Bioorganic & Medicinal Chemistry Letters 27 (2017) 4589–4596

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Digest

Lead discovery and chemical biology approaches targeting the ubiquitin proteasome system

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ARTICLE INFO

Article history:
Received 22 June 2017
Revised 24 August 2017
Accepted 28 August 2017
Available online 30 August 2017

Keywords:
Proteasome
Protein degradation
Molecular glue
Drug discovery
High-throughput
Ubiquitin

ABSTRACT

Protein degradation is critical for proteostasis, and the addition of polyubiquitin chains to a substrate is necessary for its recognition by the 26S proteasome. Therapeutic intervention in the ubiquitin proteasome system has implications ranging from cancer to neurodegeneration. Novel screening methods and chemical biology tools for targeting E1-activating, E2-conjugating and deubiquitinating enzymes will be discussed in this review. Approaches for targeting E3 ligase-substrate interactions as well as the proteasome will also be covered, with a focus on recently described approaches.

Introduction

Ubiquitin (Ub), named for its abundance in the cell, was discovered through the exploration of energy dependence and substrate specificity in intracellular protein degradation.[1,2] While certain forms of polyubiquitin chains signal degradation of a given substrate, mono-ubiquitination, ubiquitin branching, and alternative linkages regulate diverse cellular processes from gene regulation[3] to cellular trafficking.[4] The ubiquitin-proteasome system (UPS) is ATP-dependent and three classes of enzymes are required for ubiquitin chain addition. E1-activating enzymes begin the cascade by consuming ATP to form a ubiquitin-adenylate intermediate and enable covalent attachment of ubiquitin to the E1 via a thioester intermediate. E2 enzymes are responsible for ubiquitin conjugation and E3 ligases facilitate ubiquitin transfer to a substrate protein (Fig. 1). Substrate recognition and specificity is determined by the E3 ligase. Ubiquitin transfer occurs via the formation of an E3-Ub intermediate in HECT (Homologous with E6-associated protein carboxyl-terminus) E3 ligases or by a single-step transfer of ubiquitin from the E2-conjugating enzyme to the substrate in RING (Really Interesting New Gene) E3 ligases.[5] RBR (RING-Between RING-RING) E3 ligases have characteristics of both RING and HECT ligases, including a RING domain and a C-terminal catalytic domain.[6] Deubiquitinating enzymes (DUBs) cleave ubiquitin moieties from substrates after ubiquitination or prior to degradation.[7]

For decades, the UPS has been studied for a variety of reasons. The intricate specificity by which substrates are identified and degraded makes it an attractive system for therapeutic intervention. The ubiquitin proteasome system has been implicated in various biological processes including cell cycle regulation, immunity, DNA repair, phytohormone signaling, and viral replication.[5,8,9] The UPS provides a major mechanism for achieving proteostasis, which is the maintenance of a constant protein level in the cell through regulation of protein synthesis and degradation. Perturbation of this system has been implicated in cancer, and neurodegenerative diseases like Alzheimer's and Parkinson's disease.[7] For example, germline mutations in the Von Hippel Lindau (VHL) gene affect the ability of VHL protein to function as an E3 ligase. VHL is responsible for degradation of the Hypoxia Inducible Factor (HIF1A) under normoxic conditions. HIF1A has been implicated in tumorigenesis and enrichment of VHL mutations has been correlated with renal and central nervous system (CNS) hemangioblastomas.[10,11]

Most ubiquitinated substrates are targeted to the proteasome for degradation. Bortezomib, the first proteasome inhibitor approved for clinical use,[12] has paved the way for further research toward drug discovery in the UPS. Bortezomib is currently used in the treatment of multiple myeloma (MM) based on its ability to induce proteotoxicity. Since normal cells also depend on the proteasome for degradation, this approach is non-specific and many research groups are working to fine-tune the perturbation of the

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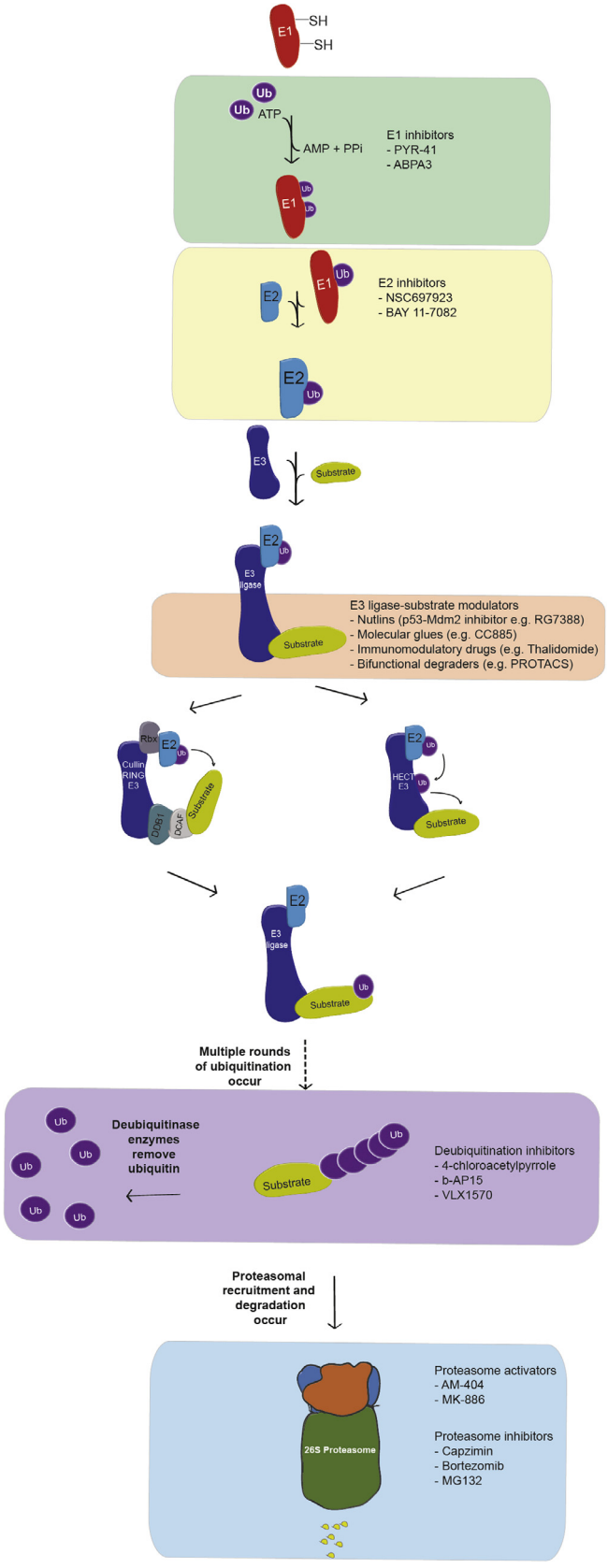
http://dx.doi.org/10.1016/j.bmcl.2017.08.058
0960-894X/

