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Immunological Aspects

Histone deacetylase inhibitors impair the host immune response against *Mycobacterium tuberculosis* infection

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ABSTRACT

Histone deacetylase inhibitors (HDACi), a novel class of anti-cancer drug, have been recently reported to suppress host immunity and increase susceptibility to infection. Tuberculosis, a leading infectious disease killer caused by *Mycobacterium* tuberculosis (*M.tb*), is basically the product of the interaction between bacterial virulence and host resistance. However, the effects of HDACi in host immunity against *M.tb* is largely unknown. In this study, we found that HDACi including Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) significantly impaired phagocytosis and killing activity of macrophage. In line with these findings, we noted that *M.tb* induced reactive oxygen species (ROS) production and autophagy are significantly suppressed by TSA. Transcriptome analysis revealed that the suppression of autophagy by TSA might due to its inhibiting autophagy-regulating genes such as *CACNA2D3*, which regulates intracellular Ca^{2+} levels. Finally, we confirmed that HDACi including TSA and SAHA significantly exacerbated the histopathological damage and *M.tb* load in the lung of *M.tb* infected mice. Taken together, our results indicated that HDACi at least TSA and SAHA significantly impaired macrophage immunity against *M.tb* and therefore increase susceptibility to TB, our findings raised the concern that the potential side effects of HDACi on latent TB reactivation should be considered in clinic.

1. Introduction

Histone deacetylases (HDACs) play important roles in regulating epigenetic states and gene expression by catalyzing the removal of acetyl groups from histones and some non-histone proteins. Based on their homology with yeast, HDACs are grouped into four classes. Specifically, class I includes HDAC-1, -2, -3 and -8, which are mainly localized to the nucleus and primarily implicated in innate immunity and regulating inflammatory cytokine production [1]. Class II includes HDAC-4, -5, -6, -7, -9 and -10, which shuttle between the nucleus and cytoplasm and are involved in controlling innate and adaptive immunity by regulating antigen presentation, B and T-lymphocyte activation and development [1,2]. Class III HDACs, also known as Sirtuins, contain an NAD^+-dependent catalytic domain that allows them to suppress gene transcription by epigenetic mechanisms [3]. HDAC-11 is the only member of Class IV HDACs.

HDAC inhibitors (HDACi) have been developed for cancer therapy based on known HDAC expression patterns and pathogenic functions [4–6]. Among them, Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), are first generation HDACi with a prototypical broad-spectrum inhibitory effect on class I, II, and IV HDACs [5]. They are also the first chemical HDAC tools available to study immune regulation in macrophages [2]. While HDACi represent a promising category of anti-cancer drugs, early clinical observations have suggested that SAHA inhibits cell-mediated immunity, raising the concern that HDACi might increase patient susceptibility to opportunistic and latent infections [7,8]. Indeed, numerous studies have shown that HDACi inhibit proinflammatory cytokine secretion and impair the innate

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immune response against infection [9]. For example, macrophages and dendritic cells treated with TSA or SAHA showed suppressed production of TLR-induced cytokines and chemokines, including *IL-12p40*, *IFN-β*, *IP-10*, *MCP5*, inducible nitric oxide synthetase, guanylate-binding protein 2 and *IL-12p35* [10]. LPS-induced *TNF-α* and *IL-6* secretion was also dramatically impaired upon prolonged pretreatment of dendritic cells with TSA or SAHA [10]. Another study showed that TSA inhibited LPS-induced *Ccl8*, *Ccl12*, *Cxcl10* and *Nos2* mRNA expression by macrophages [11].

Tuberculosis (TB) is the leading cause of human mortality caused by a single infectious agent, *Mycobacterial tuberculosis* (*M.tb*). Remarkably, about 23% of the world's population carry a latent tuberculosis infection (LTBI) [12]. Cytokines and chemokines are critical for the host defense against TB. For example, neutralizing the proinflammatory cytokine, *TNF-α*, during rheumatoid arthritis treatment can reactivate latent TB [13]. Clinical trials of several HDACi, such as SAHA, MS-275, valproate and ITF2357, in tumor patients have experienced episodes of severe infection [8,14–18]. Thus, it is important to determine the influence of HDACi on host immune response to *M.tb*.

In this study, we found that HDACi impaired *M.tb* macrophage-mediated phagocytosis and enhanced *M.tb* growth *in vitro* and *in vivo*. TSA significantly impaired cytoplasmic and mitochondrial ROS production, and inhibited autophagosome formation through down-regulation of *M.tb*-induced host immune response related gene expression, i.e. autophagy-regulating genes such as *CACNA2D3*.

2. Materials and methods

2.1. Cells and bacteria culture

The human monocyte cell line THP-1 cell was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). GFP-LC3-THP-1 cell was generated previously by Hongbo Shen [19]. Prior to infection, THP-1 cells were treated with 20 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) for 48 h to differentiate into macrophages. Virulent *M. tb* (H37Rv), attenuated *M. tb* (H37Ra) and GFP-H37Ra were grown at 37 °C in DifcoTM Middlebrook 7H9 broth with 10% oleic acid-albumin-dextrose-catalase (OADC) enrichment (Becton Dickinson), 0.05% (v/v) Tween 80 (Sigma-Aldrich), and 0.2% (v/v) glycerol (Thermo Fisher).

