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All About the Core: A Therapeutic Strategy to Prevent Protein Accumulation with Proteasome Core Particle Stimulators

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ABSTRACT: The proteasome is an essential enzyme complex in cells whose main responsibility is to degrade proteins. Proteins can be degraded through either a ubiquitin-dependent or -independent mechanism by the proteasome. A variety of small molecules have been discovered that can increase the rate of protein degradation through either mechanism. However, stimulation of the ubiquitin-independent system is likely to be the most therapeutically impactful for protein-accumulation diseases. Preliminary evidence has demonstrated efficacy of this approach for reducing proteins associated with disease. To advance the field forward, validation of this mechanism in a disease model as well as more detailed studies on how much stimulation is required to achieve a therapeutic effect must be performed.

Over the past several decades, medical technology and research has advanced to the point that humans are living longer than they ever have before. This increased longevity has led to a better understanding of how cells age but has also come with the prevalence of age-related diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD). Interestingly, aging, PD, and AD are all associated with the accumulation of toxic proteins in cells. This discovery has opened the door for therapeutic approaches aimed at reducing this load of protein. The major protein degradation systems, autophagy and proteasome-mediated degradation, have been found to be less active in these disease states, signifying them as attractive therapeutic targets.[1] However, autophagy is controlled by multiple signaling pathways and exhibits nonspecific degradation, making it a complicated system to control. Here, we discuss the role of the proteasome in these disease states and ways in which it could be therapeutically targeted to reduce protein accumulation.

UBIQUITIN-DEPENDENT PROTEASOME SYSTEM VERSUS UBIQUITIN-INDEPENDENT PROTEASOME SYSTEM

The ubiquitin-dependent proteasome system, or UPS, relies on a multiprotein pathway to target proteins for degradation by the 26S proteasome.[2] Prior to degradation, a protein is tagged with a polyubiquitin chain, which indicates to the 26S proteasome that this protein is ready to be degraded. The 26S proteasome is comprised of two protein complexes: the 19S regulatory particle (19S RP) and the 20S core particle (20S CP). The 19S RP recognizes and removes the ubiquitin tag, unfolds the protein and delivers it into the 20S CP to be hydrolyzed. The 20S CP is a barrel-shaped protease, comprised of four rings of the form $\alpha_7\beta_7\beta_7\alpha_7$. Each β-ring houses three catalytic subunits, which are responsible for performing the hydrolysis activities of the proteasome, degrading proteins into small peptides. The α-rings of the 20S CP form the "gate" of the core particle. In its latent state, the opening of the gate is 9 Å, which prevents well-folded proteins from entering the pore to be hydrolyzed. However, upon association of the 19S RP and the 20S CP, a conformational change occurs increasing the opening of the gate to allow proteins to enter more readily.[2]

The UPS is a tightly regulated system, requiring the addition of a poly ubiquitin chain in order for a protein to undergo degradation. A myriad of protein types can be degraded in this fashion. However, this process can be "hijacked" to promote the degradation of specific proteins using proteolysis-targeting chimeras, or PROTACs, which consist of an E3 ligase binder, a linker, and a molecule that binds to a protein of interest.[3] The PROTAC method increases the ubiquitination of a specific protein by the E3 ligase in order to designate that protein for degradation. This system has been applied to proteins that are difficult to target for inhibition, such as BRD4 in acute myeloid leukemia and ERRα in breast cancer.[3] It has been suggested that PROTACs may be an effective method to treat protein-accumulation diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD). However, there is a potential problem with this hypothesis.

In healthy cells, though the UPS is responsible for the majority of proteasome-mediated degradation, only 21−35% of proteasomes are in the 26S form.[4] This is adequate to maintain healthy proteostasis, or a balance between protein synthesis and degradation; but as cells age or become afflicted by a disease, such as PD or AD, there is diminished expression of the 19S RP, leaving the vast majority of the proteasome in the 20S CP form (Figure 1). In aging or diseased cells, a maximum of 10% of proteasomes are 26S and at least 90% of proteasomes are unable to perform ubiquitin-dependent degradation.[4] The inability to form the 26S proteasome, due to a lack of available 19S RP, results in diminished ubiquitin-dependent protein degradation activity. This reduced activity makes the idea of using a PROTAC in these diseases, or simply targeting the UPS, an unrealistic approach.

