A Novel Selective LSD1/KDM1A Inhibitor Epigenetically Blocks Herpes Simplex Virus Lytic Replication and Reactivation from Latency

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ABSTRACT **Cellular processes requiring access to the DNA genome are regulated by an overlay of epigenetic modifications, including histone modification and chromatin remodeling. Similar to the cellular host, many nuclear DNA viruses that depend upon the host cell's transcriptional machinery are also subject to the regulatory impact of chromatin assembly and modification. Infection of cells with alphaherpesviruses (herpes simplex virus [HSV] and varicella-zoster virus [VZV]) results in the deposition of nucleosomes bearing repressive histone H3K9 methylation on the viral genome. This repressive state is modulated by the recruitment of a cellular coactivator complex containing the histone H3K9 demethylase LSD1 to the viral immediate-early (IE) gene promoters. Inhibition of the activity of this enzyme results in increased repressive chromatin assembly and suppression of viral gene expression during lytic infection as well as reactivation from latency in a mouse ganglion explant model. However, available small-molecule LSD1 inhibitors are not originally designed to inhibit LSD1, but rather monoamine oxidases (MAO) in general. Thus, their specificity for and potency to LSD1 is low. In this study, a novel specific LSD1 inhibitor was identified that potently repressed HSV IE gene expression, genome replication, and reactivation from latency. Importantly, the inhibitor also suppressed primary infection of HSV *in vivo* in a mouse model. Based on common control of a number of DNA viruses by epigenetic modulation, it was also demonstrated that this LSD1 inhibitor blocks initial gene expression of the human cytomegalovirus and adenovirus type 5.**

IMPORTANCE **Epigenetic mechanisms, including histone modification and chromatin remodeling, play important regulatory roles in all cellular processes requiring access to the genome. These mechanisms are often altered in disease conditions, including various cancers, and thus represent novel targets for drugs. Similarly, many viral pathogens are regulated by an epigenetic overlay that determines the outcome of infection. Therefore, these epigenetic targets also represent novel antiviral targets. Here, a novel inhibitor was identified with high specificity and potency for the histone demethylase LSD1, a critical component of the herpes simplex virus (HSV) gene expression paradigm. This inhibitor was demonstrated to have potent antiviral potential in both cultured cells and animal models. Thus, in addition to clearly demonstrating the critical role of LSD1 in regulation of HSV infection, as well as other DNA viruses, the data extends the therapeutic potential of chromatin modulation inhibitors from the focused field of oncology to the arena of antiviral agents.**

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Similar to their cellular host, many DNA viruses that replicate in the nucleus are also subject to chromatin-mediated regulation of gene transcription and DNA replication (1, 2). In addition to the direct DNA binding factors that specify activation or repression of transcription, the complex overlay of nucleosome assembly, modification, and remodeling plays a critical role in determining the progression of the lytic replication cycle. For pathogens such as herpesviruses, chromatin modulation is also a regulatory component of the viral latency and reactivation cycles (3–9).

The additional layer of regulatory complexity mediated by assembled nucleosomes represents a dynamic interplay between the host cell chromatin modulation machinery and the pathogen. For the alphaherpesviruses, herpes simplex virus (HSV) and varicella-zoster virus (VZV), infection of host cells results in rapid deposition of nucleosomes (10) bearing repressive histone marks (11, 12). Expression of the viral lytic immediate-early (IE) genes and progression of lytic infection depend upon modulating these modifications to counter the accumulation of repressive marks. To do this, both viruses utilize transcriptional activators packaged in the viral particle to recruit the HCF-1 cellular coactivator complex containing both histone H3K9 demethylases (LSD1 [lysine-specific demethylase 1]) and histone H3K4 methyltransferases (Set1 or MLL family members) to the viral IE gene promoters (11, 13, 14). Recruitment of this coactivator complex thus contains the required enzymatic activities to limit or reverse the accumulation

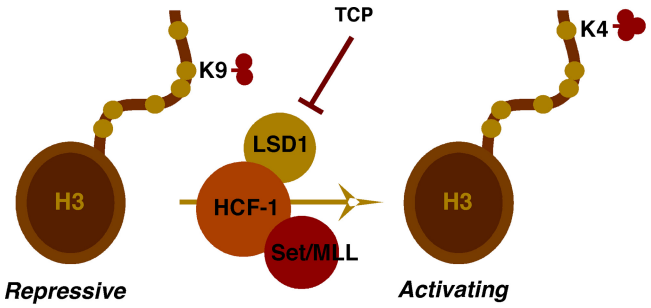

FIG 1 Inhibition of LSD1 by monoamine oxidase inhibitors (MAOIs). Upon infection, the HSV or VZV IE gene activator recruits the HCF-1 coactivator complex to viral IE gene promoters to limit the accumulation of repressive histone H3K9 methylation and promote the installation of activating marks such as histone H3K4 methylation. The HCF-1 coactivator complex consists of a set of histone modification components, including the histone H3K9 demethylase LSD1 and the H3K4 methyltransferase Set1 or MLL. MAOIs such as tranylcypromine (TCP) block LSD1 demethylation activity, resulting in stable or increased levels of repressive marks on viral IE gene promoters and suppression of IE gene transcription.

of the repressive H3K9 methylation while promoting the accumulation of the activating H3K4 methylation.

With respect to the significance of the chromatin modulation components of the HCF-1 complex, it has been clearly demonstrated that small interfering RNA (siRNA)-mediated depletion of the H3K9 demethylase LSD1 or inhibition of its activity with monoamine oxidase inhibitors (MAOIs) results in the accumulation of repressive H3K9 methylation across the viral IE gene promoters and suppression of viral IE gene expression (Fig. 1). Significantly, these inhibitors also block the reactivation of HSV from latency in sensory neurons, indicating that LSD1-mediated reversal of repressive H3K9 methylation is a crucial component of the reactivation mechanism (11). These studies demonstrate that histone modification activities such as LSD1 may represent targets for the development of novel antiviral therapeutics to control herpesvirus infection and related diseases.