2.2. RNA extraction and analysis

Total RNA was isolated from macrophages using a RNeasy kit (Omega). High RNA integrity of each sample (three biological replicates of each group) was confirmed using the Agilent 2100 Bioanalyzer prior to cDNA library construction. RNA-seq was performed by BGI (Shenzhen, China) using the BGISEQ-500 platform. The transcriptomic data were analyzed as previously described [20]. The functional enrichment analysis of differentially expressed genes in KEGG pathway database was applied using phyper in R package. The validation of differentially expressed genes analyzed by RNA-seq was performed by qPCR on a 7500 Fast Real-Time PCR System using Power SYBR Green PCR Master Mix (Bimake) and primer pairs from the PrimerBank (Supplementary Table 1).

2.3. Cell viability assay

THP-1-derived-macrophages (5×10^3) were cultured in the presence of TSA, SAHA, Entinostat, Sodium butyrate, Valproic acid or Sirtinol (Selleck) which was dissolve by DMSO at different concentration with 1:10000 dilution (10 μM, 5 μM, 2.5 μM, 1.25 μM and 0.625 μM) for 72 h. DMSO with 1:10000 dilution was used as a negative control. Cell viability was measured using WST reagent (Beytime).

2.4. Colony forming units (CFU) assays

THP-1-derived macrophages were infected with H37Ra at MOI of 10 for 6 h in the presence or absence of HDACi treatment. At 6 h post-infection, complete medium containing HDACi (TSA, SAHA, Entinostat, Sodium butyrate, Valproic acid or Sirtinol)was added to the infected macrophages for 72 h, respectively. In the reciprocal experiment, THP-1-derived macrophages were treated with HDACi for 18 h before infection with H37Ra at MOI of 10 for 6 h. Then the cells were cultured in complete media without HDACi for 72 h. At 6 h and 72 h, the infected macrophages were lysed in 200 μl sterile PBS with 0.1% SDS. The viability of the intracellular Mycobacteria was quantified by counting CFU.

2.5. Measurement of ROS production

After infection with H37Ra in the presence or absence of TSA at MOI of 10 for 24 h, the cytoplasmic ROS (cROS) and mitochondrial ROS (mROS) using 5- (and-6) - chloromethyl- 2′, 7′- dichlorodihydro-fluorescein diacetate, acetyl ester (CM-H2DCFDA) (Invitrogen) or MitoSOX™ Red mitochondrial superoxide indicator (Invitrogen), according to the manufacturer's instructions. All fluorescence intensities were measured using a FACSCalibur flow cytometer (BD) and the data were analyzed using FlowJo version 6.1 (FlowJo, LLC).

2.6. Western blotting

THP-1-derived macrophages were infected with H37Ra in the presence or absence of TSA for 24 h. Total proteins were extracted using a Minute™ total protein extraction kit (Invent). The primary antibodies anti-LC3 (Abcam), anti-P62 (Abcam), anti-Beclin-1 (Cell Signaling Technology) and HRP-conjugated secondary antibodies (Cell Signaling Technology) were used. Western blotting signals were detected using an Amersham Imager 600 (GE Healthcare) and auto-radiographic film after incubation with SuperSignal West Femto substrate (Thermo Scientific). The densitometric analysis was determined by Image J.

2.7. Immunofluorescence and confocal microscopy

GFP-LC3-THP-1-derived macrophages were infected with H37Ra at MOI of 10 containing TSA or not for 24 h. Images were acquired of cells (selected at random) containing GFP-LC3 using the Micropoint System (Andor). GFP-H37Ra infected THP-1-derived macrophages were treated with or without TSA for 24 h. After infection, complete media containing 50 nM Lyso-Tracker Red (Beytime) was added to the cells for 30 min at 37 °C to stain acidified lysosomes. Images were acquired of cells (selected at random) containing GFP-H37Ra and lysosomes using the Micropoint System (Andor). All images were captured under a 60 × oil-immersion objective.

2.8. Murine TB model

Female C57BL/6 mice (6–8 weeks old) were used for H37Rv infection. Mice were infected with H37Rv using a Glass-Col inhalation system. TSA and SAHA were formulated with 0.5% carboxymethyl cellulose (CMC). On day 14 post H37Rv infection, mice were administered TSA (5mg/kg) or SAHA (150mg/kg) by oral gavage, as previously described [21,22]. The control group only received 0.5% CMC. The mice were sacrificed on day 1 [n = 4] or day 42 [n = 6], and bacterial counts in the lungs were determined by plating 10-fold serial dilutions on 7H11 agar plates. Lungs from TSA, SAHA or CMC-control mice infected with H37Rv were stained with hematoxylin and eosin for histopathological analysis. The lung lobes were imaged digitally using a NanoZoomer Digital Pathology System (Hamamatsu Photonics).

