been assessed as latency reversal agents (LRA) to reactivate HIV-1 from cellular reservoirs and aid in viral eradication strategies. Histone deacetylase inhibitors (HDACi) have been studied *in vitro* and *in vivo* as potential candidates for HIV-1 latency reversion. Suberoylanilide hydroxamic acid (SAHA) and romidepsin (RMD) are two HDACi able to reverse HIV latency, however studies of potential off-target effects on retroelement expression have been limited. Retroelements constitute a large portion of the human genome, and some are considered “fossil viruses” as they constitute remnants of ancient exogenous retroviruses infections. Retroelements are reactivated during certain disease conditions like cancer or during HIV-1 infection. In this study, we analyzed differential expression of retroelements using publicly available RNA-seq datasets (GSE102187 and GSE114883) obtained from uninfected CD4⁺, and HIV-1 latently infected CD4⁺ T-cells treated with HDACi (SAHA and RMD). We found a total of 712 and 1,380 differentially expressed retroelements in HIV-1 latently infected cells following a 24-h SAHA and RMD treatment, respectively. Furthermore, we found that 531 retroelement sequences (HERVs and L1) were differentially expressed under both HDACi treatments, while 1,030 HERV/L1 were exclusively regulated by each drug. Despite differences in specific HERV loci expression, the overall pattern at the HERV family level was similar for both treatments. We detected differential expression of full-length HERV families including HERV-K, HERV-W and HERV-H. Furthermore, we analyzed the link between differentially expressed retroelements and nearby immune genes. TRAF2 (TNF receptor) and GBP5 (inflammasome activator) were upregulated in HDACi treated samples and their expression was correlated with nearby HERV (MERV101_9q34.3) and L1 (L1FLNl_1p22.2k, L1FLNl_1p22.2j, L1FLNl_1p22.2i). Our findings suggest that HDACi have an off-target effect on the expression of retroelements and on the expression of immune associated genes in treated CD4⁺ T-cells. Furthermore, our data highlights the importance of exploring the interaction between HIV-1 and retroelement expression in LRA treated samples to understand their role and impact on “shock and kill” strategies and their potential use as reservoir biomarkers.

Keywords: retroelement, HDAC inhibitor, HIV, shock and kill, CD4 T-cell

INTRODUCTION

Approximately half of the human genome is composed of ancient transposable elements (TE), initially termed “selfish DNA parasites” or “junk DNA.” TEs are divided in two general groups, DNA transposons and retroelements, and have shown a role in disease development and viral infection (1–3). Retroelements are endogenous sequences of eukaryotic genomes able to replicate through an RNA intermediate (2). Over 40% of the human genome is composed by retroelement sequences (2–4). They are divided into long terminal repeat (LTR) and non-LTR retrotransposons. LTR retrotransposons are a class of “fossil viruses” originated through exogenous retrovirus infection of germline million years ago, currently known as human endogenous retroviruses (HERV) (3, 5). While HERVs compose about 8% of the human genome, non-LTR retrotransposons correspond to about 33% of the genome and are divided into short interspersed nuclear elements (SINE) and long counterparts (LINE or L1) (2, 3, 6). In contrast to SINE, L1 are autonomous elements able to replicate and insert into the human genome (7–9). Both HERV and L1 expression are associated with human diseases and with viral infections, such as HIV-1 (10–13). Both HERV and HIV-1 belong to the Retroviridae family of RNA viruses that replicate through a DNA intermediate. Their complete genome is composed by *gag*, *pol* and *env* genes flanked by two LTR regions. In contrast to HERV, HIV-1 is an exogenous retrovirus, with a similar genetic structure but capable of infecting human CD4⁺ T-cells which can lead to the development of AIDS in absence of antiretroviral treatment (ART).

Over 37 million people are currently living with HIV-1 and about 27.5 million are on treatment worldwide (14). Since HIV-1 was discovered as the causative agent of AIDS, ART has been implemented to control the infection in people living with HIV (15). ART controls the viral load and suppresses HIV-1 to undetectable levels in blood (16). However, the main challenge for an HIV-1 cure is based on the ability of the virus to establish latency in T-cells that can last for decades (17, 18). The persistence of the viral cellular reservoir is ensured through clonal expansion of infected cells and the HIV-1 genome persists within the body even after prolonged periods of undetectable viral load (18). ART cessation leads to viraemia rebound and a lifelong treatment to keep the virus under control is required. The greatest barrier for HIV-1 eradication and cure consists in identifying and eliminating those persistently infected CD4 T-cells (19).

Several studies have proposed strategies to cure HIV-1 by eliminating HIV-1 reservoirs, such as the “shock and kill” strategy using latency reversal agents (LRA) (20–25). These drugs induce an HIV-1 reactivation from the persistently infected cell reservoirs (shock) followed by an intensified ART treatment (kill). LRAs induce production of viral RNA and proteins in latently infected cells, allowing the immune system to recognize them. Suberoylanilide hydroxamic acid (SAHA, also known as vorinostat) and romidepsin (RMD, also known as FK228) are histone deacetylase inhibitors (HDACi), a class of LRA. They increase the levels of histone acetylation (26–29). Addition

of acetyl groups in histones causes chromatin relaxation, which makes promoter regions accessible to the transcription machinery and transcription factors, inducing gene expression (29–31). Both agents have been approved by the United States Food and Drug Administration (FDA) for cutaneous T-cell lymphoma treatment (32, 33). They have also been assessed *in vitro* and in clinical trials to eliminate HIV-1 through latency reversion and viral reactivation (24, 34–36). SAHA and RMD are able to inhibit histone deacetylase (HDAC) enzymes, binding covalently at its active site or chelating Zn²⁺ ions. HDAC are classified into four classes (classes I to IV), but only classes I, II and IV are Zn²⁺-dependent enzymes and are inhibited by SAHA and RMD (26, 37). In contrast to SAHA, RMD is a potent inhibitor to class I HDACs, which has been reported as responsible in part for maintaining HIV in latency. This makes RMD an attractive option for HIV-1 “shock and kill” treatment (38–41).