Importantly, MAOIs such as tranylcypromine (TCP) and pargyline are not specific to LSD1 (15–17) but rather were originally designed to inhibit MAO-A and/or MAO-B for the treatment of psychiatric illnesses (i.e., severe depression). Thus, two limiting factors for the use of these compounds to inhibit alphaherpesvirus infection and reactivation are (i) the high 50% inhibitory concentration (IC_{50}) for LSD1 relative to the MAO-A/B targets (15, 16) and (ii) the broad-spectrum (i.e., non-LSD1) activity.

Therefore, in this study, a series of novel selective LSD1 inhibitors were screened for efficacy in suppressing HSV IE gene expression *in vitro*. Of these inhibitors, compound OG-L002 demonstrated significantly enhanced potency both in inhibition of IE gene expression and in suppression of viral reactivation from latency in a mouse ganglion explant model. Additionally, OG-L002 substantially suppressed HSV primary lytic infection of mice as determined by reduced viral loads in sensory ganglia. Interestingly, OG-L002 also suppressed the expression of the initial class of human cytomegalovirus (hCMV) and adenovirus genes *in vitro*, suggesting that chromatin modulation components such as LSD1 may represent common elements of multiple viral pathogens. The results, therefore, indicate that inhibition of the expression of the first class of viral genes via targeting of histone modification and chromatin-remodeling activities could hold significant potential for the control of a class of viruses and related diseases.

RESULTS

Identification of the novel LSD1 inhibitor OG-L002 as a potent inhibitor of HSV IE gene expression in cell culture. As noted, MAOIs such as tranylcypromine (TCP) were originally designed to target the MAO-A/B. While these compounds also inhibit LSD1, they have a significantly higher IC_{50} for LSD1 than for their intended targets (Fig. 2). Therefore, to identify compounds with reduced IC_{50}s for LSD1, a series of lead compounds were screened in an *in vitro* LSD1 demethylation assay using purified LSD1 protein. The IC_{50}s for each compound were determined for LSD1 and MAO-A/B as an indication of specificity (data not shown).

Compounds with low IC_{50}s for LSD1 relative to MAO-A/B were selected and tested for their efficacy in suppression of HSV IE gene expression. HeLa cells were pretreated for 5 h with the dimethyl sulfoxide (DMSO) control or 50 μM of each compound, followed by infection with HSV for 2 h. The levels of viral immediate-early (ICP4 and ICP27) and cellular control (S15) mRNAs were quantitated by quantitative reverse transcription-PCR (qRT-PCR). As shown in Fig. 2, compound OG-L002, which is highly specific to LSD1 (IC_{50}, ~0.02 μM), potently inhibited the expression of viral IE (ICP27 and ICP4) genes, but not cellular control genes. In contrast, control inhibitors that are more specific to MAO-A (clorgyline) or MAO-B (safinamide) did not show substantial inhibition of viral IE gene expression. The results further support the model that LSD1 is an integral component of the HSV IE gene regulatory paradigm and that the previously described impact of MAOI treatment on viral IE gene expression is not due to inhibition of MAO-A/B.

OG-L002 potently inhibits HSV IE gene expression and the production of progeny virus in both HeLa and HFF cells. Given the impact of the novel LSD1 inhibitor OG-L002 on HSV IE gene expression, the antiviral potential was further characterized in HeLa (Fig. 3A to C) or human foreskin fibroblast (HFF) (Fig. 3D to G) cells. Following pretreatment with the indicated concentrations of control MAOI TCP, OG-L002, MAO-A inhibitor CLG, or DMSO for 4 h, the cells were infected with HSV for 2 h (mRNA levels [Fig. 3A and B and D to F]) or 24 h (viral yields [Fig. 3C and G]). As shown, OG-L002 inhibited viral IE gene expression in both cells with a significantly reduced IC_{50} (IC_{50}, ~10 μM in HeLa cells; IC_{50}, ~3 μM in HFF cells) relative to the control MAOI TCP (IC_{50}, ~1 mM). In addition, OG-L002 potently reduced viral yields (~100-fold) in comparison to control DMSO treatment. As shown in Fig. S1 in the supplemental material, no significant toxicity was evident in HeLa or HFF cells from treatment with 50 μM OG-L002.

Specific inhibition of LSD1 by OG-L002 but not treatment with an MAO inhibitor results in accumulation of repressive chromatin on viral IE gene promoters. As previously shown, treatment of cells with the MAOI TCP resulted in accumulation of chromatin bearing repressive H3K9 methylation on the HSV IE gene promoters and suppression of viral IE gene expression upon infection (11). To confirm that the novel LSD1 inhibitor OG-L002 also results in accumulation of repressive chromatin marks, HeLa cells were pretreated with DMSO, OG-L002, MAO-A inhibitor clorgyline, or TCP for 4 h, followed by infection with HSV. The levels of dimethyl-H3K9 (H3K9-me2) and total histone H3 associated with viral IE promoters (ICP0 and ICP27) and cellular con-