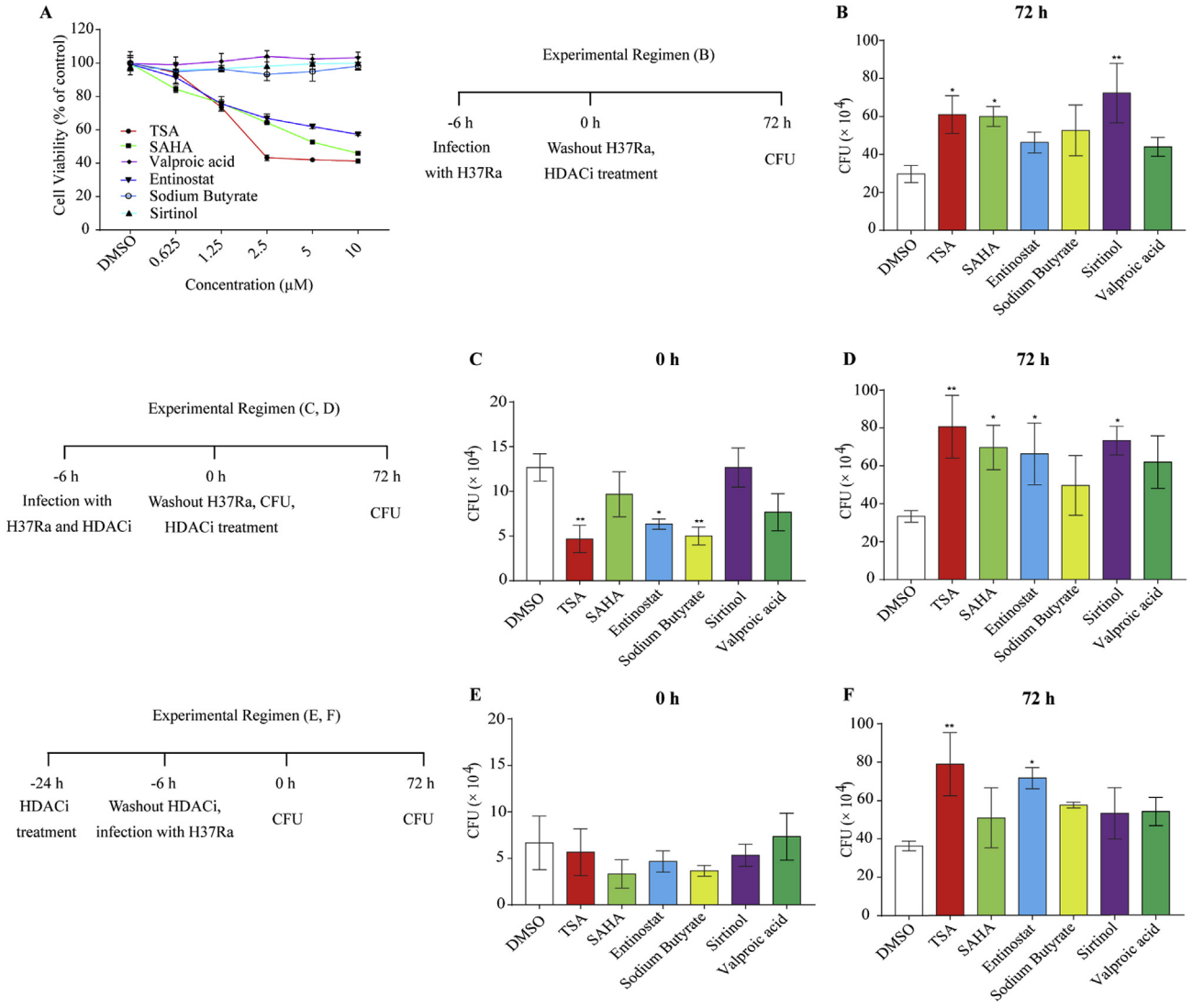

Fig. 1. HDAC inhibitors impair phagocytosis and killing of *M.tb* by macrophage. (A) PMA differentiated THP-1 cells were incubated at varying concentrations of HDACi for 72 h and cell viability was determined by an WTS-1 assay (four determinations). (B) The intracellular survival of *M.tb* were assessed post-infection with *M.tb* and treated with HDACi for 72 h. (C) The number of *M.tb* ingested by macrophage were counted at the present of HDACi. (D) The intracellular survival of *M.tb* were assessed cotreated with HDACi at the time of infection and continue treated with HDACi for 72 h. (E and F) The macrophages were treated with HDACi for 18 h prior to infection with *M.tb*, and then CFU was detected at 0 h (E) and 72 h (F) after infection. Data are presented as mean ± standard deviation (SD) of three independent replicated experiments. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

2.9. Statistical analyses

Statistical analyses were performed using GraphPad Prism 7 software. One-way ANOVA was used to assess the effects of between more than two groups. Student's *t*-test was used to assess the effects of only one parameter. Differences between groups were considered statistically significant when $p < 0.05$.

3. Results

3.1. HDACi inhibits M.tb phagocytosis and killing activity of THP-1 macrophages

Macrophage is the most important innate immune cell for host resistance against TB. Therefore, we first analyzed the effect of HDACi on bactericidal function of macrophage using differentiated THP-1. To this end, we first determined the cytotoxicity of different concentrations of HDACi in THP1-derived macrophages. We found that Sodium butyrate, Valproic acid and Sirtinol had no effect on cell viability at 10 μM, and TSA, SAHA and Entinostat had no effect on cell viability at 0.625 μM (Fig. 1A). Based on these findings, we chose the concentration of HDACi without effect on cell viability in the following experiments to determine their effects on macrophage phagocytosis and bactericidal activity against *M.tb*. We found that treatment of macrophage with TSA, SAHA and Sirtinol 72 h after *M.tb* infection significantly impaired macrophage killing capability against *M.tb* (Fig. 1B). A similar result was observed when HDACi was treated at the beginning of *M.tb* infection (Fig. 1D). TSA, Entinostat and Sodium Butyrate also significantly inhibited uptake of *M.tb* by macrophages (Fig. 1C).

