Autophagy induction by histone deacetylase inhibitors inhibits HIV-1 infection in macrophages

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Abstract

Background: Histone deacetylases inhibitors (HDACi) have been shown to induce HIV production from latently infected cells *in vitro* with the aim to enhance elimination of these cells and achieve a cure. However, the activity of these drugs in non-T-cell reservoirs is unknown. In this study, we investigated the effect of HDACi on the induction of autophagy, and in the replication of HIV within human primary macrophages.

Methods: Monocyte-derived macrophages (MDM) were treated with HDACi (romidpesin, vorinostat, belinostat, givinostat, or panobinostat) at sub-cytotoxic concentrations and assessed for autophagic flux using the expression and phosphorylation of autophagy proteins and the lipidation of microtubule-associated protein 1 light chain 3B (LC3B) by immunoblotting, qRT-PCR, and fluorescence microscopy. MDM were challenged with 10^5 $TCID_{50}$ HIV-$1_{Ba\text{-}L}$ per 5 x 10^5 cells, and replication assessed using HIV p24 antigen release into culture supernatants. The role of autophagy in the HDACi-mediated inhibition of HIV infection was investigated using small molecule inhibitors or by transducing MDM with shRNA for *BECN1* or *ATG5*. Data were analyzed using the Mann-Whitney U test.

Results: HDACi induced autophagic flux in MDM in a dose-dependent manner (belinostat < givinostat < vorinostat < panobinostat < romidepsin) as demonstrated by increased lipidation of LC3B, degradation of sequestosome and increased autophagosome formation ($P < 0.05$) in the absence of increased cell death. We also identified that HDACi induces autophagy through a ULK1 complex-dependent mechanism by suppressing MTOR activity as indicated by the dephosphorylation of RPS6KB2, EIF4EBP1 and ULK1, the most upstream and essential component of the classical autophagy pathway. HDACi induced autophagy significantly inhibited HIV-1 replication in macrophages over 10 days in a dose-dependent manner that reflected autophagy induction ($P < 0.001$) and was abrogated in the presence of RNAi for autophagy proteins or chemical inhibitors of autophagic flux ($P > 0.05$).

Conclusions: HDACi induces autophagy in human macrophages by releasing the ULK1 complex from inhibition by MTOR-mediated phosphorylation possibly as a prosurvival mechanism to counteract their cytotoxic effects. If HDACi are to form part of a multi-pronged strategy to eliminate latently infected cells with the ultimate goal of a functional or sterilizing cure for HIV, careful consideration of toxicity as well as activity in non-T-cell reservoirs is essential. These data support an important role for autophagy in the control of HIV infection, and provide new insights into novel approaches to inhibit HIV-1 replication and potentially eradicate the virus from infected cells.

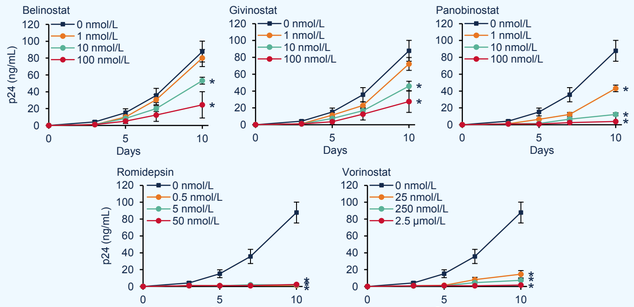
Background

- HIV-1 exploits autophagy for its replication in macrophages. HIV-1 is assembled on endocytic membranes that intersect with recycling endosomes.
- Autophagy is a major self-degradative process in mammalian cells with roles in cellular homeostasis.
- The ULK1 complex is the most upstream component in the core autophagy pathway and is essential for autophagy induction.
- MTOR suppresses autophagy by phosphorylating and inactivating the ULK1 complex. When MTOR is inhibited, ULK1 is dephosphorylated, released from inhibition and induces autophagy.
- Histone deacetylase (HDAC) inhibitors have emerged as a new class of therapeutic agents with promising outcomes during the treatment of a wide range of cancer types.

Results

Figure 1

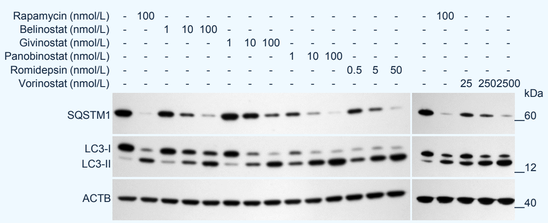
HDACi inhibit HIV-1 infection in macrophages



Macrophages were treated with increasing concentrations of HDACi for 24 h before challenge with 10^5 $TCID_{50}$ HIV-$1_{Ba\text{-}L}$ per 5 x 10^5 cells and replication followed using HIV p24 expression. All HDACi inhibited HIV-1 in a dose-dependent manner. Only the lowest doses of belinostat and givinostat failed to significantly inhibit HIV-1 replication. * $P < 0.05$, n = 5.

Figure 2

HDACi induce autophagy in macrophages



Monocyte-derived macrophages were treated with increasing concentrations of HDACi. The MTOR inhibitor rapamycin was used as a positive control. Cells were harvested and lysed after 24 h. The degradation of sequestosome 1 (SQSTM1), and microtubule-associated protein 1 light chain 3B (LC3B) lipidation were analyzed using Western blot. SQSTM1 binds LC3B-II and is degraded when autophagosomes fuse with lysosomes. All HDACi induced the dose-dependent induction of autophagy. *Top*, a representative blot. *Bottom*, densitometric analysis from 4 independent donors. * $P < 0.05$; n = 4.