However, another type of proteasome-mediated degradation is performed by the ubiquitin-independent proteasome system

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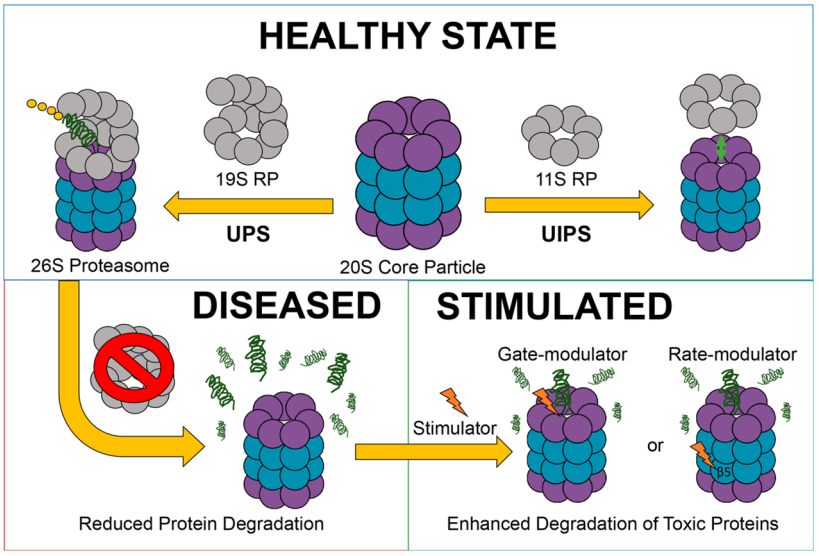

Figure 1. 20S CP can be capped with the 19S RP for ubiquitin-dependent degradation of proteins or capped with the 11S to stimulate gate opening to allow, intrinsically disordered proteins to be degraded. During a variety of disease states, the expression level of the 19S RP is decreased. Small molecules have been shown to be able to stimulate the 20S CP, with the long-term goal to develop a therapeutic approach to restore 20S CP activity to limit unwanted protein accumulation that can cause apoptosis.

(UIPS). In this system, the 20S CP can be capped by an 11S regulatory complex, which can open the gate of the 20S CP and allow untagged proteins to enter, or can remain uncapped, allowing only structurally disordered proteins to enter the gate to be hydrolyzed. Evidence indicates that proteins degraded by the UIPS are those that contain a high degree of disorder, such as intrinsically disordered proteins (IDPs), oxidatively damaged proteins, or proteins with a significant amount of intrinsically disordered regions (IDRs).[5] Recent estimates predict that up to 40% of the proteome can be degraded by the UIPS.[4] This system is regulated with the help of chaperone proteins, which bind to either the 20S CP or the protein substrate to prevent or facilitate its degradation. Despite this regulation, evidence suggests that the UIPS could be a beneficial therapeutic target in the treatment of conditions marked by protein accumulation.[1]

Premature aging can result from protein accumulation due to lack of proteasome function. One of the hallmarks of aging cells is the abundance of misfolded and oxidatively damaged proteins.[2] These proteins prevent proper cell function and contribute to the stress that can cause cellular senescence. In PD, the intrinsically disordered protein α-synuclein becomes overexpressed, accumulates, and eventually forms toxic aggregate species.[2] Aggregated α-synuclein disrupts many essential cell processes and can result in cell death. Similarly, those with AD suffer from toxic aggregates of tau and amyloid-β_{42}, two intrinsically disordered proteins.[2] These protein-accumulation conditions share a common theme: The proteins that accumulate are all preferentially degraded by the UIPS, making the 20S CP an attractive therapeutic target.[5]

