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The therapeutic potential of microbial proteasome inhibitors

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ABSTRACT

The proteasome influences cellular homeostasis through the degradation of regulatory proteins, many of which are also involved in disease pathogenesis. In particular, numerous regulatory proteins associated with tumor growth, such as cyclins, cyclin-dependent kinase inhibitors, tumor suppressors, and NF-κB inhibitors are degraded by the proteasome. Proteasome inhibitors can stabilize these regulatory proteins, resulting in the suppression of tumor development and the regulation of immune responses. Thus, proteasome inhibitors are promising candidate antitumor agents and immune-regulatory agents. Bortezomib is the first-in-class proteasome inhibitor approved for the treatment of multiple myeloma. Despite its high efficiency, however, a large proportion of patients do not attain sufficient clinical response due to toxicity and drug resistance. Therefore, the development of new proteasome inhibitors with improved pharmacological properties is needed. Natural products produced by microorganisms are a promising source of such compounds. This review provides an overview of proteasome inhibitors produced by microorganisms, with special focus on inhibitors isolated from actinomycetes.

1. Introduction

The two major protein degradation systems in eukaryotic cells are the autophagy–lysosomal system and the ubiquitin–proteasome system. The autophagy–lysosomal system was initially thought to be a bulk protein degradation system that non-selectively engulfs cytoplasmic constituents [1]. This system was viewed as a mechanism to supply nutrients during starvation. Recently, however, it has emerged that autophagy also contributes to selective protein degradation [2]. By contrast, the ubiquitin–proteasome system selectively degrades short-lived regulatory proteins involved in the homeostatic control of cells as well as abnormal proteins with misfolded structures that can be cytotoxic if allowed to accumulate [3]. The conjugation of proteins to ubiquitin polymers serves as a signal for degradation by the proteasome. Protein destruction is initiated by covalent attachment of the ubiquitin polymer (a chain consisting of more than four ubiquitin monomers) through the concerted actions of a network of proteins including the E1 (ubiquitin-activating), E2 (ubiquitin-conjugating), and E3 (ubiquitin-ligating) enzymes [4,5]. The ubiquitinated proteins are then targeted to the 26S proteasome for degradation.

The 26S proteasome, an unusually large multi-enzyme and ATP-dependent proteolytic complex, cleaves proteins into small peptides [6–8]. It consists of a hollow cylindrical 20S proteolytic core (20S proteasome) and one or two 19S regulatory particles (Fig. 1). The 19S regulatory particle recognizes ubiquitinated proteins and controls their transport into the 20S proteasome core. The 20S proteasome is a hollow cylindrical structure composed of two outer α-rings and two inner β-rings in an axial stack (αββα). These rings are composed of seven distinct α subunits and seven distinct β subunits, respectively, forming an $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ structure. The 20S proteasome possesses three distinct protease activities, caspase-like/peptidyl-glutamyl peptide hydrolyzing, trypsin-like, and chymotrypsin-like, mediated by the active sites of the β1, β2, and β5 subunits, respectively. All active sites cleave peptide bonds by an unusual mechanism in which the hydroxyl group of the *N*-terminal catalytic threonine serves as the catalytic nucleophile [9].

The proteasome can influence many basic homeostatic and pathogenic processes by controlling the degradation of regulatory proteins. For instance, proteins associated with cell cycle regulation such as cyclins, cyclin-dependent kinase inhibitors (*e.g.*, p21 and p27), tumor suppressors (*e.g.*, p53), and NF-κB inhibitors (*e.g.*, IκB-α), are degraded by the proteasome [10–13]. Thus, the proteasome is an important therapeutic target in cancer and immunological diseases. In fact, many effective proteasome inhibitors have been developed [14–17] that can stabilize these regulatory proteins and induce cell cycle arrest, endoplasmic reticulum (ER) stress, and apoptosis, thereby inhibiting tumor development [18,19]. Proteasome inhibitors are thus promising candidate antitumor agents [20,21]. In this review, we present an overview

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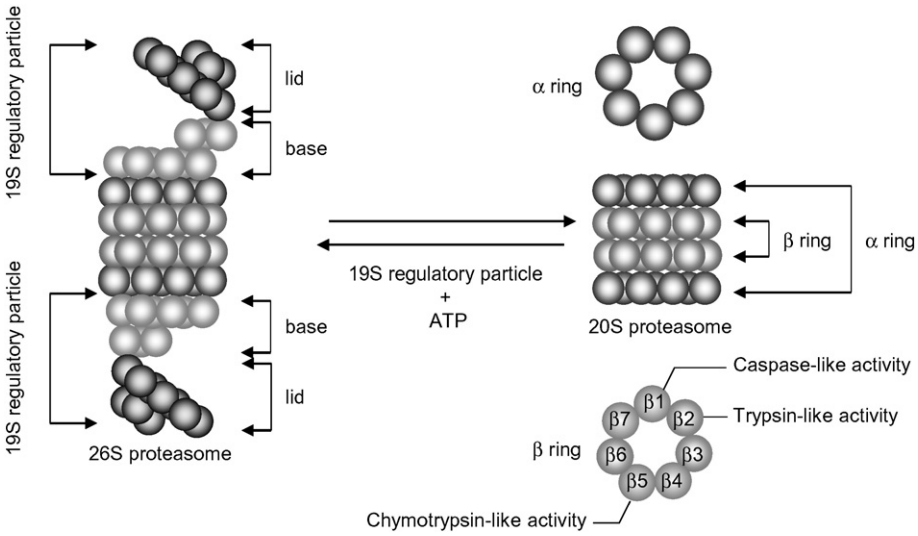

Fig. 1. Schematic model of the proteasome.

of proteasome inhibitors isolated from microorganisms and describe the benefits of natural microbial products as a source of these agents. In particular, we focus on the isolation and characterization of proteasome inhibitors produced by actinomycetes and rationally designed analogs.