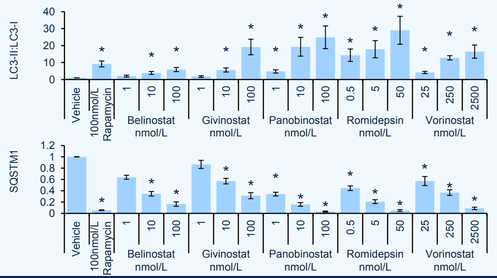
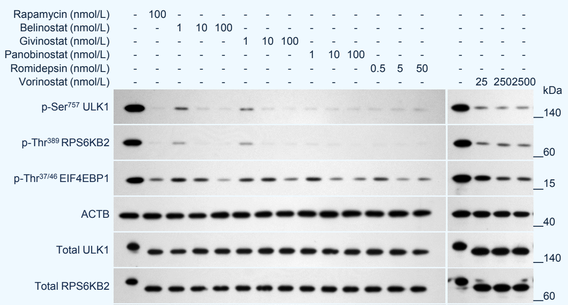


Figure 3

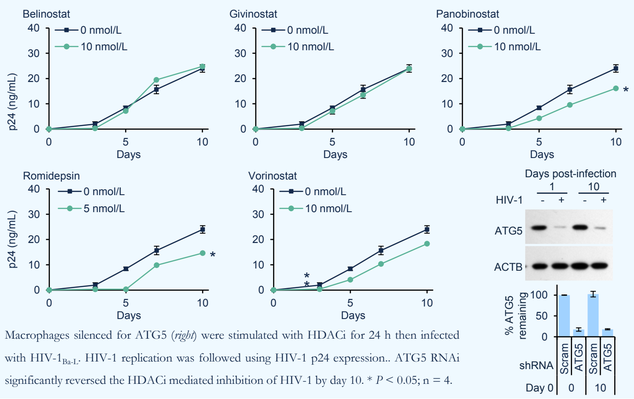
HDACi inhibit the mammalian target of rapamycin (MTOR)



Monocyte-derived macrophages were treated with increasing concentrations of HDACi. Cells were harvested and lysed after 24 h. The phosphorylation status of well known MTOR substrates ULK1, RPS6KB2 and EIF4EBP1, all upstream autophagy pathway components, was analyzed using Western blot. All HDACi induced the dephosphorylation, and thus activation of the autophagy pathway components. *Top*, a representative blot. *Bottom*, densitometric analysis from 4 independent donors. * $P < 0.05$; n = 4.

Figure 4

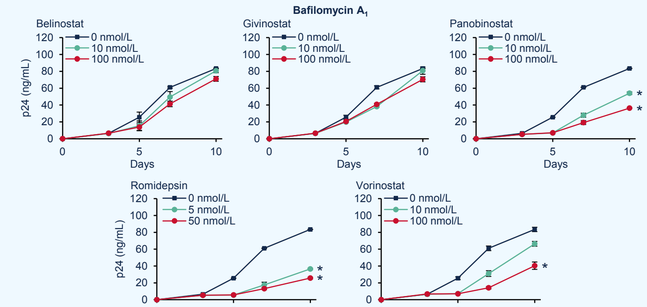
HDACi inhibition of HIV-1 requires autophagosome formation



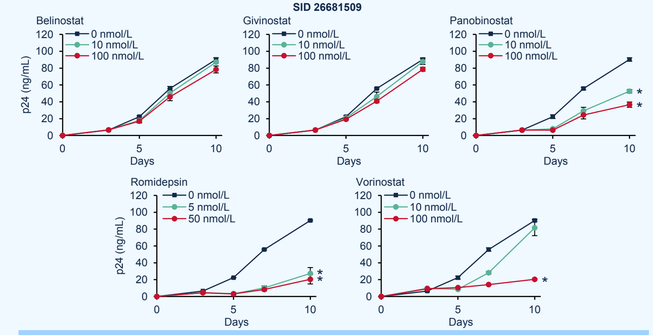
Macrophages silenced for ATG5 (*right*) were stimulated with HDACi for 24 h then infected with HIV-$1_{Ba\text{-}L}$. HIV-1 replication was followed using HIV-1 p24 expression.. ATG5 RNAi significantly reversed the HDACi mediated inhibition of HIV-1 by day 10. * $P < 0.05$; n = 4.

Figure 5

HDACi inhibit HIV-1 through the induction of autophagic flux



Macrophages were stimulated with HDACi for 24 h then treated with bafilomycin A1 *(top)*, an inhibitor of the vacuolar H+ ATPase and thus autophagosome-lysosome fusion, or SID 26681509 *(bottom)*, an inhibitor of the lysosome hydrolase cathepsin L, before infection with HIV-$1_{Ba\text{-}L}$. Both treatments significantly reversed the effect of HDACi on HIV-$1_{Ba\text{-}L}$ replication. All graphs show data from cells treated with bafilomycin A1 *(top)* or SID 26681509 *(bottom)*. * $P < 0.05$; n = 4.



Conclusions

- HDACi inhibit the replication of HIV-1 in macrophages
- HDACi trigger autophagy by suppressing MTOR and activating the autophagy kinase ULK1
- Belinostat, givinostat and vorinostat, and to a lesser extent, panobinostat and romidpesin inhibition of HIV-1 is dependent upon autophagy.

Acknowledgements

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