MECHANISMS OF TARGETING THE 20S CP

The idea of stimulating the activity of the 20S CP as a therapeutic approach to protein-accumulation diseases is not new; however, few stimulating small molecules have been reported and are only effective at low micromolar concentrations.[6−9] In theory, stimulators of the 20S CP can fall into two different categories: allosteric stimulators of the hydrolysis activity, which increase the rate of hydrolysis of one of the proteolysis activities, and gate-openers, which modify the gate to allow more proteins to enter the 20S CP. The first category of small molecule stimulators, the allosteric stimulators (Figure 2A), impact both 20S CP- and 26S-mediated hydrolysis, as the catalytic active sites are the same. Only a few small molecules with this mechanism of action have been reported.[6,10] However, impacting all proteasome-mediated degradation can cause detrimental effects. In fact, there have been reports that increasing ubiquitin-dependent degradation, i.e., the 26S activity, increases the degradation of necessary tumor-

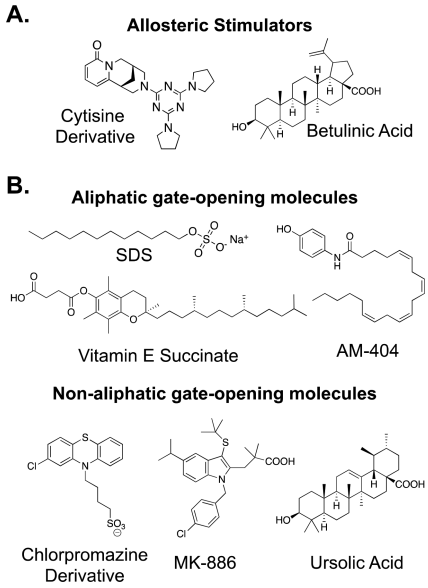

Figure 2. A few molecules have been discovered that only stimulate one of the hydrolysis activities of the 20S CP and are called allosteric stimulators (A). Additionally, a variety of small molecule stimulators have been discovered that can elicit their effect through opening or stabilizing the gate of the 20S CP (B).

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suppressing proteins, leaving the cells more susceptible to becoming cancerous.[11]

Nearly all the molecules that have been published as stimulators of the 20S CP have been classified as gate-openers, as in Figure 2B. In solution, the 20S CP fluctuates between a gate-opened state and a gate-closed state, with majority of the 20S CP in the gate-closed state.[12] The gate-opening molecules are believed to interact with the α-ring and induce a more stable gate-opened state of the 20S CP. These molecules have no impact on core particles that are associated with a regulatory complex (19S RP), as the α-ring is inaccessible in these states. On the basis of current evidence, gate-opening stimulators are not inducing an unnatural state of the 20S CP, but rather they are shifting the equilibrium in solution in favor of the gate-opened state. Such a phenomenon is believed to only increase the degradation of natural substrates of the 20S CP without causing other proteins to be degraded. As previously stated, the 20S CP is only known to degrade a specific subset of proteins, such as intrinsically disordered or oxidatively damaged proteins, allowing for this method of stimulation to have increased selectivity over allosteric stimulators. In fact, several gate-opening stimulators have been shown to increase the degradation of potentially toxic proteins, such as α-synuclein and tau, providing the first evidence of 20S CP stimulation to combat the protein-accumulation diseases, PD and AD.

MOVING FORWARD

Here, we have focused on two separate systems of proteasome-mediated degradation of proteins and analyzed how each can be targeted to promote protein degradation. Seemingly, each mechanism can provide a distinct benefit in various disease states. However, when considering age-related protein-accumulation diseases, such as aging, PD, or AD, gate-opening stimulators of the 20S CP appear to provide the greatest potential therapeutic benefit based on the current state of research. Interestingly, these stimulators display a variety of stimulating capabilities, indicating that not all gate-openers are created equal. In this respect, it is unclear what degree of stimulation would be necessary to have a therapeutic effect and to what disease state this therapy would be most aptly applied. While these molecules have been shown to increase the degradation of potentially toxic proteins biochemically and in transfected cells, validation of the therapeutic impact of these stimulators in a disease model has yet to be accomplished. Though this research area is at its beginning stages, current evidence provides an optimistic outlook for gate-opening stimulators of the 20S CP as a therapeutic approach to protein-accumulation diseases.

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Notes

The authors declare no competing financial interest.

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