Proteasome Inhibitors In Cancer Therapy: A Novel Approach To A Ubiquitous Problem

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LEARNING OBJECTIVES:

1. Describe the basic structure and function of the proteasome.
2. Identify the proteasomal subunit within the catalytic core that is crucial to cell survival.
3. List proteasome protein targets.
4. Evaluate the effect on cellular homeostasis following proteasome inhibition.
5. Detail the status of various proteasome inhibitors in pre-clinical and clinical trials.

ABSTRACT: The cellular proteasome is an important molecular target in cancer therapy and drug resistance research. Proteasome inhibitors are effective agents against multiple myeloma and mantle cell lymphoma and display great potential as treatment for a variety of other malignancies. The proteasome is a large multicatalytic, proteinase complex located in the cytosol and the nucleus of eukaryotic cells. The ubiquitin proteasome system is responsible for the degradation of most intracellular proteins and therefore plays an essential regulatory role in critical cellular processes including cell cycle progression, proliferation, differentiation, angiogenesis, and apoptosis. Cancer cells are particularly sensitive to proteasome inhibitors, indicating the utility for inhibition of the ubiquitin-proteasome pathway as an approach for cancer therapy.

INDEX TERMS: proteasome, ubiquitin, apoptosis, multiple myeloma, clinical trials

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INTRODUCTION

The cancer-preventive and anticancer effects of dietary polyphenols and flavonoids such as those in green tea are supported by results from epidemiological, cell culture, and animal studies. Several of these plant polyphenols act as proteasome inhibitors and may sensitize tumor cells to traditional chemo- and radiation therapies by interfering in pathways. While chemotherapy is an established means for cancer management, the accompanying systemic toxicity and potential for regression or complete ineffectiveness remain as real obstacles for cancer patients. To surmount these obstacles, targeting specific molecules, as opposed to the mass destruction of all mitotically active cells, has found favor as a unique treatment option in certain cancers. In fact, the proteasome has been implicated as an important molecular target in cancer and drug resistance research. Bortezomib (co-developed by Millenium Pharmaceuticals Inc., Cambridge, MA, USA and Johnson & Johnson Pharmaceutical Research and Development, L.L.C., Raritan, NJ, USA), the first and currently only proteasome inhibitor approved by the US Food and Drug Administration, is used most commonly as a combination agent for the treatment of both multiple myeloma and mantle cell lymphoma.[1] However, numerous clinical trials are underway to study the effects of bortezomib in a variety of other malignancies as well.

Bortezomib has been shown to improve clinical outcomes in certain cancers,[2,3] yet cancer cell resistance and the predictable toxicity associated with bortezomib treatment, raises the need to identify novel, synthetic or naturally occurring proteasome inhibitors. This article describes the basic physiology and biological function of the proteasome, the known proteasome protein targets and the effect of their accumulation upon proteasome

inhibition, and details the status of various proteasome inhibitors in pre-clinical and clinical trials.

The Ubiquitin Proteasome Pathway

Proteolysis in eukaryotic cells occurs predominantly within the ubiquitin (Ub)-proteasome pathway. The proper function of this pathway is a critical physiological event that maintains the intracellular concentration of a variety of proteins. Disruption of appropriate proteasome activity is disastrous to cellular homeostasis.[4] The Ub-proteasome pathway involves conjugation of activated ubiquitin molecules to a protein substrate, and the ultimate degradation of that Ub-tagged protein by the proteasome. Ubiquitin is a 76-amino acid protein that is covalently ligated to a target protein by a multi-enzymatic system.[5] Ub must be activated, conjugated, and ligated by the sequential actions of E1, E2, and E3 enzymes. E3 enzymes possess a complex mechanism of substrate specificity. The result of this pathway is a protein that is "tagged" with Ub molecules (Figure 1).[6]

Ubiquitinated proteins are recognized and discriminately degraded by the eukaryotic 26S proteasome, a massive (2000 kD) multi-catalytic, multi-subunit protease complex located in the nucleus and cytosol of nearly all cells.[7] Typically, proteins containing polyubiquitin chains (four or more Ub molecules) on lysine 48 are directed to the proteasome fordegradation.[8] Upon recognition of the Ub tagged protein by the proteasome, proteolysis of the substrate commences and the Ub molecules are released and recycled.