An important off-target effect of the shock and kill strategy is to induce expression of endogenous genes, including HERVs (42, 43). Uninfected primary CD4⁺ T-cells treated with SAHA show upregulation of HERV-9 and LTR12 as well as downregulation of ERVL (43). In addition, HERV expression also can be stimulated by HIV-1 infection in CD4⁺ T-cells, which is able to induce specific immune responses (12). Therefore, in this study, we characterized the differential expression of HERV and L1 elements in RNA-seq datasets from primary CD4⁺ T-cells (HIV-negative and HIV-1-latently infected) treated either with SAHA or RMD. We used Telescope, a bioinformatic pipeline with the ability to assign transcripts to specific genomic locations and quantify the expression of transposable elements (44). Our findings show that HDACi modulate retroelement and immune gene expression, suggesting that “shock and kill” strategy mediated by SAHA and RMD treatment induces genes expression, which could be used as biomarkers during LRA treatment.

METHODS

RNA-Seq Datasets

Herein, we analyzed RNA-seq datasets from the White et al. and the Beliakova-Bethell et al. (43, 45) studies, available in the Gene Expression Omnibus (GEO) databases under the accession numbers GSE102187 (available at: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102187>) and GSE114883 (available at: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE114883>), respectively.

In the White et al. (43) study, peripheral blood mononuclear cells (PBMCs) were collected from 4 healthy donors. Naive CD4⁺ T-cells were isolated and exposed to SAHA (10 μM) dissolved in dimethyl sulfoxide (DMSO) or untreated (DMSO solvent only) over a 24-h period. After this, RNA was extracted from cells and their untreated paired counterparts for deep sequencing. In the Beliakova-Bethell et al. study, PBMCs were obtained from four HIV-seronegative donors. Primary CD4⁺ T-cells were isolated and used to generate an *in vitro* model of latent HIV-1 infection (45). Briefly, while a portion of the CD4⁺ T cells (“bystander”) are cultivated without stimulation or infection for

5 days, another portion of cells are stained with the viable dye e-Fluor 670, infected with full-length HIV_{NL4-3} (4–6 h) and stimulated on α CD3⁺ α CD28⁻ coated plates. For establishment of latent infection in the bystander cells, after 4 days of culture, infected cells are mixed in with bystander cells and stimulated with IL-2 and IL-15. At day 7 of culture the e-Fluor negative bystander cells are recovered by cell sorting. Then, on day 10, the *in vitro* HIV-1 latency model is treated with SAHA (1 μ M), RMD (15nM), or their solvent (DMSO) for 24 h. In parallel, mock-infected cells were also cultured and treated. RNA was isolated from cells and following ribosomal RNA depletion was used to generate RNA libraries and sequenced using an Illumina HiSeq platform.

Bioinformatic Analyses

Sequence Read Archive (SRA) files from GEO were downloaded and converted into FASTQ with parallel-fastq-dump (<https://github.com/rvalieris/parallel-fastq-dump>). Host gene quantification was performed using Salmon v1.3.0 against transcript sequences from gencode v.33 plus the HIV-1 genome (K03455) as Ref. (46). Results were then merged using the tximport package in R. Additionally, reads were mapped with Bowtie2 to the human genome (hg38) with parameters—very-sensitive-local-k 100—score-min L, 0, 1.6 for multi-mappings and the resulted mappings were used as input for Telescope to accurately quantify retroelements with a single locus resolution annotation (retro.hg38.v1, available on https://github.com/mlbendall/telescope_annotation_db/tree/master/builds) (44, 47). Both Telescope and Salmon outputs were used to estimate retrotransposons differentially expressed in HDACi treated vs. untreated CD4⁺ T-cells using DESeq2 using the Wald test (48). The results were visualized by Principal Component Analysis (PCA) to check for differences between samples and group of samples using DESeq2 package (**Supplementary Figure 1**). Retrotransposons with adjusted $p < 0.05$ and absolute (\log_2 FoldChange) > 1.5 were considered differentially expressed and visualized in Volcano plots with the Bioconductor Enhanced Volcano (<https://github.com/kevinblighe/EnhancedVolcano>) and by the ggplot2 R package. In addition, Venn plots were constructed using the Venn diagram R package to show all relationships between sets of data from differentially expressed retroelements.

The five most upregulated and downregulated HERVs and L1 for each analysis were defined and shown in horizontal bar plots. The most expressed retroelements were also localized in chromosome loci and their genetic distance as well as their DNA strand orientation (sense and anti-sense) were defined using integrative genomics viewer (IGV) software and retroelement annotations (retro.hg38.v1) (49). Nearby most differentially expressed retroelement was shown in bar plot and a representative genome map was constructed using Inkscape software. Furthermore, HERV families were defined according to the retro.hg38.v1 annotation, the number of HERV loci of same family were computed and HERV family percentage was calculated and shown in pie charts.

Neighboring host genes from retroelements were defined using Telescope meta annotations (https://raw.githubusercontent.com/LLniguez/Telescope_MetaAnnotations/

main/TE_annotation.v2.0.tsv) and filtered using the list of differentially expressed retroelements in R. Over-representation analysis (ORA) was then performed with WebGestalt (available at <http://webgestalt.org/>) using gene ontology biological processes as a functional database. Genes related to immune responses were then selected and their expression was analyzed to nearby retroelement expression by fitting linear model in R.

