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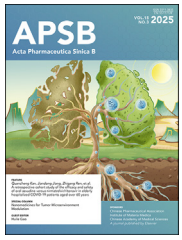

LETTER TO THE EDITOR

Lycorine hydrochloride directly targets UBA1 to suppress cellular senescence

KEY WORDS

Cellular senescence;
Lycorine hydrochloride;
Ubiquitin-activating enzyme;
UBA1

To the Editor:

Aging is characterized by progressive functional decline, with the gradual loss of proteostasis being a widely acknowledged hallmark of the aging process. Direct evidence indicates a reduction in ubiquitination levels in aged worms[1]. Therefore, the development of therapeutics that target the ubiquitination pathways could offer substantial potential for delaying the onset of aging or age-related diseases.

UBA1 and UBA6 are two major ubiquitin-activating enzymes (E1s) identified in the human genome. UBA1 is particularly crucial, being responsible for the ubiquitination of over 99% of cellular proteins. Inhibition of UBA1 triggers apoptosis, which is particularly detrimental to cancer cells[2]. Furthermore, the loss of UBA1 function is closely linked to aging. In Drosophila, mutations in the Uba1 gene lead to a shortened adult lifespan, underscoring a connection between ubiquitination and longevity[3]. Decreased ubiquitination during aging has been documented, and enhancing the ubiquitination process by targeting UBA1 could present a clinical strategy to delay the onset of aging. However, the correlation between restored ubiquitination levels and aging effects warrants further investigation.

Our earlier research was the first to reveal that treatment with lycorine hydrochloride (LH) significantly delays the onset of stress-induced premature cellular senescence (SIPS)[4]. In this work, we proved that UBA1 is a direct target of lycorine hydrochloride, functioning as an activator of the enzyme. Furthermore, lycorine hydrochloride enhances UBA1 catalytic activity by strengthening the interaction between UBA1 and the E2 enzymes. This enhancement culminates in the amelioration of ubiquitination defects associated with cellular senescence in a UBA1-dependent manner. Consequently, this intervention suppresses the expression of specific senescence-associated secretory phenotype (SASP) factors and delays the onset of cellular senescence.

1. Lycorine hydrochloride interacts with UBA1 at key residues Asp 504 and Lys 528

To address the direct molecular targets of lycorine hydrochloride on mitigating cellular senescence, we identified a cohort of 16 proteins by employing a LiP-SMap assay (Supporting Information Table S1). Among them, the ubiquitin-like modifier-activating enzyme 1 (UBA1) is implicated in the aging process. Using surface plasmon resonance (SPR) assay, we confirmed that lycorine hydrochloride binds to UBA1 with high affinity, as evidenced by a dissociation constant (K_d) of 362.7 nmol/L (Supporting Information Fig. S1A). Moreover, we conducted a cellular thermal shift assay (CETSA) to substantiate the binding between lycorine hydrochloride and UBA1 (Fig. 1A, Fig. S1B). Collectively, these findings underscore a direct interaction between UBA1 and lycorine hydrochloride.

To identify the binding sites of lycorine hydrochloride on UBA1, we first employed molecular docking to make the prediction. The results revealed that the interaction is mediated by two critical amino acid residues in UBA1, Asp 504 and Lys 528, which are postulated to be ATP binding sites on UBA1[5] (Fig. S1C). Then, we created specific point mutations, converting Asp 504 and Lys 528 to alanine (Ala) to produce UBA1-D504A and UBA1-K528A mutants. We then conducted CESTA