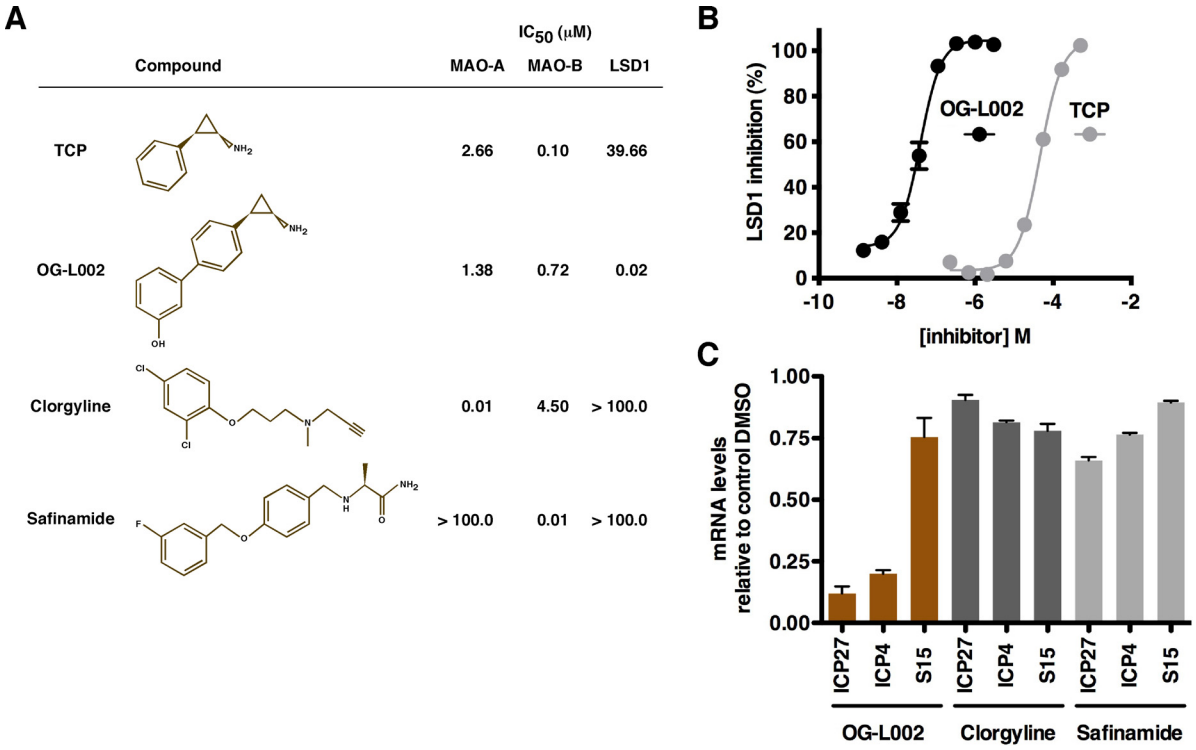

FIG 2 Screening novel LSD1 inhibitors identifies lead compounds with improved potency in the repression of HSV-1 IE gene expression. (A) Structures of TCP, OG-L002, clorgyline (CLG), and safinamide (SAF). IC_{50}s were determined *in vitro* for monoamine oxidase-A/B (MAO-A/B), monoamine oxidase-B (MAO-B), and LSD1. (B) Inhibition of LSD1 *in vitro* by TCP and OG-L002 is graphically represented. (C) HeLa cells were pretreated for 5 h with DMSO control or the indicated compound (50 μM) followed by infection with HSV-1 (0.1 PFU/cell) for 2 h. The levels of viral immediate-early (ICP4 and ICP27) and cellular control (S15) mRNAs were determined by qRT-PCR and are expressed as ratios to the levels in control DMSO-treated cells. OG-L002 and clorgyline were selected for further comparative analyses.

trols (RPL and SATa) were determined by chromatin immunoprecipitation (ChIP) assays. As shown in Fig. 4, the transcriptionally repressed cellular SATa region exhibits a high level of repressive H3K9-me2 compared to the transcriptionally active cellular RPL gene. In contrast, the total histone H3 levels associated with these regions are comparable, demonstrating the specificity of the H3K9-me2 ChIP assays (Fig. 4C and D). Notably, treatment of cells with TCP and OG-L002, but not clorgyline (CLG), substantially increased the levels of total histone H3 and H3K9-me2 (20- to 30-fold) associated with viral IE promoters. The data are consistent with increased accumulation of repressive chromatin at the IE gene promoters that results in decreased viral IE gene expression.

OG-L002 represses HSV primary infection *in vivo*. The data derived from cell culture system assays support the role of LSD1 in HSV lytic infection *in vitro* and suggest that inhibition of LSD1 activity would suppress a primary infection *in vivo* in a mouse model system. To assess this, BALB/c mice were pretreated with vehicle or OG-L002 (6 to 40 mg/kg of body weight/day) for 7 days followed by infection with a lethal dose of HSV (90% lethal dose [LD_{90}]). As an additional control, infected mice were treated with the viral DNA replication inhibitor acyclovir (ACV) (100 mg/kg/day). At various days postinfection, trigeminal ganglia were harvested from each group of mice, and viral DNA loads were determined as a measure of the progression of the primary infection. As shown in Fig. 5A, OG-L002 reduced the levels of detectable viral genomes in the ganglia in a dose-dependent manner at both 3 and 5 days postinfection.

In a second experiment, mice were treated with vehicle, ACV (100 mg/kg/day), or OG-L002 (20 mg/kg/day), followed by HSV infection at a reduced LD_{50} level. Viral DNA loads in the trigeminal ganglia were determined as indicated above. As shown (Fig. 5B), OG-L002 clearly suppressed infection as determined by reduced viral genome accumulation at days 3, 5, and 10 postinfection. Interestingly, the impact of OG-L002 was more significant than the control antiherpesvirus pharmaceutical ACV at early times postinfection (Fig. 5, day 3) and comparable to ACV at later times (days 5 and 10). While the enhanced suppression by OG-L002 relative to ACV at day 3 was reproducible in these experiments, studies with significantly larger groups of animals would be required to statistically validate this interesting observation. Most importantly, the ability to suppress primary HSV infection even at an LD_{90} challenge further supports the critical role of LSD1 in determining the progression of lytic infection.