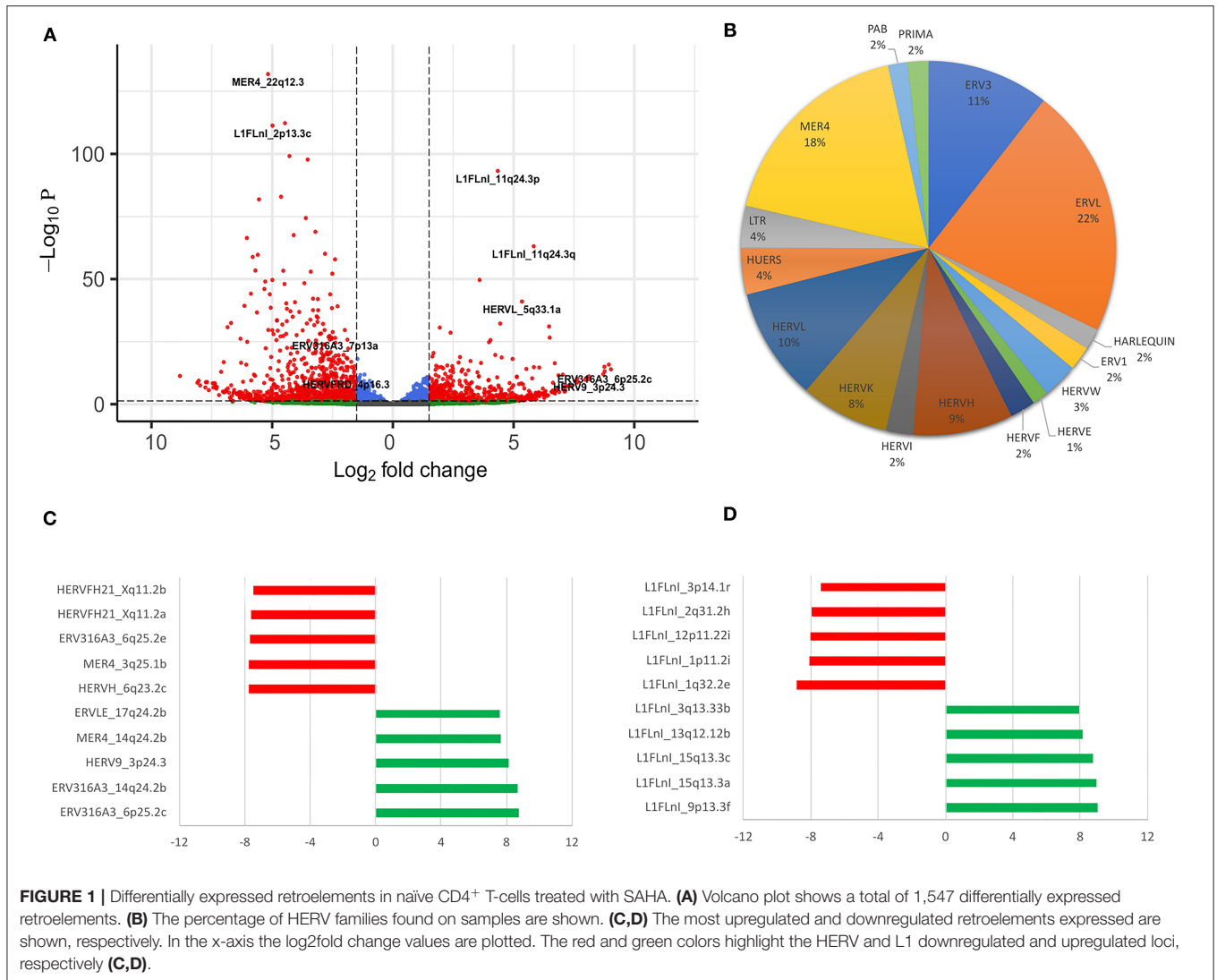
RESULTS

Retroelement Expression in Primary CD4⁺ T-Cells From Healthy Donors Treated With SAHA

To determine if LRA treatment can modulate retrotransposons expression in non-HIV-1 infected T CD4⁺ cells, we used Telescope to investigate the transcriptional modulation of retroelements in naïve CD4⁺ T-cells from four healthy HIV-1-negative donors treated with SAHA over a 24-h period (White et al. dataset). We found a total of 1,547 differentially expressed retroelements between untreated and treated samples at the 24-h time period, of which 722 corresponded to HERV sequences (303 upregulated and 419 downregulated) and 825 to L1 (285 upregulated and 540 downregulated) (**Figure 1A**). Next, we also checked if HERV expression at the family level could be affected by LRA treatment. Then, we categorized all differentially expressed HERVs according to their annotation. We found 17 different HERV families regulated by SAHA treatment (**Figure 1B**). The five most upregulated HERV loci were ERV3 (ERV316A3, 6p25.2c and 14q24.2b loci), HERVW (HERV9, 3p24.3 locus), MER4 (MER4, 14q24.2b locus) and ERVL (ERVLE, 17q24.2b locus) families (**Figure 1C**). The top downregulated HERV loci corresponded to the HERVH (HERVH, 6q23.2c locus) and HERVF (HERVFH21, Xq11.2a and Xq11.2b loci) families (**Figure 1C**). All the HERV sequences described above harbor open reading frames (ORF), allowing for potential protein coding. Proteins over 50 amino acids in length are listed for each HERV loci (**Supplementary Table 1**). Additionally, the most up- and downregulated L1 sequences were localized in chromosomes 1, 2, 3, 9, 12, 13 and 15 (**Figure 1D**).

We validated those differently expressed HERV loci in healthy cells treated with SAHA using the datasets from mock-infected CD4⁺ T-cell datasets also treated with SAHA and published by Beliakova-Bethell et al. (45). We found 172 upregulated and 235 downregulated retroelements expressed in both datasets (**Supplementary Figure 2A**). In this dataset, we found that HERVF (HERVFH21, Xq11.2a and Xq11.2b loci), HERVH (HERVH, 6q23.2c locus), ERV3 (ERV316A3, 14q24.2b locus), HERVW (HERV9, 3p24.3 locus) and MER4 (MER4, 14q24.2b locus) were also differently expressed in mock-infected cells, overlapping with the White et al. dataset (**Supplementary Figure 2B**).

Next, we analyzed nearby retroelement expression and their DNA strand orientation from most up- and downregulated HERV and L1 loci (**Figure 1C**), assessing whether genetic distance and DNA strand orientation between retroelements were able to influence their expression in the samples (**Figure 2**).