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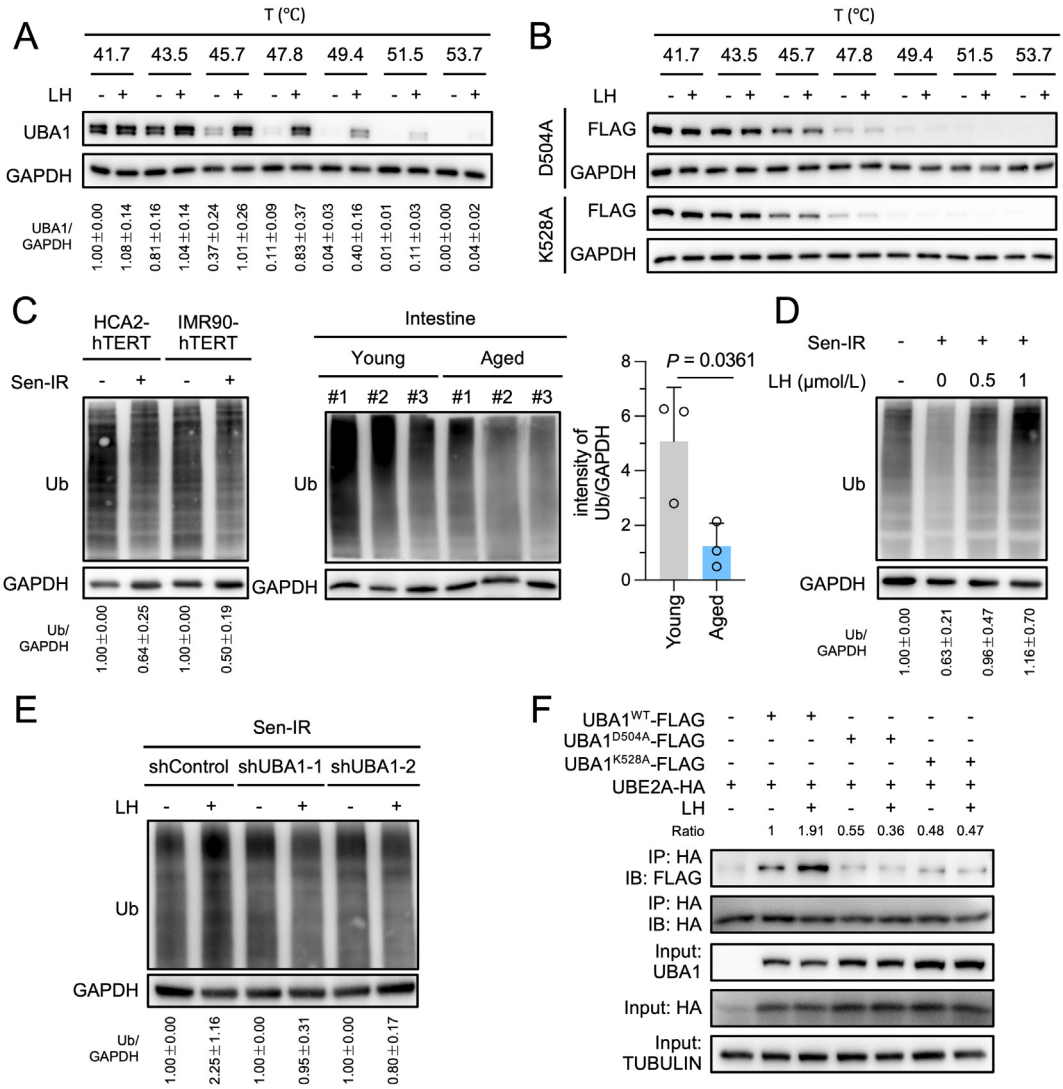

Figure 1 Lycorine hydrochloride reverses ubiquitination decline in senescent cells through activating the enzymatic activity of UBA1. (A) Cellular thermal shift assay (CETSA) conducted on HEK293 cells in the presence of 2.5 μmol/L lycorine hydrochloride (+) and DMSO (−) as controls; (B) HEK293 cells were transfected with UBA1-D504A or UBA1-K528A mutants and subjected to CETSA following treatment with 2.5 μmol/L lycorine hydrochloride (+) or DMSO (−); (C) Ubiquitination levels in senescent cells in immortalized HCA2-hTERT or IMR90-hTERT cells (left panel). Comparative Western blot analysis of ubiquitination levels in intestine isolated from young (2 months) and aged (27 months) mice (right panel); (D) Dose-dependent effect of lycorine hydrochloride on ubiquitination levels in senescent HCA2-hTERT cells; (E) Western blot analysis of ubiquitination in senescent control and UBA1 knockdown HCA2-hTERT cells; (F) Co-immunoprecipitation analysis of the interaction between UBE2A and UBA1 mutants in HEK293 cells following treatment with 1 μmol/L lycorine hydrochloride. LH, lycorine hydrochloride; Sen-IR, senescent cells induced by ionizing radiation (IR). Cells were treated with lycorine hydrochloride for 24 h, followed by 10 Gy X-ray irradiation and harvested 10 days post-irradiation. M, mol/L.