To mimic different clinic situation, for example HDACi treatment before exposure to *M.tb*, and investigate whether pre-HDACi treatment affects the susceptibility to *M.tb* infection, we also investigated whether HDACi affected macrophage phagocytosis and killing by treating with HDACi before *M.tb* infection. We found that none of HDACi affected phagocytosis activity when treating the cells 18 h before *M.tb* infection (Fig. 1E). However, cells pretreated with TSA and Entinostat significantly increased the bacterial load (Fig. 1F), indicating that TSA and Entinostat inhibited macrophage mycobactericidal activity. Taken

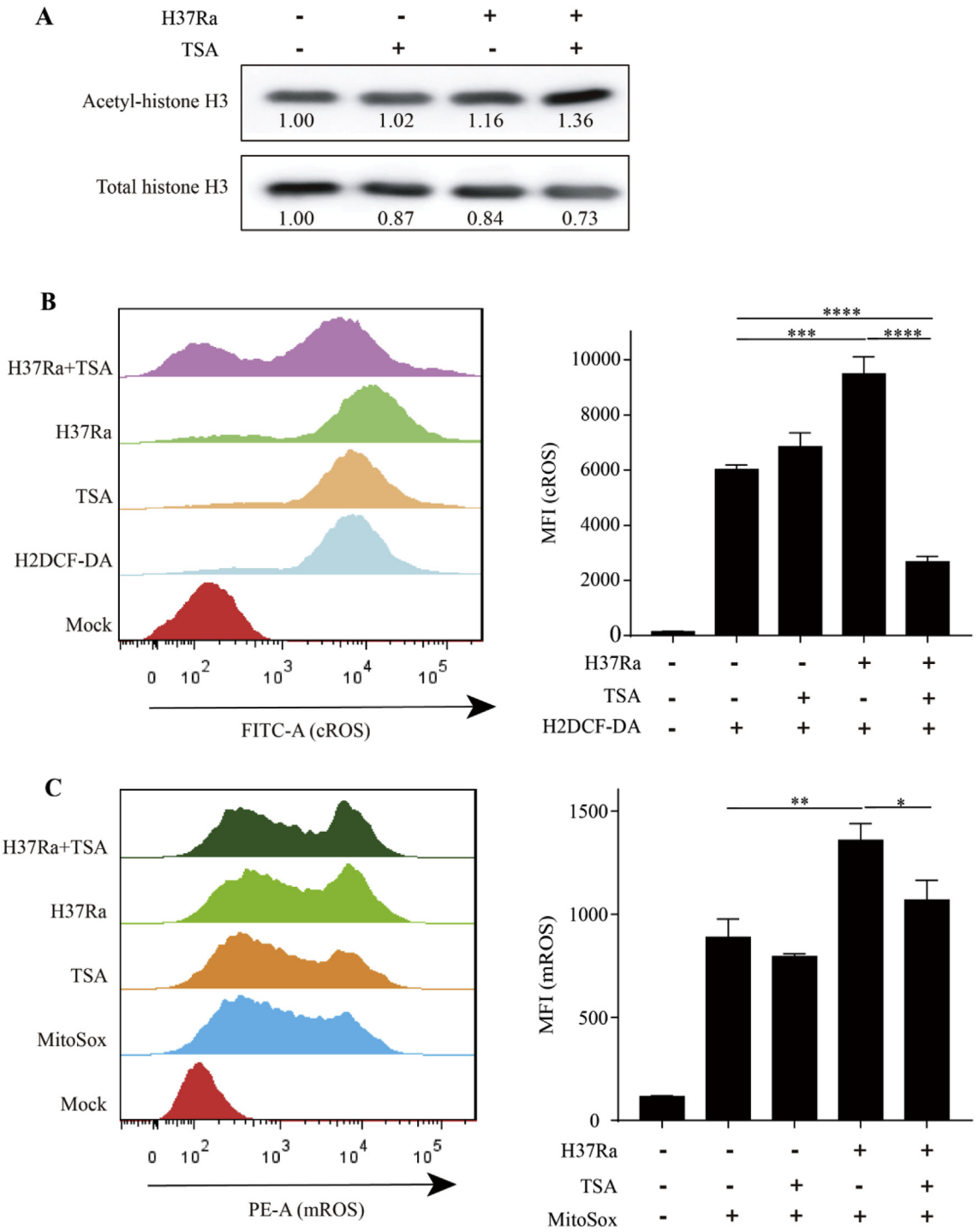

Fig. 2. TSA inhibited ROS production in *M.tb* infected macrophage. (A) TSA induced hyperacetylation of histone H3 protein with *M.tb* infection in macrophage. (B and C) At 24 h post-infection, the cROS and mROS generation were measured by flow cytometry. And the median fluorescence intensity (MFI) were used to quantitated the production of cROS (B) and mROS (C). Data are presented as mean ± standard deviation (SD) of three independent replicated experiments. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$.

together, these data indicated that HDACi significantly suppressed macrophages innate immunity against *M.tb* infection.