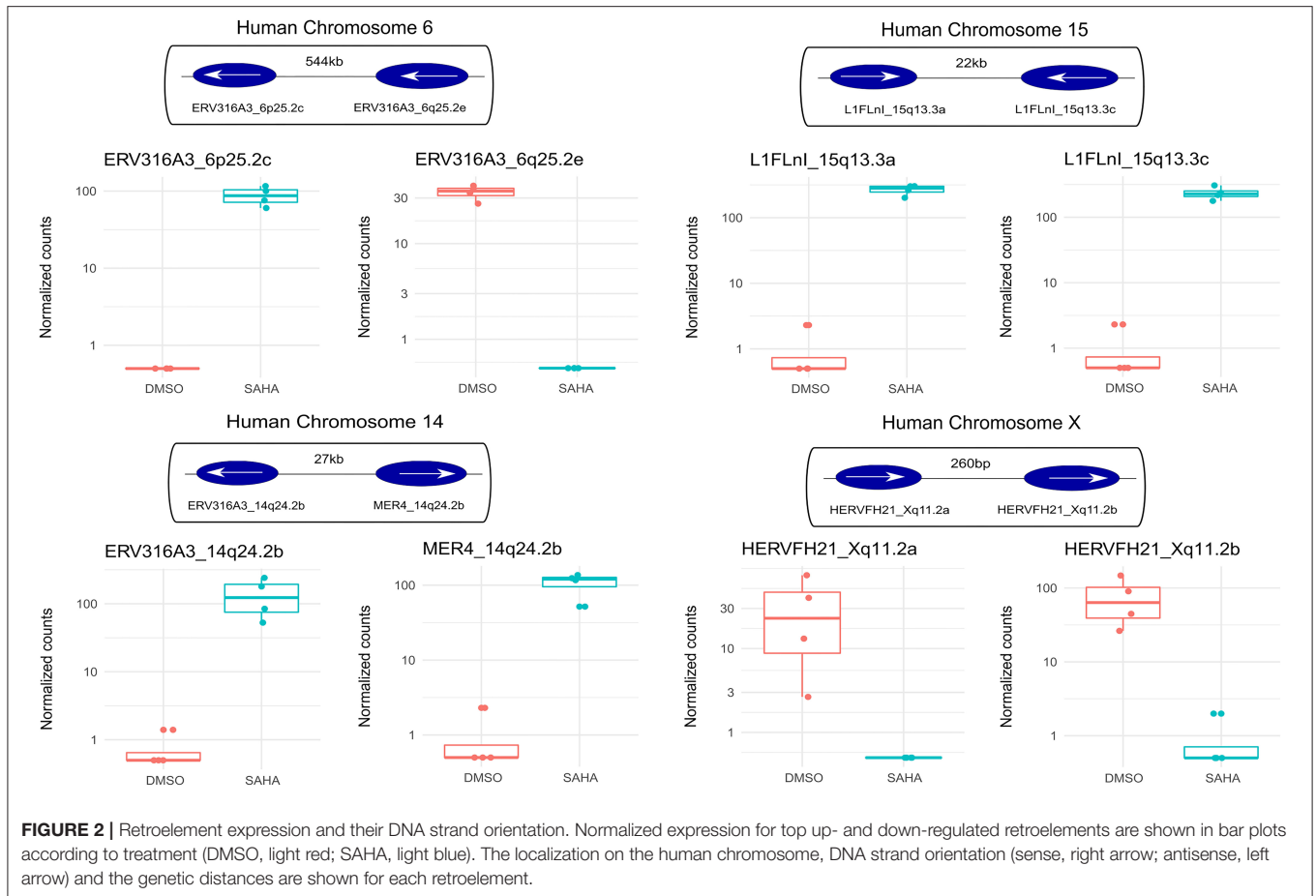
ERV316A3_6p25.2c (anti-sense) and ERV316A3_6q25.2e (anti-sense), ERV316A3_14q24.2b (anti-sense) and MER4_14q24.2b (sense), as well as LINE-1 loci L1FLNl_15q13.3a (sense) and L1FLNl_15q13.3c (anti-sense) are localized at 544, 27 and 22 kb apart, respectively. We did not find a relation in expression between these retroelement pairs when considering DNA strand orientation or genetic distance (**Figure 2**). Thus, their expression may occur independently from neighboring retroelements. In contrast, HERV FH21_Xq11.2a (sense) and HERV FH21_Xq11.2b (sense), both downregulated HERVs in SAHA-treated samples, showed a short genetic distance (~260 bp) and the same DNA strand orientation. This suggests that chromatin structure can modulate equally nearby retroelements located at a short genetic distance.

Retroelement Expression in HIV Latent CD4⁺ T-Cells Treated With SAHA and RMD

Since HDACi have been used as latency reversal agents in HIV-1 cure strategies, we assessed the impact of SAHA and RMD on

retroelement expression in CD4⁺ T cells from an HIV-1 latency model. In this model, central memory CD4⁺ T-cells latently infected with HIV-1 were treated with SAHA (1 μM) or RMD (15nM) for 24 h. Both HDAC inhibitors have been evaluated in clinical trials as latency reversal agents used in “shock and kill” strategies to eliminate HIV-1 reservoirs.

We found 712 (265 upregulated and 447 downregulated) and 1,380 (679 upregulated and 701 downregulated) differentially expressed retroelements in response to SAHA and RMD treatment, respectively (**Figures 3A–C**). The effect of SAHA and RMD on the profile of retroelement expression was different even though both drugs belong to the HDACi family (**Figures 3C–E**). While 531 HERV and L1 sequences were found differentially expressed in both treatments, a total of 1,030 HERV and L1 were specifically regulated by each drug (**Figure 3C**). We found that the level of HIV expression induced by both HDACi was limited and non-significant compared to DMSO alone (**Supplementary Figure 4**). We also aimed to analyze the impact of HIV-1 infection in these samples, comparing