UPS using more specific small-molecule or genetic approaches. Targeting E1 and E2- enzymes is perhaps more specific than targeting the proteasome, but due to the small number of these enzymes, a pleiotropic effect is still observed. The fact that E3 enzymes are responsible for substrate specificity in the UPS has driven interest in targeting E3 ligase-activity as opposed to E1 or E2 enzymes that have more ubiquitous functions.

Chemical biology and lead discovery approaches provide a means for finding new targets and small molecule tools for dissecting the UPS. In this review, we discuss recent advancements in understanding and targeting the UPS. We also highlight the assays used for UPS lead discovery. These recent reviews[7,13,14] provide additional information on the role of the UPS in disease and drug development strategies for the UPS.



Lead discovery approaches

E1- and E2- enzymes

Discovery of compounds that inhibit E1 enzymes provides an avenue for perturbing global ubiquitination and the study of downstream kinetics and alternative degradation pathways. The first cell-permeable ubiquitin E1 enzyme inhibitor, PYR-41, was identified in a fluorescence-based (QuantaBlu) *in vitro* screen for UPS modulators and was confirmed using ubiquitination affinity reactions.[15] Since the development of PYR-41,[15] significant effort has gone towards discovery of other ubiquitin and ubiquitin-like E1-enzyme inhibitors. NEDD8-activating enzyme 1 (NAE1), a non-ubiquitin E1-enzyme activates the ubiquitin-like protein, NEDD8. NAE1 is also important for Cullin-RING Ligase (CRL) activation. A specific inhibitor of NAE1, MLN4924, was developed from structure-activity analysis of the hit compound, N6-benzyl adenosine, from a HTS screen.[16] While the role of MLN4924 as an anti-tumor agent has been extensively studied, Zhou et al. showed that at low concentrations it stimulates cell proliferation through c-Myc and EGFR activation.[17] To further study the role of NAE1, An and Statsyuk generated an activity-based probe (ABP). They went on to show that ABPA3 is a potent dual inhibitor of Ubiquitin and NEDD8 E1 enzymes.[18]

Besides E1-enzyme inhibition, targeting ubiquitin E2-enzymes also provides another avenue for UPS modulation. One of the E2 enzymes that has been extensively studied is UBC13, also known as UBE2N, because of its potential role in DNA damage response through polyubiquitination chain formation of ubiquitin lysine-63 (K63-linkage).[19] UBC13 has also been implicated in NF-κB activation[19] and p53 cytosolic accumulation,[20] which makes it a potentially attractive target for both inflammatory diseases and cancer therapy. Weber and colleagues recently developed a high-throughput assay based on the AlphaScreen technology to identify inhibitors of the interaction between UBC13 and the E3 ligase RNF8.[21] AlphaScreen is a bead-based assay where targets of interest are conjugated to donor or acceptor beads. Upon excitation of the donor bead, singlet oxygen is transferred to the acceptor bead if bound ligands bring the beads into close proximity. The acceptor bead then emits light which can be measured.[22] As a proof-of-concept, Weber et al. showed that ovarian tumor domain protease ubiquitin binding 1 (OTUB1), a known UBC13 binding partner,

Fig. 1. Druggable nodes of the ubiquitin proteasome system. E1 enzymes activate ubiquitin through ATP hydrolysis and ubiquitin adenylation. Compounds referenced in the green box inhibit E1 enzymes. E2 enzymes allow for ubiquitin conjugation, and compounds referenced in the yellow box inhibit this process. The E3 ligase facilitates ubiquitin transfer from the E2 enzyme to the substrate. Compounds in the brown box modulate this process. Deubiquitinating enzymes (DUBs) cleave ubiquitin moieties from substrates after ubiquitination or prior to degradation. This process is inhibited by the compounds in the purple box. Irreversible substrate degradation occurs in the 26S proteasome, and activators or inhibitors of proteasomal degradation are referenced in the blue box.