3.2. TSA inhibits M.tb-induced ROS production by macrophage

To investigate the mechanism underlying the inhibitory effect of HDACi on macrophage bactericidal capability against *M.tb*, we first investigated the effect of TSA on *M.tb*-induced ROS production, one of the pivotal mechanism for killing of intracellular *M.tb* by macrophage, as TSA is a prototypical broad-spectrum inhibitor of class I, II, and IV HDACs that induces histone acetylation [5]. Consistent with the primary role of TSA, we confirmed that TSA increased histone H3 hyperacetylation in macrophages during *M.tb* infection (Fig. 2A). In line with the previous results in TSA inhibited ROS production in *Staphylococcus aureus*-infected macrophages [23], we found that both cROS and mROS production are significantly reduced in *M.tb*-infected macrophages in the presence of TSA at 24 h post-infection (Fig. 2B and C) These results implicated that reduced cROS and mROS production

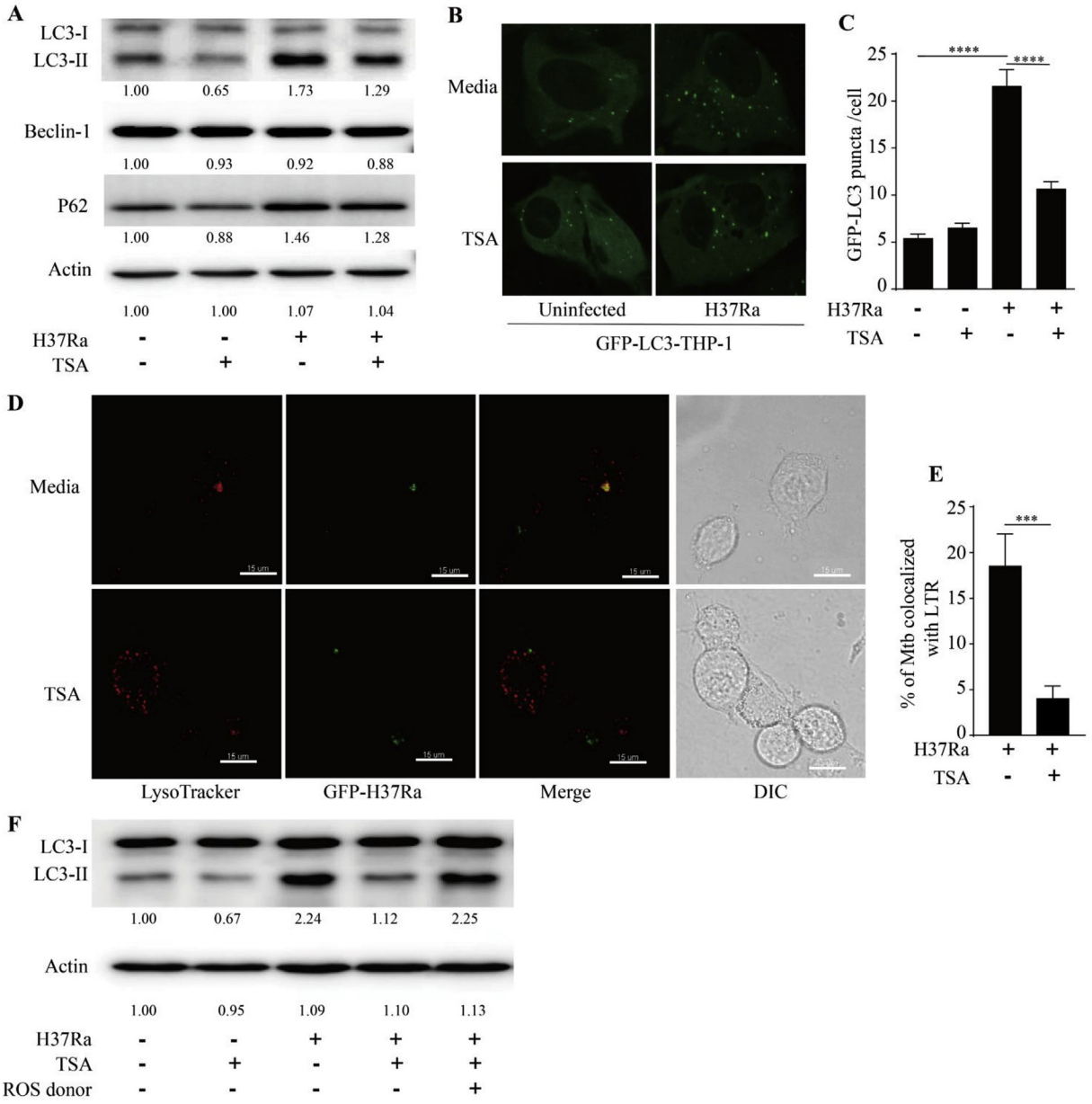

Fig. 3. TSA reduced autophagy and autophagosomes formation with *M.tb* infection. (A) Representative Western Blots displaying LC3-II and LC3-I, Beclin-1 and P62 (SQSTM1) with *M.tb* infection at the presence of TSA for 24 h. (B and C) Representative fluorescence images of individual cells with GFP-LC3 to aggregate of the autophagy machinery and bar graph showing comparative quantitation of GFP-LC3 puncta per cell more than 20 cells. (D) THP-1 cells were infected with GFP-H37Ra in the presence or absence of TSA and treated with 50 nM Lyso-Tracker Red (LTR) for half hours before fixation. (E) Quantification of LTR-positive *M.tb* from confocal more over 100 intracellular *M.tb*. (F) Representative Western Blots displaying LC3-II and LC3-I with TSA treatment and *M.tb* infection at the presence of ROS donor for 24 h. Data are presented as mean ± standard deviation (SD) of three independent replicated experiments. ***, $p < 0.001$, ****, $p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

might contribute to accelerating intracellular *M.tb* expansion during HDACi treatment.