in cells expressing these mutants in the presence of DMSO or lycorine hydrochloride. In contrast to UBA1 WT, the mutants did not show the increased thermal stability conferred by lycorine hydrochloride (Fig. 1B, Fig. S1D), confirming the direct binding of lycorine hydrochloride to UBA1 through Asp 504 and Lys 528.

2. Lycorine hydrochloride reverses ubiquitination decline in senescent cells through activating the enzymatic activity of UBA1

Studies in *Caenorhabditis elegans* demonstrating a decline in ubiquitination levels during aging. Extending this investigation to mammals, we observed a similar decrease in ubiquitination in senescent human fibroblast cell lines and aged mouse tissues in compared to their younger counterparts (Fig. 1C, Supporting Information Fig. S2A). Considering the established role of lycorine hydrochloride in inhibiting SIPS and SASP, we propose that it may modulate ubiquitination to delay the onset of SIPS by interacting with UBA1.

Next, we evaluated the impact of lycorine hydrochloride on the ubiquitination level in senescent cells. We observed that lycorine hydrochloride increased ubiquitination in a dose-dependent manner (Fig. 1D). Additionally, the treatment did not alter the protein level of UBA1 (Fig. S2B), indicating that lycorine hydrochloride binding enhances UBA1 functionality and stimulates

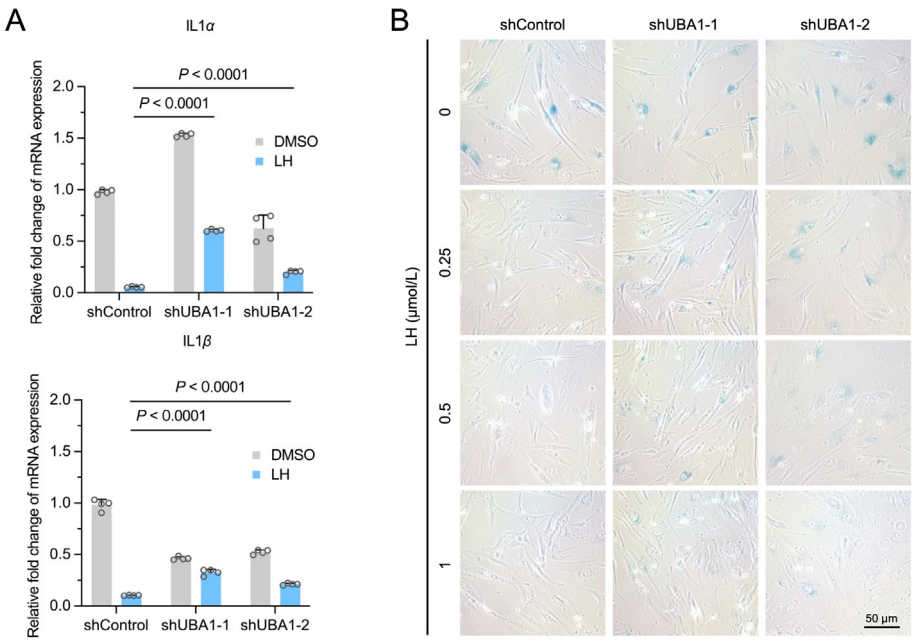

Figure 2 UBA1 knockdown abolishes the anti-senescence effects of lycorine hydrochloride. (A) Impact of 1 μmol/L lycorine hydrochloride on IL1α (upper panel) and IL1β (lower panel) expression in senescent control and UBA1 knockdown cells. (B) Representative images of senescence-associated β-galactosidase (SA-β gal) staining. Data are mean ± SD; Scale bar = 50 μm; LH, lycorine hydrochloride. Cells were treated with lycorine hydrochloride for 24 h, followed by 10 Gy X-ray irradiation and harvested 10 days post-irradiation for further investigation. M, mol/L.