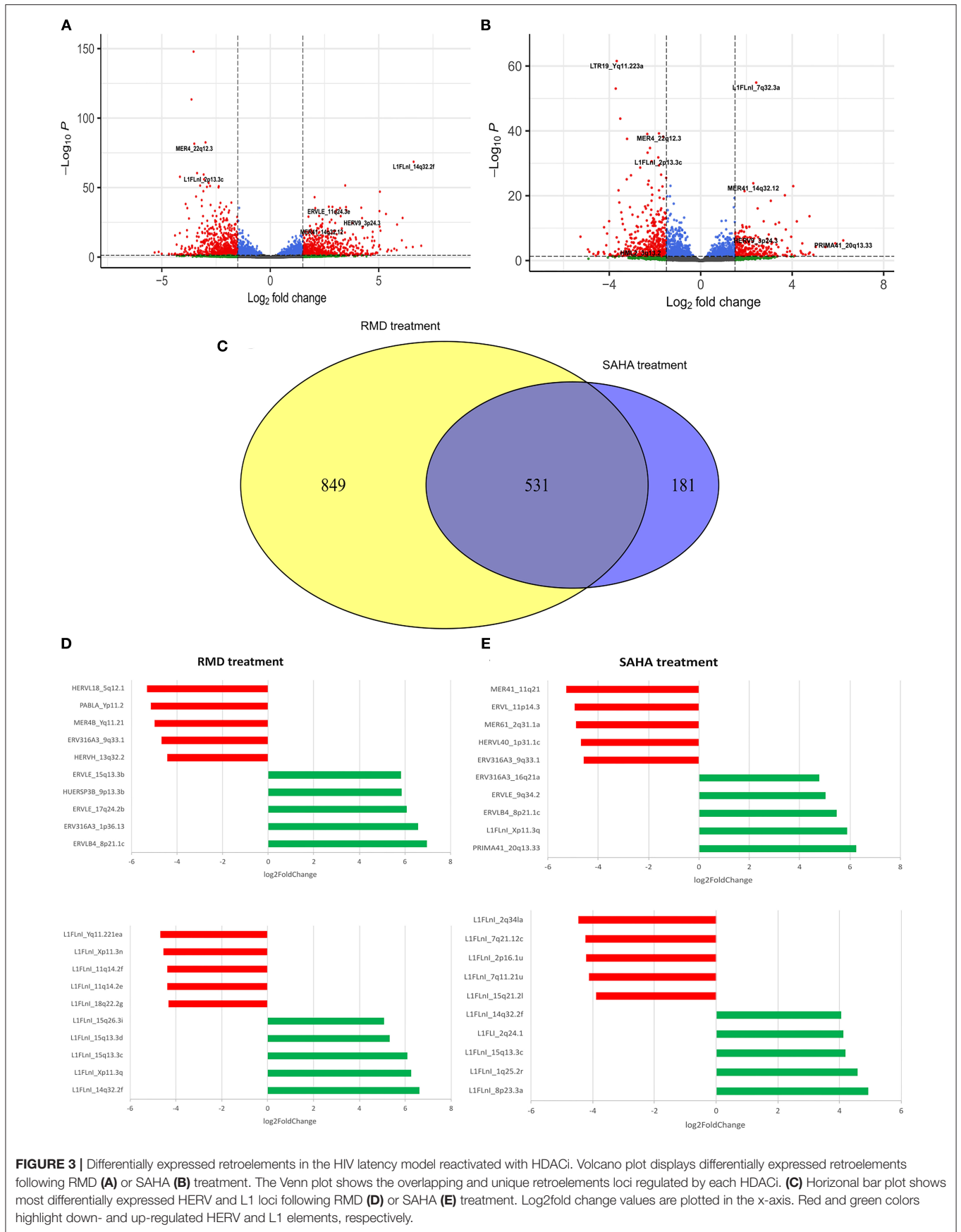
differently expressed retroelements in mock-infected CD4⁺ T-cells and HIV latent cells treated with SAHA and RMD. However, we did not find any differently expressed retroelements, suggesting that HIV-1 infection alone is not able to contribute to differential retroelement expression in cells treated with HDACi (**Supplementary Figure 3**).

Overall, at the family level, the HERV expression profile in HIV-1 latent cells treated with SAHA or RMD is similar (**Figures 4A,B**). This shows that both HDACi can regulate the expression of the same HERV families, such as full-length HERV genome families (HERV-K, HERV-W and HERV-H). In contrast, the most differentially expressed HERV elements in the SAHA treatment belong to PRIMA (PRIMA41, 20q13.33), ERV3 (ERV316A3, 16q21a and 9q33.1 loci), ERVL (ERVLB4, 8p21.1c and ERVLE 9q34.2), HERVL (HERVL40, 1p31.1c and ERVL, 11p14.3), HUERS (HUERSP3B, 9p13.3b) and MER4 (MER61, 2q31.1a and MER41_11q21) families (**Figures 3D, 4C**). With respect to RMD treatment, the most differentially expressed HERV elements belong to ERV3 (ERV316A3, 1p36.13 and ERV316A3, 9q33.1), ERVL (ERVLB4, 8p21.1c and ERVLE, 15q13.3b and 17q24.2b loci), HERVH (HERVH, 13q32.2), HERVL (HERVL18, 5q12.1), HUERS (HUERSP3B, 9p13.3b), MER4 (MER4B, Yq11.21) and PAB (PABLA, Yp11.2) families (**Figures 3E, 4D**). Additionally, these top differentially expressed

HERV loci also show ORFs, with potential protein coding capacity (**Supplementary Table 2**). These findings show that, although there are not notably differences in the general HERV family expression profile between SAHA and RMD treatment, we found that the PRIMA family in the SAHA treatment and the HERVH and PAB families in the RMD treatment are specifically most regulated by each of these HIV-1 latency reversal agents (**Figures 3, 4**). This shows that some specific phenotypes may arise from the distinct treatments and highlights the importance of analyzing retroelement differential expression at the locus-specific resolution level.