3.3. TSA inhibits M.tb-induced autophagosome formation

In addition to ROS, autophagy has been established to play critical role for killing of *M.tb* by macrophage. *M.tb* induces autophagy in infected macrophages in association with the generation of ROS from mitochondrial and NADPH oxidase sources [24]. Importantly, previous study showed that ROS is essential for autophagy-mediated *M.tb* killing by macrophage [25]. We thus investigated the effects of TSA on autophagy during *M.tb* infection. We performed western blotting to determine the proportion of LC3 presented in the LC3-I and II forms in THP-1-derived macrophages. We found that a reduction of LC3-II/Actin ratio (i.e., autophagy flux) in *M.tb*-infected macrophages treated with TSA for 24 h compared to those without TSA (Fig. 3A), while Beclin-1 and P62 levels were not significantly changed (Fig. 3A). To further confirm the effect of TSA on *M.tb*-induced autophagy, we took advantage of GFP-LC3-THP-1 to its effect on the autophagy mediated LC3 punctate structure formation.

In line with previous study [19], *M.tb* induced the formation of LC3 punctate structures in macrophages (Fig. 3B). When TSA was applied, significantly reduced LC3 puncta formation in *M.tb*-infected macrophage was observed (Fig. 3B and C). More interestingly, we found that TSA significantly inhibited colocalization of GFP-H37Ra-containing phagosomes and lysosomes in *M.tb*-infected macrophages (Fig. 3D and

E).

We further used the ROS donor (BestBio, BB-47058-1) to increase the ROS. The result showed that ROS donor could rescue the effect of TSA in *M.tb*-infected macrophage (Fig. 3F), which suggest that TSA mediated autophagy-inhibition in *M.tb*-infected macrophage was depended on the reduced ROS production. Taken together, these results suggested that suppression of *M.tb*-induced autophagy by TSA has consequently inhibitory effect on autophagosome formation, and thus resulting in escape of *M.tb* from killing.

3.4. TSA inhibits ROS-associated and autophagy-associated gene expression

The modification of histone, particularly acetylation of lysine residues of H3 and H4, plays a critical role in regulation of gene transcription [26]. This was also confirmed by our finding that TSA increased histone H3 hyperacetylation in *M.tb*-infected macrophages (Fig. 2A). To further explore the roles and underlying mechanisms of TSA in modulating host immunity against *M.tb* infection, we assessed the transcriptional profile of *M.tb*-infected macrophage treated with or without TSA by RNA-seq. We found that TSA induced the up-regulation of 220 genes and the down-regulation of 361 genes (fold change > 2, supplementary data 2) in *M.tb*-infected macrophages, compared to *M.tb* infection alone (Fig. 4A). These up-regulated genes were significantly enriched for the TNF signaling pathway, PI3K-Akt/MAPK signaling pathway, Focal adhesion and calcium signaling pathway on the other hand, the down-regulated genes were significantly enriched for Hippo signaling pathway, PI3K-Akt/MAPK signaling pathway, and calcium signaling pathway as determined by KEGG pathway analysis (Fig. 4B, supplementary data 3).

We further validated the RNA-seq results by qRT-PCR (Fig. 4C, D, 4E, 4F). Consistently, we found that TSA treatment down-regulated *NOX2*, *FLT3* and *VDAC3* expression (Fig. 4D), which are associated with ROS production [27,28]. We also found that *CAMK4* and *CDC20* (Fig. 4E and F), which are involved in autophagy [29,30], were also down-regulated in TSA-treated macrophages. Most interestingly, *CACNA2D3*, which has been recently reported to regulates Ca^{2+}-dependent autophagy in *M.tb* infection [31], was also significantly down-regulated following TSA treatment (Fig. 4E). Finally, we found that SAHA has similar effect as TSA in down-regulation of these autophagy-associated gene (Fig. S1, supplementary data 4). Together, these results suggested that TSA strongly downregulated ROS-associated and autophagy-associated gene expression in *M.tb*-infected macrophages, which is indicates inhibition of host immune responses to *M.tb*.

3.5. HDACi impairs the host defense against H37Rv in mice

To further confirm our results *in vitro* and determine the effects of HDACi *in vivo*, we treated mice with TSA and SAHA during H37Rv infection [32]. The bacterial load in the lungs of mice treated with TSA or SAHA was significantly increased compared to the control group, indicating that TSA or SAHA impaired bacterial clearance in mice (Fig. 5A). Histological examination revealed that TSA or SAHA treatment, especially TSA treatment could increase the lung pathology (Fig. 5B and C). These results suggested that TSA and SAHA treatment impaired the host defense against H37Rv infection in mouse.

4. Discussion

HDACi represent a novel class of anti-cancer drug, but accumulating data suggest that HDACi compromise the host immune response against infections. These findings raise the concern that HDACi may increase TB susceptibility, for example, reactivation of LTBI due to HDACi use.