OG-L002 blocks HSV reactivation from latency in a mouse ganglion explant model. In addition to the regulation of viral lytic replication, histone modification and chromatin remodeling play critical roles in regulating HSV latency and reactivation cycles. A number of studies have clearly correlated repressive marks associated with lytic viral genes during latency and the transition to activating marks upon reactivation (3, 4, 18–24). Notably, it was previously demonstrated that MAOIs (TCP) blocked viral IE gene expression during reactivation in a mouse latently infected ganglion explant model system. The data indicate that LSD1 was a critical component of the viral latency-reactivation cycle as well as lytic infection (11).

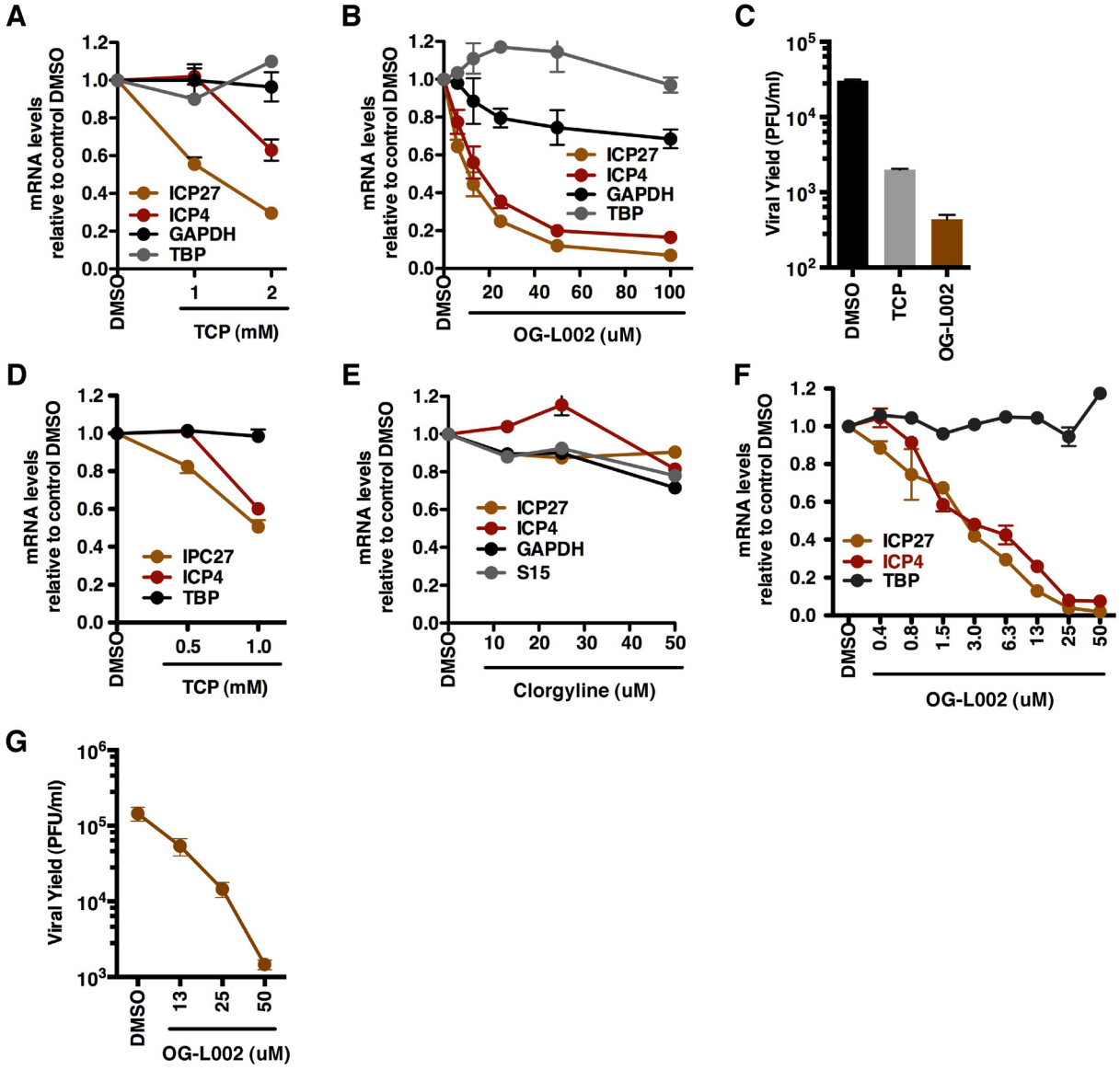

FIG 3 OG-L002 potently inhibits HSV-1 IE gene expression and viral yield in both HeLa and HFF cells. HeLa (A to C) or HFF (D to G) cells were treated with the indicated concentrations (millimolar or micromolar) of TCP, OG-L002, clorgyline, or DMSO control for 4 h, followed by infection with HSV-1 (0.1 PFU/cell) (A and B and D to F). mRNA levels of viral and cellular genes were measured by qRT-PCR at 2 h postinfection and expressed as ratios to the levels of the DMSO control (C and G). Viral yields were determined at 24 h postinfection. In panel C, 1 mM TCP and 50 μM OG-L002 were used.

To determine the efficacy of OG-L002 in repression of HSV reactivation, trigeminal ganglia from HSV latently infected mice were bisected, and the halves were explanted in the presence of DMSO and TCP (2 mM) or DMSO and OG-L002 (10 to 50 μM) for 48 h. Yields of reactivated virus (viral yields) were determined by titration. As shown in Fig. 6, OG-L002 repressed viral reactivation at both 10 μM ($P = 0.0225$) and 50 μM (~40-fold reduction; $P < 0.0001$) at a significantly reduced concentration from that required for inhibition by TCP. Thus, as previously demonstrated using TCP (11), inhibition of LSD1 with OG-L002 supports the role of this protein in HSV reactivation and further demonstrates the potency of this novel compound.

