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Proteasome inhibitors: structure and function

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Since 2003, the US Food and Drug Administration approval of bortezomib, a proteasome inhibitor, has changed the management of hematologic malignancies and dramatically improved outcomes for patients with multiple myeloma and mantle cell lymphoma. Since that time, two additional proteasome inhibitors (carfilzomib and ixazomib) have been approved, with other agents and combinations currently under investigation. Proteasomes degrade ubiquitinated proteins or substrates through the ubiquitin-proteasome pathway, a pathway that is utilized in multiple myeloma because of the high protein turnover with immunoglobulin production. Proteasome inhibitors exploit dependence on this pathway, halting protein degradation that ultimately results in apoptosis and cell death. Here we will discuss the structure of the proteasome and the mechanisms of action for proteasome inhibitors to further understand their role in hematologic malignancies.

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1. Introduction

Proteasomes play a necessary role in cell survival, DNA repair, and the proliferation of malignant cells. Choreographed degradation of cyclin dependent kinase (CDK) activators or inhibitors is needed for the cell to progress through all steps of the cell cycle, from DNA replication to mitosis [1]. Proteasomes also play an active role in normal cellular functions, and in the degradation of misfolded or mutated proteins.

Despite the essential role of the ubiquitin-proteasome pathway, proteasome inhibitors (PIs) are well tolerated in the clinic, with a limited spectrum of side effects. PIs are effective in the treatment of hematologic malignancies, including multiple myeloma and mantle cell lymphoma, improving progression free survival (PFS) and overall survival (OS). Multiple myeloma is the ideal target for PIs because of the large amount of IgG production in plasma cells. The high protein turnover in myeloma cells results in a favorable therapeutic window for PIs in this disease with preferential susceptibility of the malignant cells relative to normal cells. In 2003, the US Food and Drug Administration approved the first PI, bortezomib (Velcade, PS-341, Takeda Oncology, Cambridge, MA) for the treatment of relapsed and refractory multiple myeloma (Fig. 1). Since then, two other agents, carfilzomib (Kyprolis, Amgen, Thousand Oaks, CA) and ixazomib (Ninlaro, Takeda Oncology, Cambridge, MA) have secured regulatory approval for the treatment of multiple myeloma, and the indication for bortezomib has expanded to include first-line treatment of multiple myeloma and mantle cell lymphoma. Other PIs are still under investigation, including marizomib, which may be beneficial in patients with glioblastoma. Herein we will discuss the proteasome structure and function and explore the mechanisms that allow PIs to be so effective in hematologic malignancies.

2. The ubiquitin-proteasome pathway

The ubiquitin-proteasome pathway (UPP) regulates cellular functions, removing proteins that are damaged, misfolded, or otherwise not wanted in the cell in a well-orchestrated manner. The protein is first targeted for degradation through ubiquitination. Ubiquitination requires three distinct steps to achieve a polyubiquitinated product that is easily identified by the proteasome. The first step occurs when ubiquitin becomes activated by E1, the ubiquitin-activating enzyme. Activated ubiquitin is then transferred to E2, the ubiquitin-conjugating enzyme and E3, the ubiquitin-protein ligase that transfers ubiquitin to proteins. This process is repeated multiple times until a polyubiquitin chain emerges, targeting the protein for degradation in the proteasome [1,2].

The 26S proteasome is a multiprotein complex made of a 20S catalytic core and one or two 19S regulatory subunits on either end of the 20S core (Fig. 2). The 19S subunits bind the polyubiquitin chain, cleaving it from the target protein. The protein then passes through the 20S core where it is degraded to small oligopeptides, less than 25 amino acids. The 19S subunits commonly flank the 20S core. However, the 20S core can additionally act alone to cause ubiquitin-independent protein degradation. This core is a barrel

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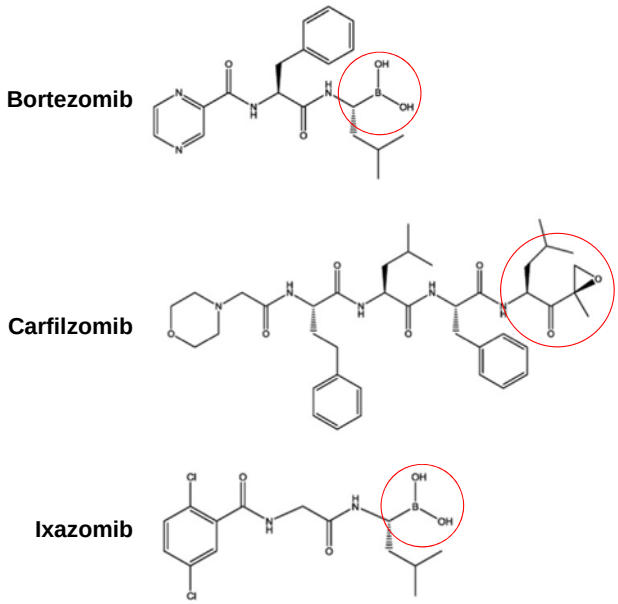

Fig. 1. Chemical structures of bortezomib, carfilzomib and ixazomib. The circles highlight the active moieties of the individual PIs.

structure composed of four heptameric rings. The two alpha rings sandwich the two beta rings. The beta rings each contain three active sites for protein degradation: chymotrypsin-like (β5), trypsin-like (β2), and caspase-like (β1). The chymotrypsin-like site on β5 is the primary target of PIs [3], although at higher drug concentrations of drug, the other two trypsin- and caspase-like sites are also inhibited.

