

The use of proteasome inhibitors in anti-cancer therapy

Marzena Baran¹, Paulina Miziak¹, Katarzyna Bonio²

¹Chair and Department of Biochemistry and Molecular Biology, Medical University of Lublin

²Department of Cell Biology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin

Abstract

Proteins are the basic building blocks of the human body. Most proteins, after fulfilling their function, are degraded with the help of a multi-catalytic proteolytic complex - the 26S proteasome, thanks to its prior labeling with a poly-ubiquitin chain. The ubiquitin-proteasome complex is involved in the control of many important cellular processes. It is involved in the cell cycle, cell proliferation and growth, apoptosis, control of receptor function, quality of proteins in the endoplasmic reticulum, immune response, stress response and other extracellular factors. The dysfunction of the ubiquitin-proteasome pathway plays an important role in the development of many neurodegenerative and neoplastic diseases, as well as diseases with an immune and infectious origin. Accordingly, research is being conducted where the UPS complex is the target of therapeutic activities. Specific proteasome inhibitors are used in cancer therapy. The first to be approved by the FDA was bortezomib, which is a peptide derivative of boric acid. It has the ability to initiate the process of apoptosis by inhibiting the activity of the NF- κ B protein, which is a transcription factor. This compound is used in the treatment of multiple myeloma and malignant lung and breast cancers. There are also studies on the combination of proteasome inhibitors with other chemotherapeuticam. There is still growing interest in research aimed at obtaining substances that allowed to control the mechanisms of proteolytic degradation.

Key words: Ubiquitin, proteasome, proteasome inhibitors, anti-cancer therapy

Introduction

Two complementary pathways of intracellular degradation are present in eukaryotic cells[1]. One is the lysosomal pathway, which involves the degradation of endogenous proteins by endocytosis, pinocytosis, or by autophagocytosis or selective transport. The second of these is the extrasosomal pathway, which involves proteolysis in the cytosol by 26S proteasomes and ubiquitin[2]. The ubiquitin-proteasome system is responsible for the breakdown of proteins important for the proper functioning of cells: enzymes regulating biosynthetic pathways, proteins regulating the course of the cell cycle, many transcription factors, proteins encoded by oncogenes and suppressor genes or proteins involved in the immune response, as well as structural proteins[5]. It is indirectly responsible for the regulation of the cell cycle and the apoptosis process[10]. The proteasome is the main component of the ubiquitin-proteasome (UPS) pathway[4]. The 26S proteasome is an ATP-dependent, multi-catalytic complex responsible for the degradation of polyubiquitin-modified intracellular proteins. Its presence has been detected in the cytoplasm and in the cell nucleus of all eukaryotic organisms.[2]. The proteasome is the major component of the ubiquitin-proteasome (UPS) pathway. The eukaryotic proteasome, ie 26S, consists of a 20S core and two 19S regulatory elements[4]. The formation of this complex is an ATP-dependent process[2].

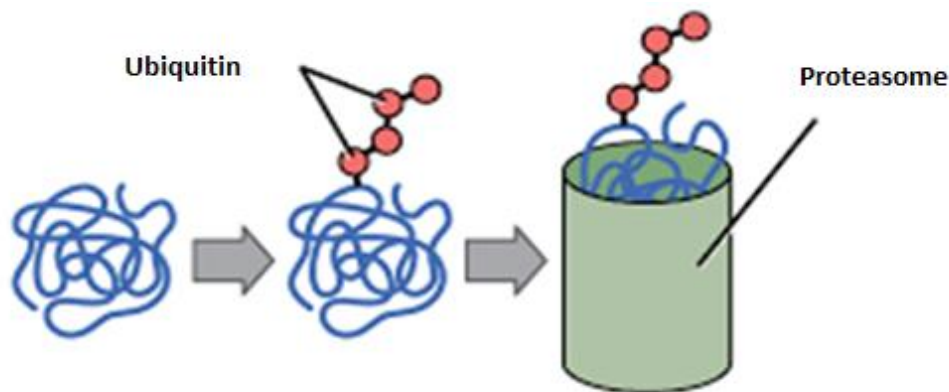


Figure 1. Structure of the proteasome [<https://pl.khanacademy.org/>]