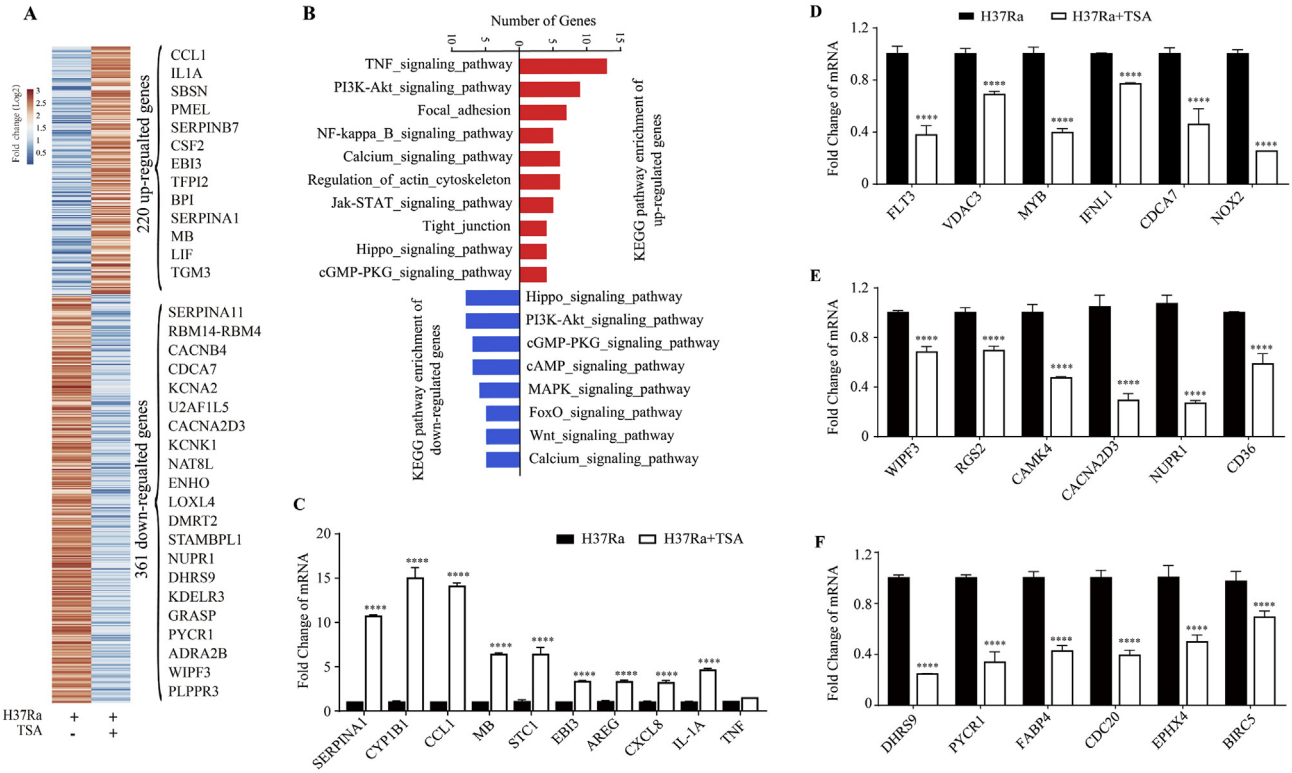

Fig. 4. Trichostatin A regulated genes expression induced by *M.tb* in macrophages. (A) Number of differentially expression genes either up-regulated (red) or down-regulated (blue) (fold change > 2)induced by TSA cotreatment *M.tb* compare with *M.tb* alone. Representative related genes are listed vertically. (B) The KEGG pathway enrichment of difference expression genes. Quantitative RT-PCR validated mRNA expression of up-regulated genes (C) and down-regulated genes (D) by TSA cotreatment with *M.tb*. mRNA levels were normalized to β-Actin. Data are presented as mean ± standard deviation (SD) of three independent replicated experiments. ***, $p < 0.001$, ****, $p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

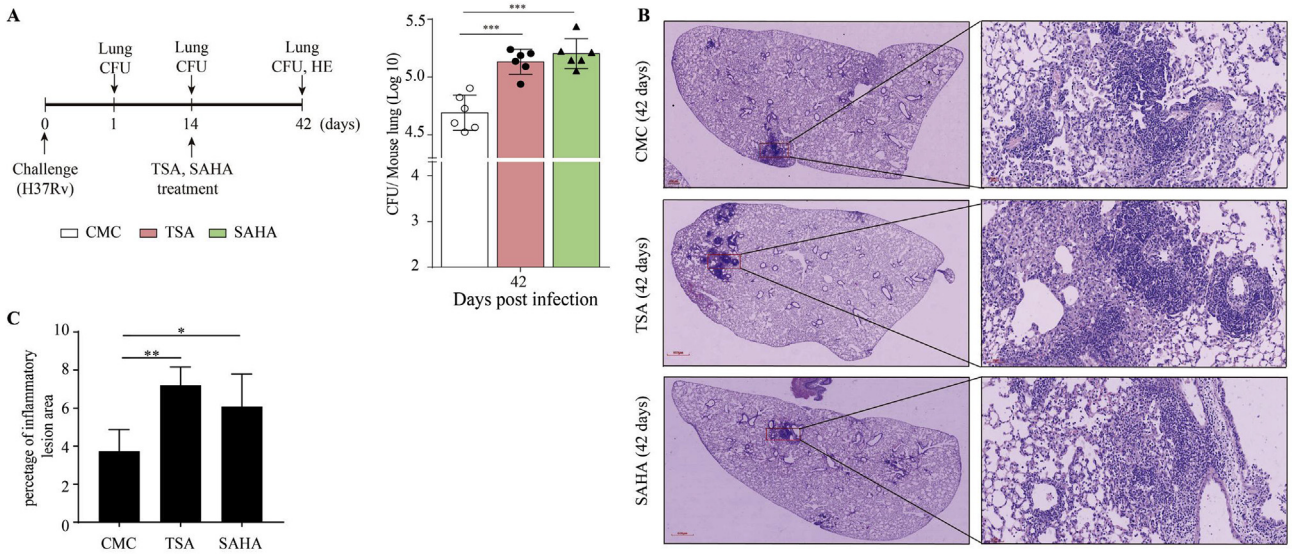

Fig. 5. TSA assist H37Rv growth and tissue pathology in mice. (A) Bacterial burden (CFU) were shown in lung tissue from individual animals (n = 6) at 42 days post-infection. (B) Histopathology of lungs from CMC-control, TSA or SAHA treatment mice infected with H37Rv, stained with hematoxylin and eosin. The lung lopes of HE staining were photographed in digital form using NanoZoomer Digital Pathology System and outlined areas in main images are enlarged in insets (50 μm). (C) The percentage of inflammatory lesion area of each lung. Data are presented as mean ± standard deviation (SD). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

In this study, we found that HDACi including TSA, SAHA, Entinostat and Sirtinol, significantly inhibit macrophage bactericidal activity against *M.tb*. Whole genome RNA-seq revealed that TSA has profound effect on *M.tb* induced gene expression in macrophage with significantly downregulated ROS-associated and autophagy-associated gene expression.