3. Mechanism of PI-mediated cellular cytotoxicity

The mechanism by which PIs lead to cell death is diverse and affects many pathways utilized in cancer cells (Fig. 3). One putative mechanism of cytotoxicity is inhibition of the NF-kB pathway, a pro-survival pathway for many cell types, especially those of hematopoietic lineages. IκBα is an endogenous protein inhibitor of NF-κB that is degraded by the proteasome when the cell receives a stimulus to activate the pathway. Its degradation is necessary for the p50/p65 NF-kB transcription factors to become active and translocate to the nucleus [4]. When the proteasome is inhibited, IκBα remains intact and bound to the p50/p65 NF-kB heterodimer, preventing activation of the NF-kB pathway. Inhibition of the NF-kB pathway was initially thought to be the principal mechanism of anti-cancer activity of PIs because this pathway plays a role in cell proliferation, invasion, metastasis, and angiogenesis. However, a potent IκB kinase inhibitor, PS-1145, which blocks NF-kB activation proximal to the IκBα step, does not emulate the cellular toxicity profile of PIs, suggesting other mechanisms are equally, if not more, important [5].

Multiple putative mechanisms of cellular toxicity have been proposed for PIs (PIs). Included amongst these is direct induction of apoptosis through c-Jun NH_2-terminal kinase (JNK) and p53. For example, proteasome inhibition leads to activation of JNK, resulting in programmed apoptotic cell death via caspase 8 and caspase 3. The expression of p53, a known tumor suppressor important in activating cell death under diverse circumstances, is induced by treatment with PIs, which can induce apoptosis even in the presence of mutant p53 [6]. Alternatively, proteasome inhibition can induce interaction of p53 with MDM2, followed by activation of the JNK pathway, shifting the cell toward apoptosis [7]. Additionally, PIs can act indirectly to cause apoptosis by preventing degradation of pro-apoptotic family proteins. Normally, the pro-apoptotic proteins Bim, Bid, and Bik are regulated through rapid ubiquitination and proteasomal degradation. With proteasomal inhibition, these proteins accumulate, triggering caspase activation and cell death [8–10]. NOXA, a pro-apoptotic member of the Bcl-2 family that interacts with p53 in the setting of DNA damage [11], can be induced by hypoxia, cytokine signaling, or mitogenic stimulation, but is normally rapidly degraded through the proteasome [12]. Proteasome inhibition increases levels of NOXA, activates caspase-9, and consequently leads to apoptosis [13]. Proteasome inhibition can also induce expression of NOXA independently of p53, inducing further cell death [14].

Following synthesis on ribosomes, proteins are usually folded and assembled in the endoplasmic reticulum (ER) before being released. In multiple myeloma, for example, plasma cells produce large quantities of immunoglobulins resulting in a high protein burden for the ER. The ER has a quality control mechanism to monitor for misfolded proteins that cannot be properly refolded, and targets those proteins for degradation by the proteasome [15]. When the cell produces large amounts of proteins, the ER becomes stressed. This ER stress initiates the unfolded protein response (UPR), activating intracellular signal transduction pathways to maintain homeostasis in the ER by reducing protein synthesis; alternatively, the UPR can cause cell cycle arrest and induce apoptosis, depending on the severity of the ER stress [16]. PIs prevent the degradation of ubiquitinated proteins, effectively blocking the translocation of misfolded proteins out of the ER to the proteasome. The accumulation of misfolded proteins in the ER

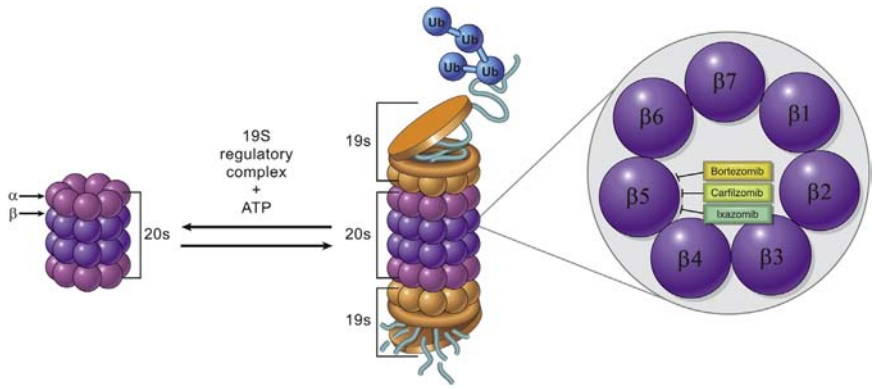

Fig. 2. Proteasome structure. The 20S catalytic core binds to the 19S regulatory complex to form the 26S proteasome structure. Proteins that are tagged with ubiquitin bind to the 19S complex and are degraded at the proteolytic β subunits. Bortezomib, carfilzomib, and ixazomib all inhibit the β5 subunit, thereby inhibiting the catalytic activity of the proteasome.