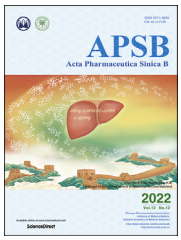
Acta Pharmaceutica Sinica B 2022;12(12):4519–4521

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



HIGHLIGHT

SMIP-30, a potent and selective PPM1A inhibitor with potential to treat tuberculosis

KEY WORDS

PPM1A;
Tuberculosis;
Drug discovery;
Compound library

Recently, a collaborative research led by Weibo Yang and Jim Sun[1] published in *Cell Chemical Biology* identified a potent and selective small molecule inhibitor (SMIP-30) for human protein phosphatase Mg^{2+}/Mn^{2+}-dependent 1 A (PPM1A). They applied this chemical probe to determine the autophagy receptor p62 as a new substrate of PPM1A, and proved that SMIP-30 could enhance the selective autophagy of macrophages to limit the survival of intracellular *Mycobacterium tuberculosis* (Mtb) (Fig. 1). These findings should promote new therapeutic modalities to overcome tuberculosis (TB)[1].

TB is a global devastating infectious disease caused by Mtb that is the second-leading infectious killer after corona virus disease 2019 (COVID-19). Although there are some effective drugs against TB, the current therapies have shortcomings like a lengthy treatment cycle, poor patient compliance, significant toxicity and side effects, as well as the continuous emergence of new drug-resistant strains, highlighting the necessity of finding novel anti-TB therapies[2]. Mtb is an intracellular pathogen that employs a number of tactics to obstruct the normal operation of host cells in order to evade host immune response and achieve long-term survival in host cells. The interactions between pathogen and host provides new ideas and specific targets for anti-TB treatment and drug development[3]. Host-directed therapy (HDT) is an emerging concept aimed at enhancing host immune responses through the use of drugs/compounds with or without antibiotics against Mtb infection, which has the potential to curtail the course of treatment, avert drug resistance and lessen lung injury[1,3]. PPM1A has been identified as a possible host target for Mtb infection, and inhibition of PPM1A may be a feasible therapy for TB HDT[1]. However, since the development of phosphatase inhibitors is challenging and undergoes poor selectivity, the potent and selective PPM1A inhibitor does not exist.

To explore effective PPM1A inhibitors, Yang, Sun and their colleagues[1] constructed a pseudo-natural product library applying a biomimetic modularization strategy (BMS). Through high-throughput screening (HTS) of compound library, they identified five small molecule inhibitors of PPM1A (SMIPs) with moderate potency. To further improve these compounds, they used function-oriented synthesis (FOS) and obtained a potent PPM1A inhibitor SMIP-30 (IC_{50} = 1 μmol/L), which possessed higher activity than sanguinarine (a known PPM1A inhibitor) and synthetic accessibility. It is noteworthy that SMIP-30 was a selective PPM1A inhibitor with IC_{50} value more than 30 μmol/L for PPM1B, the closest homologue of PPM1A. Next, kinetics of inhibition experiment indicated that SMIP-30 acted as an uncompetitive inhibitor of PPM1A. This could explain that although PPMIA and PPM1B share a conserved catalytic core region, the formation of different enzyme–substrate complexes might result in the higher selectivity of SMIP-30 for PPM1A than PPM1B.

Macrophages are the main target cells of Mtb. In order to establish whether inhibition of PPM1A can influence the survival of Mtb in macrophages and prove that PPM1A is the real host protein of TB HDT, infection of macrophages knocked out PPM1A (ΔPPM1A) or treated with SMIP-30 with Mtb strains revealed reduced Mtb burden in both cells. Given the potency of SMIP-30 in limiting the survival of Mtb in macrophages, the authors then performed cytotoxicity assays and found that SMIP-30 was well tolerated *in vivo* and did not cause hyperinflammatory responses, supporting its safety and compliance with HDT. Additionally, combining first-line TB antibiotic rifampicin with SMIP-30 substantially enhanced host responses and accelerated Mtb clearance in macrophages. The data presented above