OG-L002 results in reduced expression of hCMV IE genes and adenovirus E1A gene. In addition to alphaherpesviruses, gene transcription of other DNA viruses (i.e., human cytomegalovirus [hCMV], adenovirus, and papillomavirus) is also subject to chromatin-mediated regulation (7, 25, 26). For hCMV, initial repression of the genomes is also evident via the assembly of nucleosomes bearing repressive marks (7), and viral IE gene expression is suppressed by the nonspecific LSD1 inhibitor TCP (27). These observations raise the possibility that chromatin modulation components such as LSD1 might be a required component for the initiation of lytic infection by multiple distinct viral pathogens. To test this hypothesis, cells were treated with control DMSO or the indicated concentrations of OG-L002, followed by infection with hCMV or adenovirus type 5. The levels of the viral mRNAs and cellular controls were determined by qRT-PCR and expressed as ratios to the levels for the DMSO control. The results

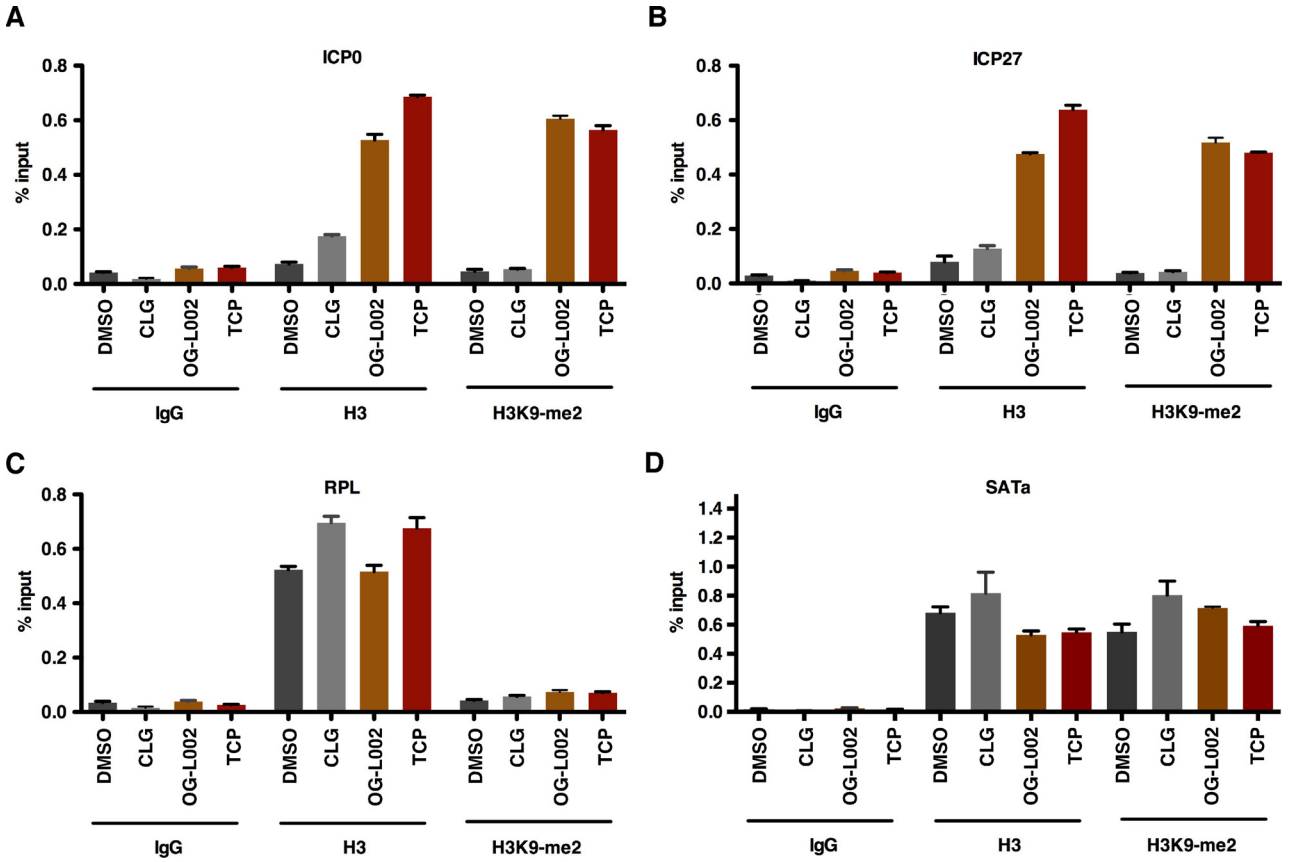

FIG 4 Specific inhibition of LSD1 by OG-L002 but not treatment with an MAO inhibitor results in accumulation of repressive chromatin on viral IE gene promoters. HeLa cells were pretreated with DMSO, OG-L002 (50 μM), clorgyline (50 μM), or TCP (2 mM) for 4 h, followed by infection with HSV-1 (0.1 PFU/cell) for 3 h. ChIP assays were performed using control IgG, H3K9-me2, or histone H3 antibodies. The levels of H3K9-me2 and histone H3 associated with viral IE promoters (ICP0 and ICP27) and cellular controls (RPL and SATa) are expressed as the percentage of input. RPL is a transcriptionally active cellular gene, and SATa is a transcriptionally repressed cellular region. The data shown are representative of two independent experiments.

clearly demonstrate that the expression of both hCMV (Fig. 7A) IE and adenovirus (Fig. 7B) early genes are substantially repressed by the LSD1 inhibitor. Thus, despite the distinct classes of these viruses, they share the requirement for some common chromatin modulation components, such as LSD1, to activate their early genes.

