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Darci J. Trader, Scott Simanski, Paige Dickson, Thomas Kodadek

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Establishment of A Suite of Assays That Support the Discovery of Proteasome Stimulators

Darci J. Trader, Scott Simanski, Paige Dickson Thomas Kodadek*

Departments of Chemistry and Cancer Biology, The Scripps Research Institute, 130 Scripps Way Jupiter, FL 33458

*To whom correspondance should be addressed: kodadek@scripps.edu

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ABSTRACT

Background: The proteasome catalyzes the degradation of many mis-folded proteins, which are otherwise cytotoxic. There is interest in the discovery of proteasome agonists, but previous efforts to do so have been disappointing.

Methods: The cleavage of small fluorogenic peptides is used routinely as an assay to screen for proteasome modulators. We have developed follow-on assays that employ more physiologically relevant substrates.

Results: To demonstrate the efficacy of this workflow, the NIH Clinical Collection (NCC) was screened. While many compounds stimulated proteasome-mediated proteolysis of the pro-fluorogenic peptide substrates, most failed to evince activity in assays with larger peptide or protein substrates. We also show that two molecules claimed previously to be proteasome agonists, oleuropein and betulinic acid, indeed accelerate hydrolysis of the fluorogenic substrate, but have no effect on the turnover of a mis-folded protein in vitro or in cellulo. However, two small molecules from the NCC, MK-866 and AM-404, stimulate the proteasome-mediated turnover of a mis-folded protein in living cells by 3- to 4-fold.

Conclusion: Assays that monitor the proteasome-mediated degradation of larger peptides and proteins can distinguish bona fide agonists from compounds only able to stimulate the cleavage of short, non-physiologically relevant peptides.

General significance: A suite of assays has been established that allows the discovery of bona fide proteasome agonists. AM-404 and MK-866 can be useful tools for cell culture experiments, and can serve as scaffolds to generate more potent 20S stimulators.

Introduction

The synthesis, folding, trafficking and degradation of proteins are all carefully coordinated to maintain proteostasis in healthy cells (1,2). Defective proteostasis, due to chemical damage or mutation, leads to the accumulation of mis-folded proteins, which can form highly cytotoxic amyloids. These species are central to the development of many diseases, including neurodegenerative conditions such as Alzheimer's and Parkinson's diseases (AD and PD). An important component in maintaining proteostasis is the rapid digestion of mis-folded species. While autophagy plays a role in this process, particularly under certain pathological conditions such as heart failure (3), the bulk of this "janitorial function" in healthy cells is mediated by the proteasome (4). Low levels of proteasome activity exacerbate protein-folding diseases, while higher proteasome activity ameliorate them (5-14). Furthermore, high levels of proteasome activity are correlated with long life (15).

The simplest form of the proteasome is the 20S core particle (CP), which is comprised of two copies each of 14 unique proteins (seven α sub-units and seven β sub-units) arranged as four stacked heptameric rings (Figure 1) (16). The three catalytic sites of the proteasome are located in the interior of the barrel-like 20S CP. Substrate entry must occur through a channel that is so narrow only unstructured peptide chains can pass (17). The 20S CP can associate with capping complexes on one or both ends of the barrel. For example, the 26S proteasome is comprised of the 20S CP and one or two copies of the 19S regulatory particle (RP). The 19S RP binds poly-ubiquitylated proteins, unfolds them in an ATP-dependent fashion, and recruits deubiquitinases to remove the ubiquitin chains so that they can be recycled (18). An alternative cap, the 11S complex is present in specialized immunoproteasome complexes (19) that process proteins into peptides suitable for transport to the cell surface and display in the form of MHC complexes.