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences

https://doi.org/10.1016/j.apsb.2022.10.001

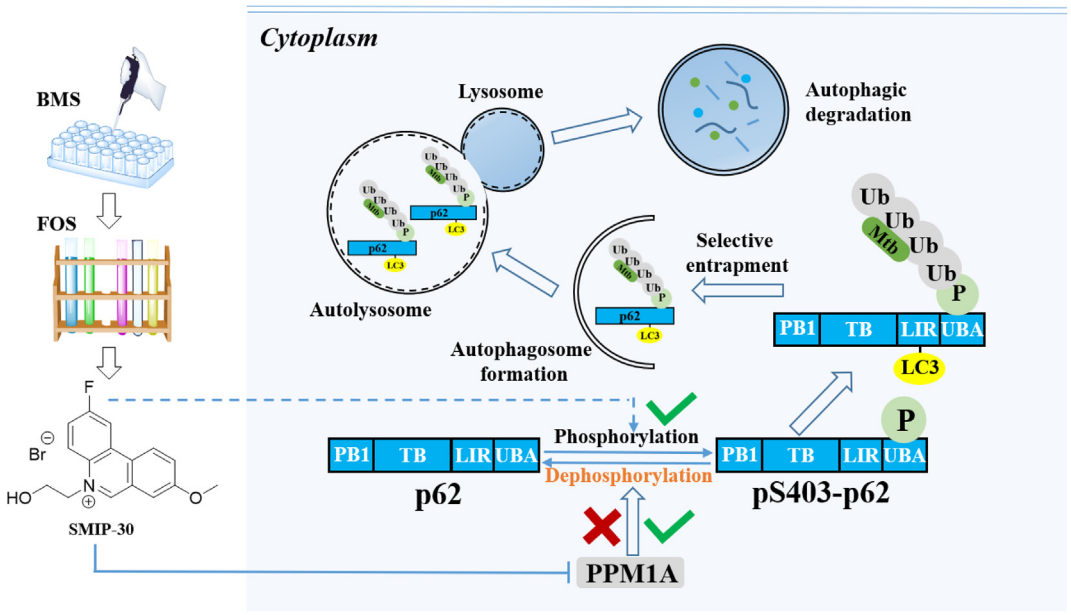


Figure 1 The PPM1A inhibitor SMIP-30 enhances the phosphorylation of p62, making it more accessible to label Mtb exposed to the cytoplasm and target it to autophagosomes.

demonstrated that targeting PPM1A is an effective strategy to treat TB by regulating host immune response.

After Mtb invades the body, macrophages can initiate immune mechanisms such as apoptosis, autophagy, necrosis, and Toll-like receptor (TLR) signaling pathway-mediated inflammatory response, among which autophagy is considered to be beneficial to host defense. Activation of autophagy can promote the maturation of Mtb phagosomes in macrophages and facilitate the clearance of Mtb[4]. Therefore, to explain how inhibition of PPM1A accelerates Mtb clearance in macrophages, Yang, Sun and their colleagues[1] focused on the relationship between PPM1A and autophagy. Firstly, genetic perturbation experiments applying wild-type (WT) macrophages, ΔPPM1A and macrophages with high expression of $PPM1A^{+}$ showed that Mtb infection increased the expression of the autophagy marker LC3B-II in ΔPPM1A cells, but not in $PPM1A^{+}$ cells, which indicated that the inhibition of PPM1A can activate autophagy in Mtb-infected macrophages. Significantly, treatment with SMIP-30 could induce the expression of LC3B-II in WT macrophages, but LC3B-II not increase in ΔPPM1A or $PPM1A^{+}$ macrophages, suggesting that SMIP-30 induces selective autophagy in a PPM1A-dependent manner during Mtb infection. Consistent with the genetic perturbation results, immunofluorescence experiments confirmed that the autophagy pathway of Mtb-infected ΔPPM1A cells and SMIP-30-treated WT macrophages was activated. Conversely, SMIP-30 treatment did not show significant effects when PPM1A was deleted or overexpressed. These data demonstrated that SMIP-30 activated the autophagy of macrophages infected with Mtb by inhibiting PPM1A.

P62, also called sequestosome 1 (SQSTM1), includes four domains: phox/bem 1p (PB1), TRAF6-binding (TB), LC3-interacting region (LIR) and ubiquitin-associated domain (UBA), acting as a bridge to connect autophagy receptor protein LC3 and ubiquitinized proteins. Selective autophagy (xenophagy) of intracellular pathogens is modulated by p62, whose UBA domain can especially identify ubiquitin-coated intracellular bacteria, and phosphorylation of serine 403 (S403-p62) in the UBA domain can increase affinity for polyubiquitin chains and degradation of Mtb-containing autophagosomes[5]. Based on this principle, the authors further explored the possible mechanism of activating autophagy. The phosphorylation of S403-p62 increased in macrophages knocked out of PPM1A or treated with SMIP-30, suggesting that inhibiting PPM1A might stabilize the sequestosomes containing ubiquitinated proteins or bacteria, thus activating autophagy by the mechanism dependent on p62 phosphorylation, which may explain why SMIP-30 has a high Mtb clearance rate. The above experimental results indicated that there might be biochemical interactions between PPM1A and p62, so Yang, Sun and their colleagues[1] designed a phosphosynthetic peptide including the amino acid sequence around S403 and used recombinant PPM1A for *in vitro* phosphatase assay. The results showed that in the presence of metal cofactors, PPM1A could specifically and dose-dependently dephosphorylate p62 on S403, affirming that p62 was the substrate of PPM1A. Finally, with autophagy inhibitor and apoptosis inhibitor as controls, it was further proved that induction of autophagy activation was mainly responsible for the antibacterial activity of SMIP-30.