It was found that the 20S catalytic core has several peptidase activities which differ in their specificity in the hydrolysis of peptide bonds:

- chymotrypsin-like (CHTL), which is responsible for breaking peptide bonds formed by the carboxyl group of hydrophobic amino acids
- trypsin-like (TL), responsible for the hydrolysis of peptide bonds formed by the carboxyl group of basic amino acids
- caspase-like (CL) responsible for the hydrolysis of peptide bonds formed by the carboxyl group of acidic amino acids[2,4].

In addition to these three activities, the proteasome has two more that are less well understood, namely the activity - branched-chain amino acid-preferring (BrAAP), which hydrolyzes amino acids with branched side chains, and the activity of small neutral amino acid-preferring (SNAPP), which prefers low-molecular neutral amino acids[4]. Regulatory complexes (19S) are designed to recognize the polyubiquitin chain, unfold the protein and transport it to the interior of the catalytic core, where the substrate protein will be

degraded[2]. Another element of the extrasomal pathway is the small molecular protein ubiquitin. This protein is found in the cytoplasm of all eukaryotic cells. This protein carries out the process of ubiquitination, that is, the determination of misfolded proteins that are later degraded in the extrasosomal pathway. The ubiquitination process is multi-stage and requires the cooperation of many enzymes and energy derived from high-phosphate bonds of ATP[3, 6].

The process includes the following steps:

- activation of ubiquitin with the participation of the E1 enzyme (activating ubiquitin) and ATP, thanks to which a high-energy thioester bond is created between ubiquitin glycine and the E1 enzyme cysteine
- transfer of activated Ub to cysteine in the E2 conjugating enzyme
- transfer of Ub from E2 and lysine, thanks to E3 ubiquitin ligase[2,10].

Disruptions in the protein control process through the proteasome-ubiquitin system lead to changes in the intracellular response network, and this affects cell physiology. Disruptions in this process may affect the development of many diseases, such as cancer, neurodegenerative or metabolic diseases[8]. In the cancer cells can see uncontrolled growth and lack of susceptibility to factors directing them to the apoptotic pathway. It is related to a dysregulated cell cycle and accelerated metabolism. Therapeutic strategies focus on the regulation of the cell cycle and its processes. Many basic pathways are involved in the function of the ubiquitin-proteasome complex. Therefore, the use of proteasome inhibitors is justified in anti-cancer therapy[3,7]. In the process of cancer formation, cells lose their genetic stability, which causes them to synthesize a large amount of misfolded proteins. By using proteasome inhibition, the degradation of such proteins can be blocked, which will accumulate in the endoplasmic reticulum. This will lead to the activation of caspases and natural cell death[1]. Cancer cells show increased proteasome activity, which protects them from apoptosis. The aim of the therapy aimed at blocking the activity of proteasomes is to induce the process of apoptosis by activating the mitochondrial and receptor pathways[2]. Proteasome inhibitors can act in several ways, one of them is to block the binding site of degraded proteins, and the other is to modify the N-terminal structure of the proteasome's threonine[7]. The majority of inhibitors act by inhibiting chymotrypsin-like activity, which is dominant in comparison to other proteasome activities[2]. Proteasome inhibitors can be divided into 2 groups. Of synthetic origin, which are a uniform group of compounds with the structure of small peptides. They have a peptide backbone that differs in its C-terminal structure. This group includes: peptide α -ketoamides, peptide α -ketoaldehydes, peptide aldehydes, peptide derivatives of vinyl sulfonate and peptide derivatives of boronic acid. The second group includes inhibitors of naturally derived proteasomes, which are a heterogeneous group of compounds synthesized by various groups of organisms. These include γ -lactam thiol esters, linear peptide epoxyketones or macrocyclic peptides[2,9]. Currently, peptide derivatives of boronic acid are used for therapeutic purposes due to their unique properties. They are characterized by selectivity in action and metabolic stability[2]. The most popular proteasome inhibitor used in cancer therapy is bortezomib[7].