The proteolytic core of the 26S proteasome, the 20S proteasome, is composed of four heptameric, stacked rings (α7, β7, β7, α7) that form a barrel-like structure and possess multiple peptidase activities (Figure. 1).[9] Within the α-rings, the α-subunits ensure that only unfolded polypeptides are allowed exposure to the central β-rings. Proteolysis results from substrate contact with the β-subunits. The β5 subunit possess chymotrypsin-like activity and cleaves after amino acids

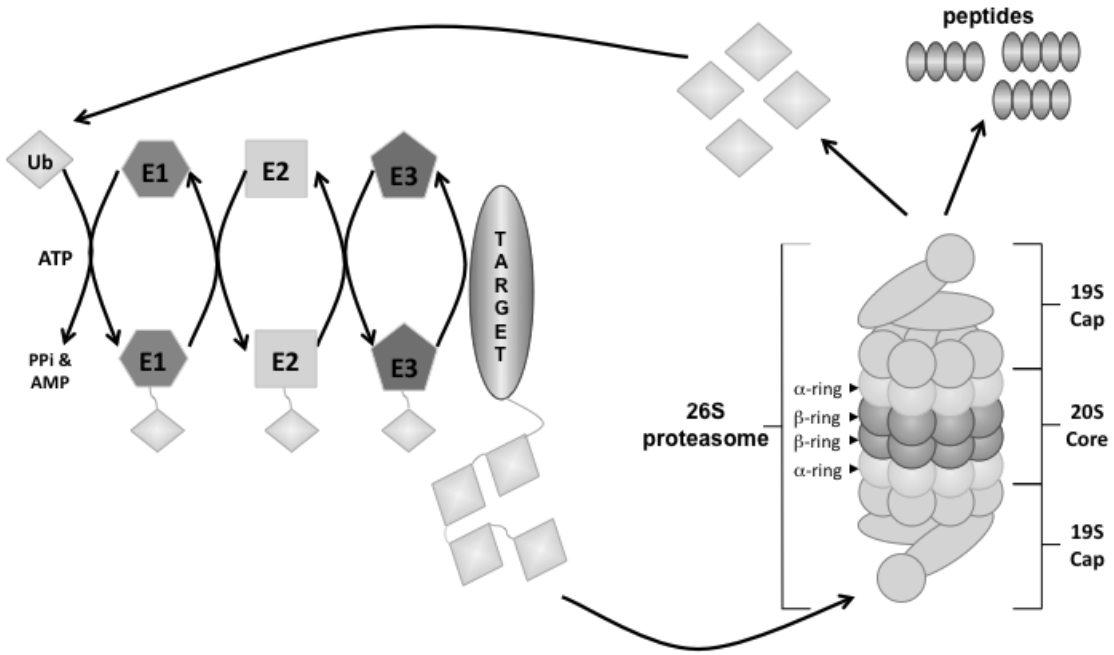

Figure 1. The ubiquitin-proteasome pathway. Ubiquitin (Ub) is ligated to target proteins by a multi-enzymatic system consisting of Ub-activating (E1), -conjugating (E2), and -ligating (E3) enzymes. Ubiquitinated proteins are escorted to the 26S proteasome, through the 19S cap and to the catalytic 20S core for degradation into individual amino acids or oligopeptides. The Ub is released and recycled to begin the process anew. Adapted from, Landis-Piwowar KR et al.[1]

with hydrophobic side chains. The β1 subunit confers peptidylglutamyl peptide hydrolyzing-like and cleaves after amino acids with acidic side chains. The β2 subunit has trypsin-like activity and cleaves after amino acids with basic side chains.[9] As the polypeptide is sequentially degraded, the by-products formed consist of short polypeptides that range in length from as little as 4 amino acids to 25 amino acids with the average being 7-9 amino acids.[10] This very carefully controlled system that determines the demise of proteins via ubiquitination, followed by proteasomal degradation is crucial for cellular homeostasis.

Proteasome Substrates and Cellular Consequences of Inhibition

Because the proteasome is fundamental to maintaining the appropriate balance of cellular proteins it is rather intuitive that disruption of proteasome function would be catastrophic to the fate of a cell. The ubiquitin-proteasome pathway is responsible for the degradation of proteins linked to the cell cycle,[11-14] to apoptosis[15] and to the accumulation of abnormal proteins that result from cellular stress such as oxidative damage.[16] Inappropriate proteasome activity can alter cellular homeostasis by progressive degradation of regulatory proteins or stabilization of inappropriately produced proteins. In either case, the proteasome may be implicated in the pathogenesis of various disease states including cancer.[17] Proteasome inhibition is an established means for the treatment of multiple myeloma, mantle cell lymphoma, and non-Hodgkin lymphoma.[3] Furthermore, the collection of empirical data for the use of proteasome inhibition as a treatment for various solid tumors is underway.[18]

To fully understand the molecular consequences of proteasome inhibition, a discussion of the specific classes of proteins that are ubiquitinated and degraded by the Ub-proteasome pathway must be included. Proteasome inhibition leads to an almost immediate decrease in the rate of cellular proteolysis. Numerous proteins involved in the formation and the survival of cancer cells have been identified as proteasome substrates and include cell cycle-promoting (cyclins A, B, D and E)[11-14] and inhibiting (p27) proteins,[19] the "guardian of the genome" protein (tumor suppressor protein p53),[20] pro-apoptotic protein Bax,[15] and the inhibitor of NFkB, IkB-a.[21] Some of these target proteins are discussed below.