From the perspective of HDT against TB, PPM1A represents a novel target of autophagy-induced pathway, and its selective inhibitor SMIP-30 activates autophagy *via* a mechanism dependent on p62 phosphorylation, consequently restricting the survival of Mtb in macrophages and infected mice lungs. Overall, this work overcomes the challenges of phosphatase inhibitor design and opens up new avenues for TB treatment.

As reported, HDT based on autophagy activation shows efficient therapeutic manipulation of host immunity against Mtb and its drug-resistant strains infection. Currently, the mechanism of activating autophagy mainly includes indirect activation of signal transduction pathways or acting at the later stages of autophagy pathway[6]. Notably, the inhibition of PPM1A by SMIP-30 directly acts on the early stage of autophagy, providing a unique way to

induce autophagy in Mtb-infected cells and expanding the available library of druggable targets. However, due to the promiscuous of most phosphatases and the effect of p62 phosphorylation on multiple cellular pathways, it is necessary to further investigate whether SMIP-30 and other PPM1A inhibitors will have off-target and related side effects.

Macrophages can limit the survival of Mtb through autophagy, but Mtb also adopts multiple strategies during infection to circumvent autophagy-related immune clearance. Elucidating the dialogue mechanism between Mtb and macrophage autophagy will better understand the process of Mtb escaping immune detection and inducing drug resistance in the process of TB. For instance, Mtb-secreted acid phosphatase that targets the host Rab 7 can prevent autophagosome-lysosome fusion[7], which suggests HDT drugs that modulate autophagy should not only enhance autophagy activation, but also overcome Mtb-induced blockade of autophagosome-lysosome fusion. Additionally, to reduce exposure to non-target cells and undesired side effects, developing immunomodulatory nanoparticle-based therapies for Mtb infection will achieve more targeted drug delivery, reduce harmful side effects, and increase drug concentrations in target cells.

References

1. Berton S, Chen L, Liang YC, Xu Z, Afriyie-Asante A, Rajabalee N, et al. A selective PPM1A inhibitor activates autophagy to restrict the survival of *Mycobacterium tuberculosis*. *Cell Chem Biol* 2022;**29**:1126−39.e12.
2. Kuang W, Zhang H, Wang X, Yang P. Overcoming *Mycobacterium tuberculosis* through small molecule inhibitors to break down cell wall synthesis. *Acta Pharm Sin B* 2022;**12**:3201−14.
3. Chai Q, Wang L, Liu CH, Ge B. New insights into the evasion of host innate immunity by *Mycobacterium tuberculosis*. *Cell Mol Immunol* 2020;**17**:901−13.
4. Paik S, Kim JK, Chung C, Jo EK. Autophagy: a new strategy for host-directed therapy of tuberculosis. *Virulence* 2019;**10**:448−59.
5. Matsumoto G, Wada K, Okuno M, Kurosawa M, Nukina N. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol Cell* 2011;**44**:279−89.
6. Pellegrini JM, Tateosian NL, Morelli MP, García VE. Shedding light on autophagy during human tuberculosis. A long way to go. *Front Cell Infect Microbiol* 2022;**11**:820095.
7. Chai Q, Zhang Y, Liu CH. *Mycobacterium tuberculosis*: an adaptable pathogen associated with multiple human diseases. *Front Cell Infect Microbiol* 2018;**8**:158.

Shujing Xu, Xinyong Liu*, Peng Zhan*

Department of Medicinal Chemistry, Key Laboratory of Chemical Biology, Ministry of Education, School of Pharmaceutical Sciences, Shandong University, Ji'nan 250012, China

*Corresponding authors.

E-mail addresses: xinyongl@sdu.edu.cn (Xinyong Liu), zhanpeng1982@sdu.edu.cn (Peng Zhan)

Received 22 August 2022
Revised 22 September 2022
Accepted 4 October 2022