SMER28 binding to VCP/p97 enhances both autophagic and proteasomal neurotoxic protein clearance

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Abstract

The ability to maintain a functional proteome by clearing damaged or misfolded proteins is critical for cell survival, and aggregate-prone proteins accumulate in many neurodegenerative diseases, such as Huntington, Alzheimer, and Parkinson diseases. The removal of such proteins is mainly mediated by the ubiquitin-proteasome system and autophagy, and activity of these systems decline in disease or with age. We recently found that targeting VCP/p97 with compounds like SMER28 enhances macroautophagy/autophagy flux mediated by the increased activity of the PtdIns3K complex I. Additionally, we found that SMER28 binding to VCP stimulates aggregate-prone protein clearance via the ubiquitin-proteasome system. This concurrent action of SMER28 on both degradation pathways resulted in the selective decrease in disease-causing proteins, but not their wild-type counterparts. These results reveal a promising mode of VCP activation to counteract the toxicity caused by aggregate-prone proteins.

Keywords: Aggregate-prone proteins, autophagy activation, PI3P, SMER28, ubiquitin-proteasome system, VCP/p97

In mammalian cells, misfolded proteins are efficiently removed by quality control systems, including the ubiquitin-proteasome system (UPS) and autophagy. Soluble monomeric proteins are mainly targeted to the UPS and oligomeric and smaller aggregated species are degraded by autophagy. The accumulation of misfolded and aggregated cytoplasmic proteins is a hallmark of many neurodegenerative diseases, such as Huntington, Alzheimer, and Parkinson diseases. Therefore, enhancing the degradation of misfolded proteins by modulation of the UPS and autophagy activity is an attractive therapeutic strategy.

Induction of autophagy using small molecules reduces the accumulation of toxic proteins and importantly ameliorates signs of neurodegeneration in diverse animal models. Many of these compounds induce autophagy by inhibiting MTOR complex 1 or through activation of AMPK. However, direct modulation of these central kinase complexes may not be ideal due to their broad impact on cellular homeostasis.

Previously, our lab had identified a small molecule, SMER28, as an MTOR-independent inducer of autophagy, which enhances clearance of autophagic substrates, like mutant HTT (huntingtin) and A53T SNCA/α-synuclein in cellular and fly disease models. Subsequent studies showed that SMER28 treatment also accelerates clearance of the APP (amyloid beta precursor protein)-derived fragment in cell lines and primary neuronal cultures and is neuroprotective in the Parkinson disease rat model. SMER28 is well tolerated by various animal models and crosses the blood-brain barrier; however, its molecular target was unknown.

We recently identified VCP/p97 (valosin containing protein) as a binding target for SMER28 [1]. VCP is an abundant hexameric ATP-driven chaperone which governs key steps in protein quality control networks, including the UPS and autophagy. To better understand SMER28 binding to VCP, we employed limited proteolysis-coupled mass spectrometry (LiP-MS) and showed that SMER28 binds VCP in the cleft formed between its substrate-binding domain and ATPase domain 1. The binding of SMER28 causes a selective increase in the ATPase activity of the D1 domain without affecting VCP D2 domain activity or its hexameric structure. Using a panel of SMER28 analogs we further confirmed that the modulation of VCP ATPase activity in the D1 domain upon binding correlates with the autophagy-inducing properties of SMER28.

The induction of autophagy by SMER28, measured for example by the increased formation of LC3-positive structures, is dependent on VCP and VCP ATPase activity. We confirmed these findings using a novel autophagic-flux reporter, SRAI-LC3B, which we recently developed and characterized in this study. Interestingly, SMER28 enhances the

recruitment of early autophagic markers, including WIPI2 (WD repeat domain, phosphoinositide interacting 2) and ATG16L1.

In our previous study, we revealed a novel role for VCP in the early events of autophagosome formation, where VCP ATPase activity enables proper assembly of the PtdIns3K complex I to produce PtdIns3P. We found that SMER28 binding to VCP enhances PtdIns3P synthesis in a PtdIns3K complex-dependent manner (Figure 1). The observed increase in PtdIns3P upon SMER28 treatment is caused by enhanced assembly and activity of the PtdIns3P-producing PtdIns3K complex I, composed of BECN1, ATG14, NRBF2, PIK3C3/VPS34 and PIK3R4/VPS15.

In addition, we showed that SMER28 binding to VCP enhances degradation of soluble misfolded proteins through the UPS. Using a ubiquitin fusion degradation (UFD) proteasome reporter (Ub-G76V-GFP), we found that SMER28 treatment enhances proteasome-dependent clearance without affecting intrinsic proteasome activity. This indicates that SMER28 treatment enhances the clearance of the misfolded species by both the proteasome and autophagy routes, most probably by concurrent targeting of the monomeric substrates for proteasome degradation and oligomers/aggregates via autophagy.

SMER28 treatment induces degradation of polyQ expanded mutant proteins in mouse striatal cells and in fibroblasts derived from Huntington disease and spinocerebellar ataxia type 3 patients. Importantly, SMER28 does not reduce the levels of wild-type HTT or ATXN3, suggesting that SMER28 binding to VCP enables preferential clearance of the mutant/misfolded species and preserves the levels of the wild-type/normally-folded counterparts. Enhancing the selective removal of aggregate-prone toxic proteins is a desirable therapeutic strategy. Thus, we think this mode of VCP activation may be a very attractive target for number of neurodegenerative diseases.

References:

[1] Wrobel, L., Hill, S.M., Djajadikerta, A. *et al.* Compounds activating VCP D1 ATPase enhance both autophagic and proteasomal neurotoxic protein clearance. *Nat Commun* **13,** 4146 (2022). https://doi.org/10.1038/s41467-022-31905-0

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Competing Interest. DCR is a consultant for Aladdin Healthcare. Technologies Ltd., Mindrank AI, Nido Biosciences, Drishti Discoveries and PAQ Therapeutics. None of the other authors have conflicts of interests.

Figure 1. SMER28 acts by binding VCP and selectively stimulating ATPase activity of its D1 domain. SMER28 binding to VCP stimulates assembly and activity of the PtdIns3K complex I to increase the levels of PtdIns3P, which results in enhanced autophagosome biogenesis. In addition, SMER28 binding to VCP stimulates clearance of soluble misfolded proteins through the ubiquitin-proteasome system.

Functional protein

soluble aggregate-prone protein

N

D1

SMER28

VCP/p97

D2

PtdIns3K complex I

Phagophore

insoluble species

Proteasome

Proteasome mediated degradation

Autophagy mediated degradation