DISCUSSION

Epigenetic modifications, in conjunction with the cell type milieu of DNA binding regulators, control the transcriptional activity in healthy tissues and contribute to the temporal and spatial interpretation of the genome (28–30). Additionally, recent advances have demonstrated that this epigenetic overlay is often dysregulated in various cancers (31, 32), immunoinflammatory diseases (i.e., diabetes [33], lupus [34, 35], asthma [36]) and a variety of neurological disorders (37, 38). Importantly, epigenetic modifications, represented by DNA methylation, histone modifications, ATP-dependent nucleosomal remodeling, and the involvement of noncoding RNAs and organizational complexes (i.e., CTCF), are reversible, and changes in the epigenetic profile often precede disease pathology. Thus, components regulating the epigenetic landscape of the genome represent valuable indicators for disease progression and prognosis (39) and more importantly, new therapeutic targets.

In recent years, significant advancements have been made in epigenetic drug discovery in oncology (40–42) and inflammatory immunology (43, 44). At this time, several inhibitors of histone deacetylation or DNA methylation are in clinical trials or have been approved for clinical use to treat hematological malignancies or myelodysplastic syndrome, respectively (39, 45, 46). More recently, histone methyltransferases and demethylases have emerged as new therapeutic targets, as these enzymes have been found to be overexpressed, altered, or mutated in some cancers or other diseases (47, 48).

LSD1, the first histone demethylase identified, is overexpressed in a number of malignancies, including some leukemia, breast, small-cell lung, colorectal, prostate, neuroblastoma, and bladder cancers (48–50). Furthermore, preclinical studies (51, 52) have demonstrated that the MAOI TCP is useful in the treatment of acute myeloid leukemia, likely via inhibition of the activity of this enzyme. Importantly, in addition to its role in these diseases, LSD1 is also a critical component of the transcriptional regulatory complexes that activate the initial stages of HSV and HIV gene expression (11, 53).

These results highlight the interesting possibility that LSD1 represents a novel target for the treatment of viral diseases. However, as noted, the LSD1 inhibitors presently utilized are members of the MAOI pharmaceuticals and have high K_i values for LSD1

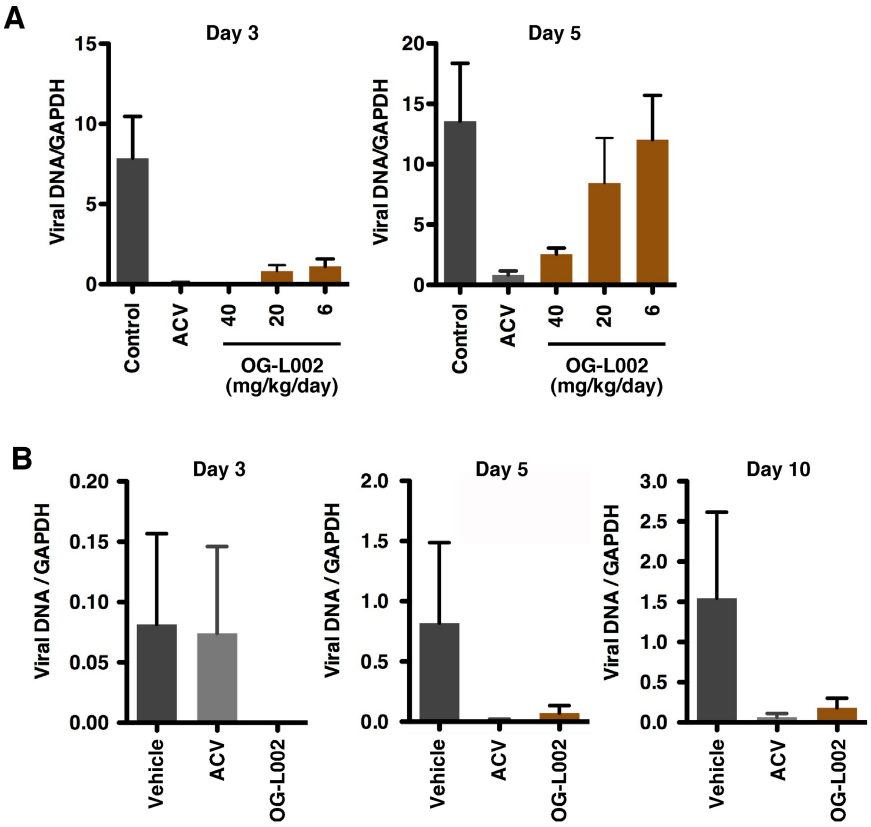

FIG 5 Treatment of mice with OG-L002 reduces HSV primary infection *in vivo*. (A and B) BALB/c mice were treated with vehicle, acyclovir (ACV) (100 mg/kg/day), or OG-L002 (indicated amounts) and infected with HSV-2 intranasally. At the indicated days postinfection, viral loads in the trigeminal ganglia were determined by qPCR. (A) Viral loads in trigeminal ganglia at days 3 and 5 postinfection at an LD_{90} (1.1×10^5 PFU/mouse) ($n = 3$). (B) Viral loads in trigeminal ganglia at days 3, 5, and 10 postinfection at an LD_{50} (1.1×10^4 PFU/mouse). Mice ($n = 5$) were treated with OG-L002 at 20 mg/kg/day.

and a broader spectrum of enzyme targets. Thus, for both oncology as well as for the treatment of infectious viral diseases, there remains a critical need to develop potent and specific LSD1 inhibitors.

In this report, a set of novel LSD1 inhibitors that were identified in *in vitro* inhibition assays were assessed for the ability to repress HSV IE gene transcription and lytic infection *in vitro*. One potent and specific inhibitor, OG-L002, was further characterized and demonstrated to (i) inhibit HSV IE gene expression in cell culture, (ii) increase repressive chromatin assembly on viral IE gene promoters, (iii) suppress viral lytic infection *in vivo*, and (iv) suppress HSV reactivation from latency in a mouse ganglion explant model system at significantly reduced compound levels relative to the MAOI TCP.