Retroelements and Immune Gene Expression in HIV Latent CD4⁺ T-cells

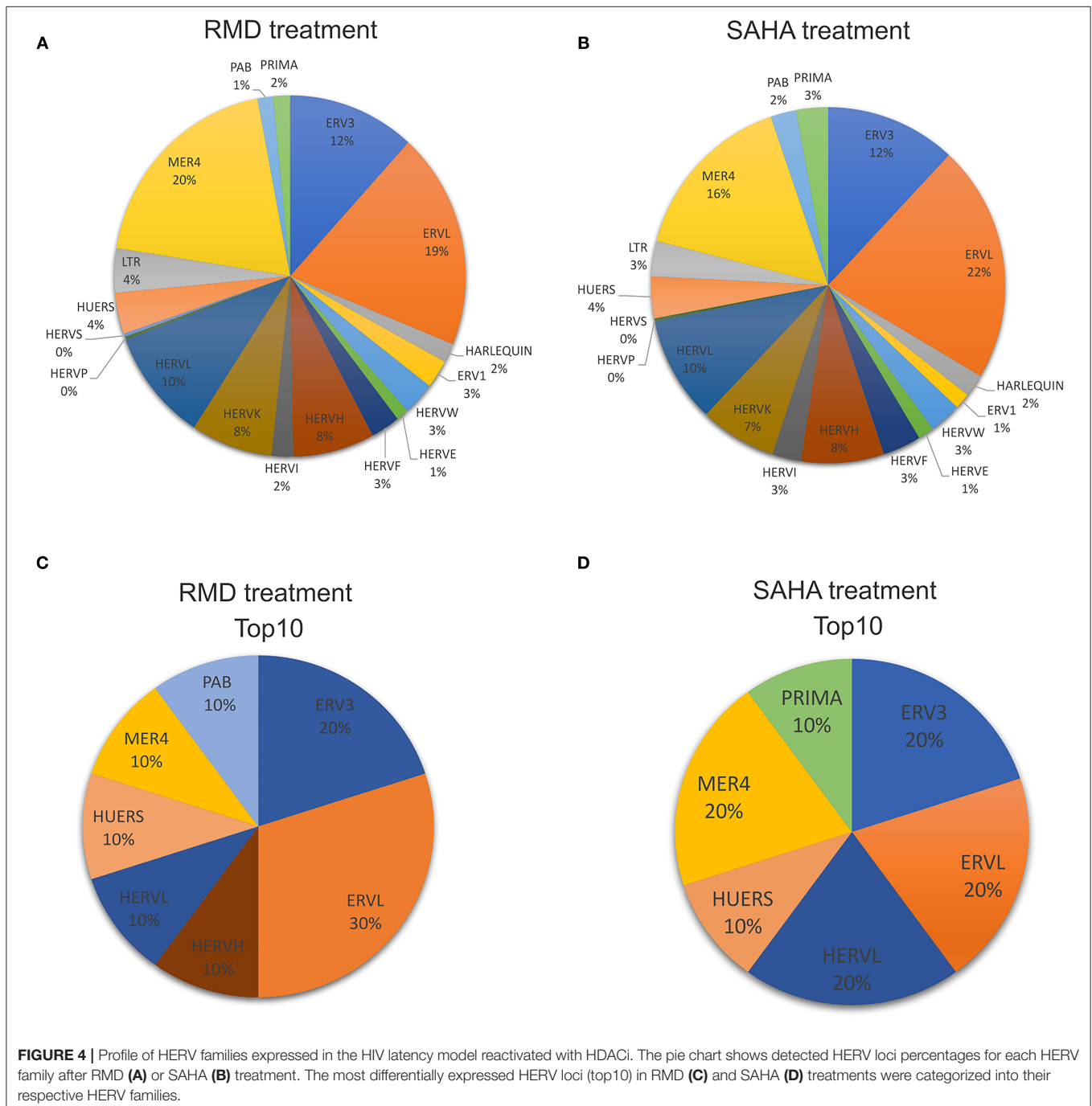
As retroelements are potentially able to control the expression of host nearby genes (or genes impacting HERVs) and also induce immune responses, we performed an exploratory analysis to find differentially expressed retroelements close to immune response-related genes. We found 64 immune genes by the vicinity of differentially expressed retroelements (**Supplementary Table 3**). We then analyzed the relationship between retroelement and nearby immune gene expression upon HDACi treatment. We selected eight immune genes that present a key role on an HIV infection context (ESR1, GBP5,



IFI16, TRAF2, TRIM5, RUNX1, THEMIS and TNFAIP3). TRAF2 and GBP5 were upregulated in the samples treated with HDACi (**Supplementary Figures 5, 6**) and correlated with HERV (MERV101_9q34.3) and L1 (L1FLnI_1p22.2k, L1FLnI_1p22.2j, L1FLnI_1p22.2i) expression (**Figure 5**). This suggests that HDACi may modulate immune gene and retroelement expression as well as affect immune responses in HIV latent CD4⁺ T-cells treated with SAHA or RMD.

DISCUSSION

Different drugs have been reported to reverse HIV-1 latency such as HDACi, but they may also have off-target effects (43, 45, 50, 51), including effects on retroelement expression (42, 43), which can potentially lead to cellular dysfunction (5, 10, 52, 53). Therefore, in this study we analyzed HERV and L1 differential expression at the single locus resolution level using Telescope bioinformatic pipeline in RNA-seq datasets from uninfected and