the ubiquitination process. Furthermore, we established a stable UBA1 knockdown cell line (Fig. S2C), the knockdown of UBA1 abolished the ubiquitination increase induced by lycorine hydrochloride (Fig. 1E), thereby confirming UBA1's essential role in mediating the restoration of ubiquitination levels in senescent cells by lycorine hydrochloride.

The conjugation of ubiquitin to E1 and its subsequent transfer to E2 are ATP-dependent steps critical to the ubiquitination process. We aimed to determine how lycorine hydrochloride, by binding to the ATP binding sites on UBA1, could enhance this process. An *in vitro* ubiquitination assay revealed an increase in ubiquitin conjugation to UBA1 in the presence of lycorine hydrochloride, while UBA1 mutants abolished the enhancement (Fig. S2D). We then identified E2 enzymes, specifically UBE2A, UBE2B, reported to act downstream of UBA1[6], UBE2D2[7], UBE2E3[8], reported to be associated with cellular senescence. Co-immunoprecipitation assays showed that lycorine hydrochloride specifically strengthened the interaction between UBA1 and UBE2A, UBE2D2 and UBE2E3, with no effect on the UBA1−UBE2B interaction. Notably, the UBA1-D504A and UBA1-K528A mutants eliminated the enhancing effect of lycorine hydrochloride on this interaction (Fig. 1F, Fig. S2E and F), indicating that lycorine hydrochloride facilitates the transfer of ubiquitin from UBA1 to E2s by enhancing the protein-protein interaction, thereby rescuing the decline in ubiquitination associated with cellular senescence.

3. UBA1 knockdown abrogates the suppressive effects of lycorine hydrochloride on cellular senescence

As lycorine hydrochloride presented potent effect on the onset of SIPS and the expression of SASP factors, we then conducted RT-qPCR and senescence-associated beta-galactosidase (SA-β gal) assay to elucidate the function of UBA1 in modulating the effects of lycorine hydrochloride on cellular senescence. RT-qPCR analysis revealed that lycorine hydrochloride markedly reduced the expression of SASP factors IL1α and IL1β in senescent cells, an effect that was partially reversed by UBA1 depletion (Fig. 2A). β-Gal staining further confirmed UBA1's role, showing an increase in positive cells upon UBA1 knockdown after lycorine hydrochloride treatment (Fig. 2B, Supporting Information Fig. S3A), indicative of a UBA1-dependent inhibition of SIPS by lycorine hydrochloride.

In conclusion, our study identifies UBA1 as a direct target of lycorine hydrochloride. By rescuing the decline in ubiquitin levels associated with aging, lycorine hydrochloride enhances UBA1's catalytic activity, impacting cellular senescence. A previous study identified auranofin, a small molecule compound, that binds to UBA1 and augments its enzymatic activity by promoting interactions with E2 enzymes[6]. This aligns with our own findings, suggesting a shared mechanism by which small molecules can regulate UBA1 activity. Given UBA1'grffs critical role at the onset of the ubiquitination cascade, the enhancing effects of these small molecules on UBA1 could potentially amplify its function. Consequently, this underscores UBA1 as a promising therapeutic target. Most notably, this research highlights the intricate relationship between ubiquitination and senescence, clarifies UBA1 as the molecular target of lycorine hydrochloride, and proposes a potential therapeutic strategy for treating senescence-related diseases.

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Author contributions

Jiaqing Yang: Writing − original draft, Data curation. Junhao Xu: Investigation, Data curation. Ziheng Qiu: Investigation. Zhiyong Mao: Writing − review & editing, Conceptualization. Xiaojun Xu: Writing − review & editing, Conceptualization. Ying Jiang: Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. Guizhu Wu: Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Conflicts of interest

The authors declare no competing interests.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.01.026.

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