The Ub-proteasome pathway closely regulates degradation of proteins (called cyclins) involved in progression through interphase of the cell cycle. For example, both cyclin D and cyclin E are degraded by ubiquitin-mediated proteolysis and their destruction allows for cell passage out of G_1 and through to S phase.[12,13] Likewise, degradation of cyclin A allows passage from S into G_2. Finally, for the cell to mitose, cyclin B must be rapidly degraded by the Ub-proteasome pathway.[11] Accumulation of these proteins following proteasome inhibition leads to a halt in cell cycle progression and ultimately to cell death.

Cell survival is critically dependent upon the proper ratio of pro-apoptotic (proteins that incite cell death) to anti-apoptotic proteins (proteins that block cell death). The pro-apoptotic protein Bax is involved in mitochondrial cytochrome c release that induces the mechanisms of cell death.[22] In normal cells, Bax is maintained at relatively low concentration and degradation of Bax by the Ub-proteasome pathway ensures that cells survive to maintain tissue homeostasis. However, in pathological conditions such as cancer, degradation of Bax may be increased[15,23-26] to promote survival and proliferative ability of the cell[15] under conditions that would normally be unfavorable. Proteasome inhibitors have been shown to increase the concentration of Bax in cancer cells[27] and since highly proliferating cells (cancer cells) are more sensitive to proteasome inhibition than non-proliferating cells,[28-32] this accumulation of Bax leads to apoptosis induction.[1,33]

The tumor suppressor protein p53 functions in cell cycle regulation and prevention of genome mutation to the extent that it has taken the moniker, "guardian of the genome." p53 is vitally important to blocking cell cycle progression at the G_1/S restriction point in the presence of DNA damage and in doing so, promotes apoptosis. Much like Bax, p53 is maintained at a constant, but low level in normal cells under normal conditions and is also targeted by the Ub-proteasome pathway for degradation.[34] The mechanisms of cancer cell transformation are known to involve altered cell cycle regulation and ineffective DNA repair beyond the control of p53. However, an accumulation of p53 is observed in the presence of proteasome inhibitors[35,36] and leads to the induction of apoptosis.[36]

Finally, NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a transcription factor that regulates genes involved in cell survival and proliferation and is aberrantly expressed in numerous conditions, but most importantly for this discussion, in cancer cells. Importantly, the Ub-proteasome pathway does not regulate NF-kB directly, but does so indirectly by degradation of an inhibitory molecule called IkBa (inhibitor of NF-kB). Very simply, IkBa complexes with NF-kB and in doing so, inhibits the translocation of NF-kB into the nucleus where it could promote transcription of cell survival genes. IkBa is a target of the Ub-proteasome pathway and is accumulated upon proteasome inhibition. The accumulation of IkBa sequesters NF-kB in the cytoplasm and in doing so, contributes to apoptosis in cancer cells.[37-39]

Proteasome inhibitors

Proteasome inhibitors can be categorized as reversible and irreversible and their inhibition is predominately and most commonly accomplished by interaction with the b-subunits. More precisely, inhibition of the N-terminal threonine of the proteasomal β5-subunit (chymotrypsin-like activity) has been specifically implicated in conferring apoptosis induction in cancer cells.[30] Some proteasome inhibitors, including bortezomib, bind and inhibit other β-subunits in conjunction with β5, while other compounds bind the a-subunits or even to the enzymes involved in ubiquitination.

As of September 2011, the National Institutes of Health described 188 different clinical trials in a state of active recruitment, involving applications of the proteasome inhibitor bortezomib (Velcade) to cancer therapy and can be found at: http://clinicaltrials.gov/ct2/results?term=bortezomib+cancer&recr=Open&no_unk=Y. However, common toxic side effects as well as native or acquired resistance to bortezomib has been described in human cancers and in various cell lines. For this reason, new classes of compounds are in continuous states of empirical design and analysis. Thirteen clinical trials are underway that include proteasome inhibitory compounds other than bortezomib and are described at: http://clinicaltrials.gov/ct2/results?term=proteasome+inhibitor+cancer+NOT+bortezomib&recr=Open. The vast majority of these trials (combination therapies that include bortezomib or a variety of other proteasome inhibitors) are in the earliest state of human testing (phase I trials).