Importantly, LSD1 is critical at the initial stages of HSV infection, as the protein is required to limit the cell-mediated deposition of histone H3K9 repressive methylation of the invading viral genome. Corollary regulatory mechanisms in other viral pathogens (hCMV and adenovirus) led to the demonstration that OG-L002 was also effective at suppressing viral early gene expression from each of these distinct pathogens. Taken together, these results clearly define LSD1 as an important component of the lytic replication cycle of multiple DNA viruses in addition to the role in reactivation of HSV from latency.

Inhibition of initiation of infection or reactivation has distinct advantages in that it: (i) represents a stage of the viral infection that is distinct from the later viral DNA replication that is targeted by presently utilized inhibitors (i.e., ACV and derivatives); (ii) prevents the expression of viral gene products that may be toxic or oncogenic or result in inflammatory disease; (iii) may act synergistically with replication inhibitors for a more potent inhibition of lytic infection; and (iv) represents a cellular target that may circumvent the issues of viral escape and drug resistance. Most significantly, LSD1 represents a novel antiviral therapeutic target to suppress initiation of infection of a number of nuclear DNA viruses.

MATERIALS AND METHODS

Cells and viruses. HeLa, MRC-5, and Vero cell lines were obtained from American Type Culture Collection (ATCC) and maintained according to standard procedures. Telomerase reverse transcriptase gene (TERT)-immortalized human foreskin fibroblast (HFF) cells were a gift of T. Shenk (Princeton University). Human simplex virus 1 (HSV-1) strains 17 and F were obtained from N. Fraser (University of Pennsylvania) and B. Roizman (University of Chicago), respectively. HSV-2 strain MS has been described elsewhere (54). Human cytomegalovirus (hCMV) Towne strain RC256 (VR-2356) and adenovirus type 5 (VR-1516) were obtained from ATCC, respectively.

Antibodies and primers. Antibodies and the sequences of primers used in these studies are listed in Table S1 in the supplemental material.

MTT cytotoxicity assays. HeLa or HFF cells were treated with the indicated concentrations of saponin (positive control) or compound OG-L002 for 12 h. Cytotoxicity was determined using conditions recommended by the manufacturer (BioAssay Systems) and expressed as ratios to the cytotoxicity of the DMSO control.

qRT-PCR. cDNA was produced from equal amount of total RNA using RNAqueous-4PCR and RETROscript kits (Ambion) according to the manufacturer's recommendations. cDNA was quantitated by qPCR using appropriate primers listed in Table S1 in the supplemental material and SYBR green master mix (Roche) on an ABI 7900HT system (Applied Biosystems; SDS 2.3 software). Primer sequences are listed in Table S1.

Inhibitors. The structures and IC_{50}s of the relevant inhibitors are shown in Fig. 2A. Cells were pretreated with control DMSO, TCP (tranylcypromine) (catalog no. P8511; Sigma), or the indicated concentration(s) of inhibitors for the indicated times, followed by infection with HSV, hCMV, or adenovirus as described in the appropriate figure legends. Viral yields were determined by determining the titers of the homogenate/lysate of total infected cells on Vero cell monolayers.

ChIP assays. Histone ChIP assays were done essentially as described previously (13, 55) with modifications. Briefly, HeLa cells pretreated with DMSO control, 2 mM TCP, 50 μM OG-L002 or 50 μM clorgyline (CLG) were infected with 0.1 PFU/cell of HSV for 3 h. The infected cells were cross-linked with 1% formaldehyde in phosphate-buffered saline (PBS). Then, chromatin from 5×10^6 cells was prepared, sheared by sonication, clarified, and incubated with 4 μg of the appropriate antibodies (Abs) (see

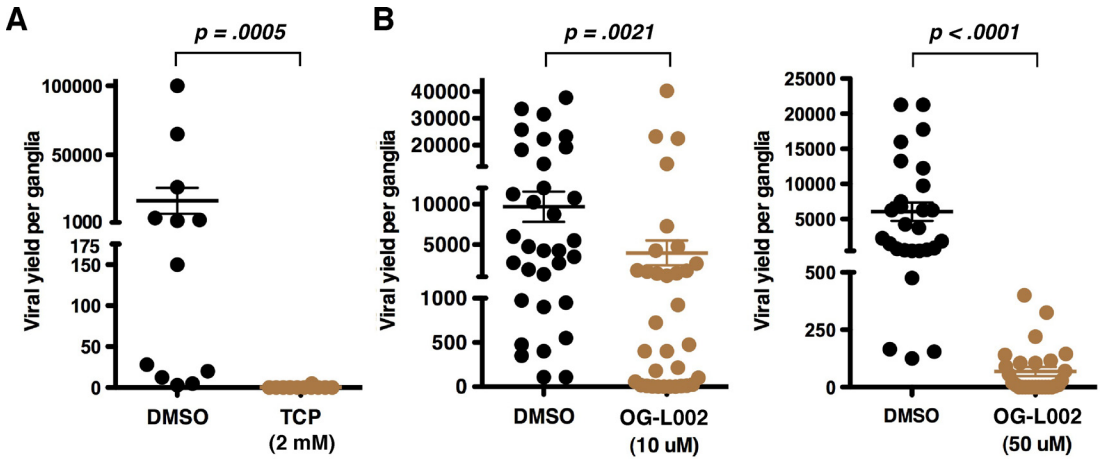

FIG 6 OG-L002 blocks HSV-1 reactivation from latency. (A and B) Halves of bisected trigeminal ganglia from HSV-1 latently infected mice were explanted in the presence of control DMSO or TCP (2 mM) (A) and DMSO or OG-L002 (10 μM or 50 μM) (B) for 48 h. Viral yields were determined by titration on Vero cells. Statistical analyses were done using Wilcoxon matched-pair signed-rank test (DMSO versus TCP, $P = 0.0005$, $n = 12$; DMSO versus OG-L002 [10 μM]), $P = 0.0021$, $n = 33$; DMSO versus OG-L002 [50 μM], $P < 0.0001$, $n = 27$).