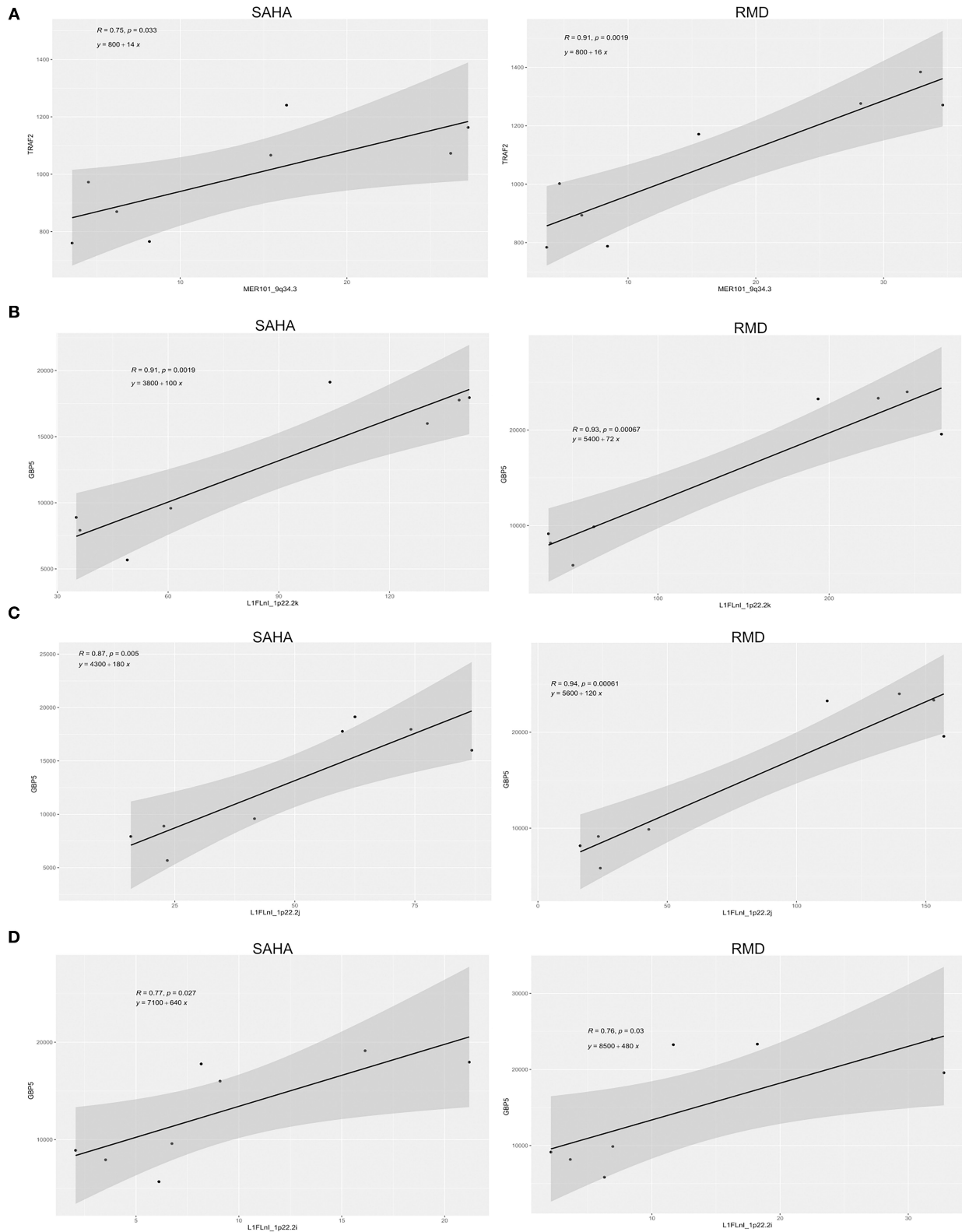


FIGURE 5 | Relationship between retroelement and nearby immune gene expression. The association between TRAF2 (A) and GBP5 (B–D) expression with their respective nearby retroelement expression is shown. Pearson’s correlation coefficients and their associated *p*-values are displayed as well as linear equation in each plot.

HIV-1 latently infected CD4⁺ T-cells, treated with SAHA and RMD, two widely used HDACi. We performed PCA to check for transcriptional differences between individual samples and group of samples. The results showed a variation of about 12% between donors and of around 85% between groups (DMSO, SAHA and RMD) (**Supplementary Figure 1**). Thus, despite inherent genetic differences between donors, data from each donor in each group are homogeneous, showing similar differential gene expression.

Our findings from uninfected CD4⁺ T-cells treated with SAHA showed a total of 722 HERV and 825 L1 loci differentially expressed, categorized in 17 HERV families, such as the ERV3, HERVW, MER4, ERVL, HERVH and HERVF families. These data are in agreement with a previously published study, which showed that HERVW (HERV-9 or LTR12) and ERVL families were differentially expressed (43). We also validated the differentially expressed HERV loci using a dataset of uninfected CD4⁺ T-cell treated with SAHA (45). We found a 56% congruence between datasets, which represents 407 differential HERV expressed in both datasets, such as HERVF, HERVH, ERV3, HERVW and MER4 families. The contrast between datasets may be explained by a difference in the concentration of SAHA used between studies (10 μ M in the White et al. study vs. 1 μ M in the Beliakova-Bethell et al. study), as well as differences between the CD4⁺ T-cell subset analyzed and the culture protocol (43, 45). While the Beliakova-Bethell et al. study analyzed a central memory CD4⁺ T-cell model of HIV latency, using uninfected CD4⁺ T-cells (mock-infected) and *in vitro* HIV-infected (latent HIV cell), the White et al. study used naive CD4⁺ T-cells (uninfected) treated with SAHA or DMSO. In addition, mock-infected cells from the Beliakova-Bethell et al. study were cultured and stimulated with anti-CD3/CD28 antibodies and also incubated with IL-2 and IL-15 before SAHA and DMSO treatment, which is likely to explain the difference in retroelements expression between these two datasets and emphasizes the importance of culture conditions in differential TE expression analyses.

SAHA regulates gene expression by HDAC inhibition (54, 55). HDACs are involved in epigenetic regulation, removing the acetyl groups from histones. Their inhibition become chromatin more relaxed, permitting transcriptional activation (56, 57). In humans, there are 18 HDAC enzymes divided in four classes (I – IV) (58). Classes I, II and IV HDACs are zinc-binding enzymes and inhibited by SAHA, inducing gene transcription. In addition, retroelement promoters and their DNA orientation may also regulate nearby gene expression, such as neighbor HERVs and L1 (3, 59, 60). We found two neighboring HERVs downregulated in the same DNA strand orientation [HERV FH21_Xq11.2a (sense) and HERV FH21_Xq11.2b (sense)], localized in ~260 bp of genetic distance in an intergenic region. This piece of data suggests that chromatin structure can modulate equally nearby retroelements located at a short genetic distance and highlights the importance of chromatin structure and genome accessibility on HERV promoters and regulation of nearby gene expression.