TSA significantly inhibits *M.tb*-induced ROS production and autophagy, which are important for intracellular *M.tb* clearance. Finally, we confirmed the inhibitory effects of HDACi on macrophage bactericidal activity by showing that TSA and SAHA treatment of mice *in vivo* significantly increase susceptibility *M.tb*. To the best of our knowledge, these data provide the first *in vivo* evidence that HDACi impair the host immunity against TB. Further clinical investigation is warranted to determine whether screening and preventing reactivation of LTBI, a strategy currently used for anti-TNF-α treatment in autoimmune diseases, should also be applied to the clinical use of HDACi.

A recent epidemiological study estimated that about 23% of the world's population carry a latent TB infection [12]. In China, about 40% of the population is infected with *M.tb*. Nevertheless, the majority infected patients efficiently contain the *M.tb* infection and assume LTBI status, demonstrating the critical role of the host immune response in determining the progression of *M.tb* infection. In line with this, both animal and clinical studies have confirmed that cellular immunity is essential for TB protection. In recognizing the immune suppressive activity of HDACi, it is important to determine to what extent HDACi treatment might affect the outcome of *M.tb* infection. Indeed, some research has focused on the effects of HDACi on *M.tb* infection. Schiebler et al. reported that valproic acid (VPA), a selective HDAC1 inhibitor, increases macrophage killing ability of *M.tb* by inducing mTOR-independent autophagosome formation [33]. However, the minimum inhibitory concentration of VPA (0.5 mM) on *M.tb* in macrophages is much higher than the upper limit allowed to be used in humans. Rao et al. found that VPA (5.9 mM) and SAHA (6 μM) enhanced the effects of first-line TB drugs against intracellular *M.tb* [34]; however, again the concentrations are too high to clinic use and the effect of SAHA at 6.0 μM might be confound by its direct killing of *M.tb* in host cell free medium. When a lower concentration (6 μM) of VPA was used in our study, we did not observe any effect of VPA on macrophage killing capability against *M.tb*.

Recently, Tubastatin A, a highly selective HDAC6 inhibitor, significantly enhances resistance to *M.tb* infection in mice by increasing *TNF-α*, *IL-12* and *IFN-γ* and inhibiting *IL-10* production [35]. Conversely, Moreno-Gonzalo et al. recently reported that *HDAC6*$^{-/-}$ mice are highly susceptible to intracellular *Listeria monocytogenes* infection due to an inhibited innate immune and autophagy response to TLR-mediated signaling [36]. Thus, further investigations are needed to clarify the role Tubastatin A in TB using HDAC6$^{-/-}$ mice. By contrast, activation of SIRT1, a class III HDAC, reduces intracellular *M.tb* growth by inducing autophagy and phagosome-lysosome fusion [37]. Thus, the effects of HDACi on TB might vary depending on their selectivity and concentration. At the concentrations required for clinical application (e.g. cancer treatment), non-selective HDACi such as SAHA and TSA, significantly impair innate immunity and increase susceptibility to TB.

In line with the broad effects of HDACi, we found that TSA has profound suppressive effects on macrophage ROS production, phagocytosis and autophagy that should be induced by *M.tb* infection. Our results show that TSA inhibits ROS production during long-term (24 h) TSA treatment. Notably, we found that TSA-inhibited autophagy and autolysosome formation, which is critical for killing *M.tb*. We also found that TSA significantly inhibits the expression of intracellular calcium modulators, such as *CACNA2D3*, *CACNB4* and *CAMK4*, which are associated with the functional autolysosome pathway in autophagy [31,38]. Functional inhibition of Ca^{2+} signaling is associated with reduced phagosome-lysosome fusion, through which *M.tb* survival is increased in human macrophages. A recent study identified that *CACNA2D3*, which regulates ER Ca^{2+} signaling to inhibit autophagy, is directly down-regulated by *M.tb*-induced miR-27a [31]. Although we did not find that the expression of miR-27a was increased by TSA, we noted numerous microRNAs (e.g. miR3128, miR767-5p, miR29b-2-5p et al.) other than miR-27a that target *CACNA2D3*, which might also contribute to reduced *CACNA2D3* expression [39]. We also found that TSA inhibited PI3K-Akt and MAPK signaling and ROS production, both of which are important in regulating cytosolic Ca^{2+} release [40]. Taken together, our findings suggest that TSA significantly inhibits *M.tb*-induced autophagy and autolysosome formation by modulating calcium release. Further investigations to clarify the exact mechanisms are now warranted.

5. Conclusion

In this study, we reported that HDACi, such as TSA and SAHA, impair macrophage-mediated killing of *M.tb* and moderately increased susceptibility to TB *in vivo*. This impairment of the host defense against TB by HDACi through multiple ways which was associated with inhibited *M.tb*-induced ROS production and autophagy.

Funding

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.101861.

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