Bortezomib

Bortezomib is an FDA-approved reversible proteasome inhibitor that primarily functions as an inhibitor of b5 activity.[40] The most common toxicities associated with bortezomib treatment include thrombocytopenia and neuropathy.[2] Recent application of bortezomib in cancer therapy has focused on its efficacy as a combination agent for myeloma in which patients experienced improved time to progression and overall survival when treated with melphalan, prednisone, and bortezomib than when treated with melphalan and prednisone alone.[41] Other studies highlight the addition of bortezomib to established therapies in newly diagnosed myeloma and include such combinations as lenalidomide and dexamethasone,[41] melphalan, prednisone, and thalidomide, and dexamethasone, cyclophosphamide, and lenalidomide.[42,43]

While these studies describe the promise of bortezomib for myeloma and there have been successes in the treatment of mantle cell lymphoma,[44] other malignancies, hematological and solid-tumor, have shown meager responses to bortezomib treatment. Furthermore, bortezomib response followed by resistance (refractory malignancy) remains a clinical problem. To identify mechanisms of resistance, the proteasomal β5-subunit has been characterized in various cell culture models and was found to be mutated and/or over-expressed,[45,46] indicating the potential for clinically relevant resistance mechanisms to bortezomib.

Carfilzomib

A variety of novel proteasome inhibitors have been developed to circumvent bortezomib resistance and potentially decrease toxicity. Carfilzomib is an irreversible inhibitor of the proteasomal β5-subunit and is therefore more potent than the reversible bortezomib.[47] Because of its irreversible nature, a cell must synthesize new proteins to form new proteasome molecules to overcome carfilzomib's mechanism of action. Importantly, carfilzomib is an effective proteasome inhibitor in certain cell lines resistant to bortezomib[48,49] and in myeloma patients who display disease progression after bortezomib treatment.[50] Carfilzomib is currently in phase II clinical trials and may become an important alternative to bortezomib

treatment in a variety of malignancies.

Marizomib (NPI-0052)

A second irreversible proteasome inhibitor, marizomib also inhibits the proteasomal b5-subunit in addition to the β1- and β2-subunits. In cell culture and in mouse models of leukemia and myeloma, marizomib induces apoptosis and slows tumor cell proliferation.[51,52] Prolonged treatment with marizomib, or with analogs with similar structure, has been suggested to promote apoptosis induction more potently in leukemia cells than compounds such as bortezomib because multiple proteasome activities are inhibited.[53] Because of promising findings in early studies, marizomib is under continued investigation in phase I clinical trials for various malignancies that include, relapsed and/or refractory myeloma, non-small cell lung carcinoma, pancreatic cancer, and others.

Nutritional Compounds

The cancer-preventive and anticancer effects of dietary polyphenols and flavonoids such as those in green tea are supported by results from epidemiological, cell culture, and animal studies. Several of these plant polyphenols act as proteasome inhibitors and may sensitize tumor cells to traditional chemo- and radiation therapies by interfering in pathways that lead to drug resistance.[54] The green tea polyphenols epigallocatechin-3-gallate (EGCG) has proven an effective proteasome inhibitor in numerous cell-based and animal-model studies.[55] Likewise, the soy isoflavone, Genistein,[56] the active ingredient of tumeric, curcumin,[57] the Chinese medicinal compound, Celastrol[58] and several other flavonoids[59] and nutritional compounds have all been implicated as specific and potent proteasome inhibitors. Compounds such as genistein, and green tea polyphenols have shown potential in phase II clinical trials.[1]

While nutritional compounds appear to possess promising anticancer effects, oral dietary polyphenols often exhibit poor bioavailability, possibly due to their inability to pass through the gut and into the circulation intact. Generally, the proteasome inhibitory activities of dietary polyphenols depend on their susceptibility to biotransformation reactions such as methylation, sulfonation, and glucuronidation.[1] Additionally, it should be noted that while EGCG is an effective proteasome inhibitor on its own, EGCG at very high doses is antagonistic to compounds such as bortezomib in myeloma cell lines.[60] While it is unlikely to be able to achieve a clinically relevant, bortezomib-inhibiting, dose of EGCG, it is an important issue that a full patient history is obtained when monitoring any therapy.

CONCLUSIONS

The Ub-proteasome pathway is responsible for the vast majority of proteolysis in eukaryotic cells and disruption of this pathway alters the normal concentration of cell-cycle regulatory proteins, proteins involved in apoptosis, and ultimately, cellular homeostasis. The reversible proteasome inhibitor bortezomib has been successful as a treatment option for multiple myeloma and mantle cell lymphoma, but cancer recurrence is a major clinical problem. Interestingly, a novel strategy to overcoming bortezomib resistance focuses on inhibition of the enzymes that generate the ubiquitin tag. By blocking the activity of the E1, E2 and E3 enzymes, proteins that would normally be destined for proteasomal destruction accumulate and disturb cellular homeostasis.[61] These novel concepts may lead to the development of new synthetic Ub-proteasome pathway inhibitors to achieve the best possible outcomes for cancer patients.

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