SAHA and RMD are both approved by the FDA for cutaneous T-cell lymphoma treatment and have been assessed in clinical trials to reverse HIV latency (34–36, 61–63). Viral latency is the main challenge for an HIV-1 cure. HIV-1 latently infected

cells can evade the immune response, establishing viral cellular reservoir that last for years (64–67). ART cessation leads to viraemia rebounds even after prolonged period of viral undetectable levels (18). Several studies have proposed strategies for HIV-1 cure by eliminating HIV-1 reservoirs, such as the “shock and kill” strategy, using LRA (20–25). Therefore, for assessing latency reversal in context of HIV-1 infection, we also analyzed the expression of retroelements (HERV and L1) in HIV-1 latent CD4⁺ T-cells treated with HDACi. We found 712 and 1,380 differentially expressed retroelements in response to SAHA and RMD treatment, respectively. Five hundred and thirty-one of retroelements were found differentially expressed in both treatments, while 1,030 were differently regulated according to type of treatment. In addition, it is known that HIV-1 infection is able to induce HERV expression by its viral proteins, such as Tat protein (68). Therefore, we also compared differentially expressed retroelements between HIV-1 latent CD4⁺ T-cells and mock-infected CD4⁺ T-cells treated with SAHA and RMD. However, we did not find any differently expressed HERV or L1 under this comparison. These data agree with Beliakova-Bethell et al. study, which found only six and eleven host genes differently expressed between mock-infected and HIV-1 latency cells treated with SAHA and RMD, respectively (45). It also is important to highlight that the frequency of latently infected cells in the *in vitro* model was low (8–20%), thus any difference in their response to HDACi between a mock-infected cell and a HIV latently infected cell would unlikely be detectable, unless it was very robust. This agrees with the idea that the effect of HIV reactivation at altering gene expression pathways induced by HDACi is minimal and that gene modulations were similar in mock-infected cells and the model of HIV latency (uninfected vs. infected). These data further shows that HIV-1 reactivation in the latent cell model was unable of independently regulate the expression of retroelements in CD4⁺ T-cells treated with SAHA or RMD, suggesting that retroelement expression is mostly regulated by HDACi.

The comparison between SAHA and RMD treatment in HIV-1 latently infected CD4⁺ T-cells shows no different expression profiles of general HERV families. Both drugs were able to regulate full-length HERV genomes, such as those of the HERV-K, HERV-W and HERV-H families, as well as partial HERV genomes which are able to encode viral proteins, such as those of the PRIMA4, HERVF, ERV3 and MER4 families. The full-length HERV element is about 9.5 kb in length, showing two long terminal repeats (LTRs) and three essential viral genes (gag, pol, and env) which encode four viral proteins (Env, PR, RT and Gag). Both partial and full-length HERV genomes are able to modulate cellular pathways and stimulate immune responses (5, 10, 69). On the other hand, comparison of the top ten most differentially expressed HERVs by SAHA and RMD showed that the PRIMA family was mostly regulated by SAHA treatment and the HERVH and PAB families were mostly regulated by RMD treatment. This suggests that each HIV shock-and-kill strategy regulates exclusive components that may provide specific biological phenotypes in each treated cell.

Retroelements are also able to control the expression of nearby host genes and also induce immune responses (3, 5, 10, 59, 60, 69). Herein, we found 64 immune genes nearby differentially

expressed retroelements, including eight immune genes with known key roles on HIV-1 infection (ESR1, GBP5, IFI16, TRAF2, TRIM5, RUNX1, THEMIS and TNFAIP3). TNF receptor associated factor 2 (TRAF2) and guanylate binding protein 5 (GBP5) proteins were upregulated in the samples treated with HDACi and correlated with HERV (MERV101_9q34.3) and L1 (L1FLnI_1p22.2k, L1FLnI_1p22.2j, L1FLnI_1p22.2i) expression localized in intergenic region. TRAF2 is a member of the TNF receptor associated factor (TRAF) protein family and mediate the signal transduction from members of the TNF receptor superfamily (70). TRAF2 is required for TNF- α -mediated activation of NF- κ B and MAPK8/JNK (71, 72). Also, HIV-1 Nef is able to activate TRAF2, thus mediating activation of NF- κ B and increasing HIV-1 replication in macrophages (73). GBP5 belongs to the guanylate binding protein family, which is an interferon-inducible subfamily of guanosine triphosphatases. It is able to enhance endogenous IFN expression by interacting with the NF- κ B complex, stimulating its signaling and inducing antiviral responses (74, 75). Several studies have associated HERV expression with NF- κ B activation (68, 76–78). In addition, HIV-1 Tat protein can induce HERV expression through regulating the NF-AT and NF- κ B pathways (79), suggesting an interplay between HIV-1 and HERV expression through cellular pathways.

In summary, our findings show that off-target effects of HDAC inhibitors, such as SAHA and RMD, may induce retroelement gene expression and also modulate host genes in treated CD4⁺ T-cells. In addition, several studies have shown that HERV expression is able to induce antibodies and cytotoxic immune responses, leading to cellular death (10, 80–82). Its potential roles as immunotherapeutic and as a biomarker have been discussed for different type of diseases, including HIV-1 infection (10, 12, 80, 82). Thus, our findings suggest that retroelements expression might constitute a biomarker for “shock-and-kill” clinical trials

using HDAC inhibitors. In addition, the data presented here highlight the importance of exploring the interaction of HIV-1 and HERV interaction from trial subjects for understanding its impact on “shock and kill” treatments.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102187> and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE114883>.

AUTHOR CONTRIBUTIONS

GC and MM designed the study and wrote the manuscript. GC and LI performed the data analysis. MS and DN helped with study design and wrote the manuscript. All authors revised the final draft and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fviro.2021.756635/full#supplementary-material>

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