Table S1 in the supplemental material) absorbed to 50 μl of Dynabeads (Invitrogen) at 4C for 4 h. The immunoprecipitated DNA was recovered and quantitated by qRT-PCR using appropriate primers listed in Table S1. The ChIP data shown are representative of at least two independent experiments.

Impact of compounds on HSV primary infection *in vivo*. Groups of 4-week-old BALB/c female mice were pretreated with vehicle or OG-L002 (2 to 40 mg/kg of body weight/day; intraperitoneal) for 7 days followed by intranasal infection with HSV-2 (strain MS) at an LD_{50} or LD_{90} as described previously (54, 56, 57). Control animals were treated with acyclovir (ACV) (100 mg/kg/day; oral) beginning at 1 day postinfection according to standard protocols. Vehicle, OG-L002, or ACV treatments were continued daily for 8 to 10 days postinfection. Trigeminal ganglia were harvested from animals in each group at the indicated days postinfection. Total genomic DNA was isolated, and viral genomic DNA levels were determined, in triplicate, by qPCR using SYBR green mix (Roche) on an Eppendorf Realplex^4 master cycler. The levels of viral DNA in each sample were normalized to the level of the cellular glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene to control for variances in the isolated ganglion tissue.

Mouse ganglion explant-reactivation model. BALB/c mice were infected with 2.5×10^5 PFU HSV-1 (strain F) per eye following corneal scarification. The trigeminal ganglia of latently infected mice were harvested 30 days after clearance of the primary infection. Rapidly explanted ganglia were divided in half, and each half was cultured in the presence of control DMSO or LSD1 inhibitor TCP or OG-L002 for 48 h. The resulting viral yields were determined by determining the titers of the homogenate of total trigeminal ganglia on Vero cell monolayers. All animal care and handling were done in accordance with the NIH Animal Care and Use Guidelines and as approved by the NIAID Animal Care and Use Committee. Statistical comparisons were made by using the Wilcoxon signed-rank test with a statistical significance of <0.05 (Prism V5.0a).

LSD1 demethylation assay. Human recombinant LSD1 protein (BPS Bioscience Inc.) was incubated with dimethylated H3K4 peptide (Millipore) as the substrate in the presence of various concentrations of lead compound inhibitors (0 to 75 μM) or control tranylcypromine. The de-

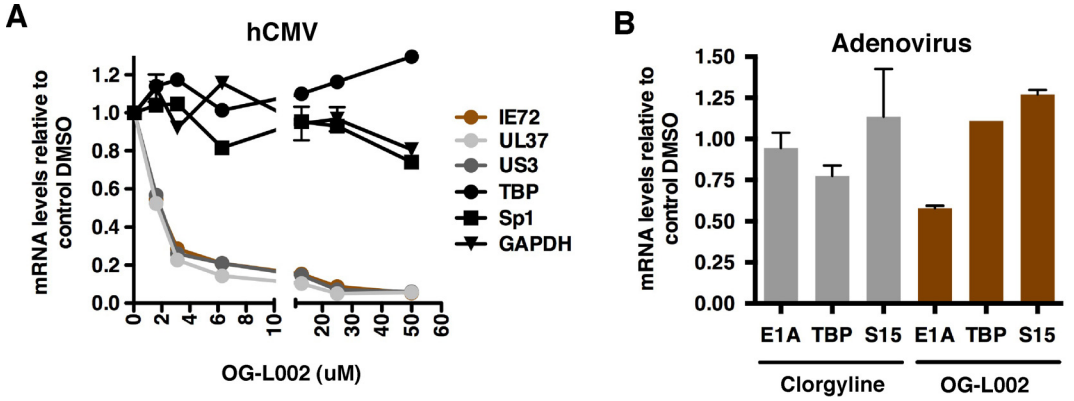

FIG 7 Inhibition of LSD1 by novel inhibitor OG-L002 results in reduced expression of hCMV IE genes and adenovirus E1A gene. (A) MRC-5 cells were treated with control DMSO or the indicated concentrations (micromolar) of OG-L002 for 3 h, followed by infection with hCMV (0.1 PFU/cell) for 5 h. The mRNA levels of the viral IE genes (IE72, UL37, and US3) and control genes (Sp1, GAPDH, and TBP) were determined by qRT-PCR and expressed as ratios to the levels in DMSO-treated cells. (B) HeLa cells were pretreated with control DMSO, OG-L002 (50 μM), or clorgyline (50 μM) for 4 h, followed by infection with adenovirus 5 (5.6×10^3 normalized infectious units [NIU]/cell) for 2 h. The mRNA levels of the viral E1A gene and cellular control genes (S15 and TBP) were determined by qRT-PCR and expressed as ratios to the levels of the DMSO control.

methylase activity was measured by the release of H_2O_2 produced during the catalytic process, using the Amplex red peroxide/peroxidase-coupled assay kit. Each reaction was done in triplicate. The maximum LSD1 demethylase activity was obtained in the absence of inhibitor and corrected for background fluorescence. The K_i (IC_{50}) of each inhibitor was calculated as half-maximal activity.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00558-12/-/DCSupplemental.

Figure S1, TIF file, 1.1 MB.

Table S1, TIF file, 1.3 MB.

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The National Institutes of Health has the following patent application: Methods of preventing or treating viral infection or reactivation from latency in a host using inhibitors of the LSD1 protein. T. M. Kristie and Y. Liang, U.S. patent application 61/083,304; international patent application PCT/US2009/051557.

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