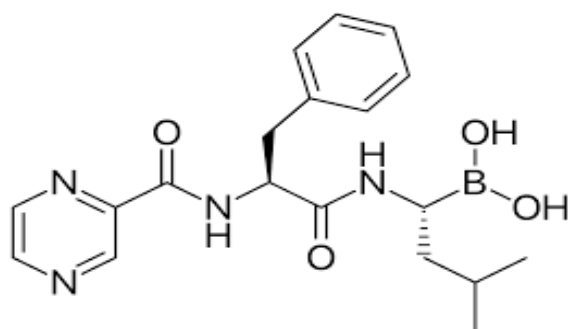


Figure 2. Structural formula of bortezomib. (<https://pl.wikipedia.org/wiki/Bortezomib>)

The mechanism of its action is based on the induction of the apoptosis process as a result of inhibition of the activity of the NF- κ B protein[4,7]. NF- κ B results in increased transcription of genes responsible for the production of pro-inflammatory cytokines, adhesins, anti-apoptotic proteins and proteins involved in angiogenesis[2]. Moreover, the action of bortezomib causes the stimulation of stress response proteins, the production of reactive oxygen species, which induces the internal pathway of cell death and inhibits signaling of protein kinase activated by PI3K[7]. Bortezomib was the first proteasome inhibitor used in cancer therapy. Currently, this compound is used in the treatment of multiple myeloma, non-Hodgkin's lymphoma and malignant neoplasms[9]. Attempts have been made to use bortezomib in combination therapy with other drugs, e.g. epoxomicin, pegylated doxorubicin, docetaxel and irinotecan. These combinations reduce the process of cell proliferation and direct them to the apoptotic pathway[3]. Ixazomib and delanzomib are among the group of peptide derivatives of boric acid that are currently in clinical trials[2].

Bibliography:

1. Podgórska J., Komórkowy system sprzątający – degradacja proteasomalna i jej rola w terapii onkologicznej, *Biotechnologia.pl*, 2017
2. M. Staszczak (2017), Szalaki ubikwityna- proteasom, jako cel strategii terapeutycznych, *Postępy Biochemii* 53 (4), 289-291
3. Bubko I. i inni, Rola proteasomu w terapii chorób nieuleczalnych, *Postępy Hig Med Dosw.*, 2010; 64: 314-325
4. Maliński M., Cichocki M., Inhibicja aktywności proteasomu, jako nowa strategia w terapii i chemioprewencji nowotworów, *Postępy Hig Med Dosw* 2013; 67 : 90-106
5. N. M. Weathington, R. K. Mallampalli (2014), Emerging therapies targeting the ubiquitin proteasome system in cancer, *The Journal of Clinical Investigation*, 124,1
6. Dees E.C, Orłowski R.Z., 2006, Targeting the ubiquitin–proteasome pathway in breast cancer therapy, *Future Oncol.* (2006) 2(1), 122
7. Kazula A., Kazula E., Proteasomy a nowe kierunki terapii, *Terapia i Leki.*, (2009)., tom 65, nr.7
8. Henshall F.N., Kumar S. T, Ubiquitination and the regulation of membrane proteins. *Physiol. Rev.* (2017). 97: 253-281
9. Stój J., Karpowicz P., Proteolityczny kombinat i jego regulatory, *Wiadomości chemiczne*, (2012), 66
10. Romaniuk R. i inni, Inhibitory proteasomów w terapii onkologicznej, *Postępy Hig Med Dosw.*, (2015) 69: 1443-1450