2. Bortezomib, a first-in-class proteasome inhibitor

MG-132, the peptide aldehyde benzyloxycarbonyl-Leu-Leu-leucinal [22], was designed as the first proteasome inhibitor (Fig. 2A). It was developed as a substrate mimetic peptide inhibitor for the 20S proteasome and suppresses proteasome activity by forming a hemiacetal with the hydroxyl of the active site threonine in the 20S proteasome [9].

Bortezomib (formerly known as PS-341) is a dipeptide boronic acid (Fig. 2B), MG-132 analog, that potently inhibits the chymotrypsin-like activity of the 20S proteasome [23,24]. It demonstrated potent cytotoxicity pattern against a National Cancer Institute panel of 60 cancer cell lines and suppressed the growth of xenograft tumors in animal models [24]. Phase I clinical trials were conducted on a variety of solid tumors and hematologic malignancies [25,26]. Therapeutic responses were observed in patients with multiple myeloma (MM), and the efficacy of bortezomib for MM was subsequently confirmed by a phase I to phase III clinical trials [26–29]. Bortezomib was approved as a first-in-class proteasome inhibitor for third-line treatment of relapsed and refractory MM by the United States Food and Drug Administration (FDA) in 2003 and was approved as first-line treatment for newly diagnosed MM in 2008 [30–32].

Bortezomib is particularly active against MM due to the high protein turnover. The main function of the ubiquitin–proteasome pathway is the quality control of newly synthesized proteins. Myeloma cells produce and secrete large amounts of protein, including immunoglobulins. The high rate of immunoglobulin biosynthesis by MM cells imposes an unusually high burden on the proteasome because these proteins fail to properly fold and so must be degraded by the proteasome *via* the ER degradation pathway. Therefore, the unfolded protein response (UPR) can be easily induced by proteasome inhibition [33]. Moreover, extensive immunoglobulin production by MM cells increases their sensitivity to proteasome inhibition [34]. As a result, the partial inhibition of MM proteasome *in vivo* by bortezomib sufficient to kill MM cells while having no toxic effect on normal cells.

Mantle cell lymphoma (MCL) is an aggressive form of B-cell non-Hodgkin lymphoma. In 2006, bortezomib was approved for the treatment of relapsed and refractory MCL [35]. Although the precise mechanism of action of bortezomib on MCL is poorly understood, recent studies have revealed that the transcription repressor PR domain zinc finger protein 1 (PRDM1, Blimp1) is required for the apoptotic effect of bortezomib on MCL [36]. Bortezomib induces PRDM1 functions, at least in part, through direct repression of MKI67 and proliferating cell nuclear antigen (PCNA) and inhibits NOXA activity.

Antibody-producing plasma cells, the nonmalignant precursors of MM cells, are also very sensitive to proteasome inhibitors [37,38], suggesting possible effects against autoimmune diseases. Several groups have demonstrated the efficacy of proteasome inhibitors in animal models of autoimmune diseases, including lupus nephritis [39], myasthenia gravis [40], multiple sclerosis [41], streptococcal cell-wall induced polyarthritis [42], rheumatoid arthritis [43], irritant sensitivity [44], psoriasis [45], asthma [46], and colitis [47].

However, bortezomib also induces many side effects, including painful peripheral neuropathy, orthostatic hypotension, pyrexia, cardiac and pulmonary disorders, adverse gastrointestinal events, myelosuppression and thrombocytopenia asthenia [27,48–50]. Of these potential adverse events, peripheral neuropathy is the most common dose-limiting side effect. Although the pathophysiology and molecular mechanisms of bortezomib-induced peripheral neuropathy are not completely understood, recent studies have revealed that bortezomib inhibits HtrA2/Omi, an ATP-dependent stress-inducible mitochondria serine protease [51] involved in neuronal survival [52]. The inhibition of HtrA2/Omi is now considered one of the most likely causes of bortezomib-induced peripheral neuropathy. Furthermore, most MM patients treated with bortezomib rapidly develop resistance [53]. Thus, more effective antitumor treatment requires the development of novel proteasome inhibitors with improved side effects profiles and sustained efficacy.

3. Proteasome inhibitors from microorganisms

3.1. Proteasome inhibitors produced by actinomycetes

Natural microbial products often have therapeutic activities that can improve general health and be used to treat diseases. They have great chemical diversity and are a promising resource for drug discovery. Umezawa and Aoyagi [54,55] isolated many protease inhibitors from microorganisms, including leupeptin, antipain, pepstatin, and chymostatin, that are still the widely used due to their low cost. Actinomycetes, in