

PROTEASOME INHIBITION: RECENT ADVANCES IN ANTITUMORAL THERAPY

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Abstract

The major pathway for degradation of intracellular proteins is the ubiquitin-proteasome pathway. There is increasing evidence revealing that the dysregulation of the ubiquitin-proteasome pathway are involved in pathogenesis of many diseases such as cancer and neurodegenerative disorders. Many studies are focused now on developing new drugs that target ubiquitin-proteasome pathway, based on the observation that proteasome inhibition induces apoptosis in many tumor cells.

This review exposes recent data concerning the use of proteasome inhibitors in cancer therapy.

Rezumat

Proteinele intracelulare sunt degradate în principal pe calea ubiquitină-proteazom, numeroase cercetări dovedind că perturbări ale acestei căi sunt implicate în patogenia multor afecțiuni, precum cancerul și bolile neurodegenerative. Multe studii sunt axate acum pe dezvoltarea de noi medicamente care vizează calea ubiquitină-proteazom, bazându-se pe observația că inhibitorii proteazomului induc apoptoza în celulele tumorale.

Această lucrare prezintă date recente referitoare la utilizarea inhibitorilor de proteazom ca agenți antitumorali.

Keywords: ubiquitin, proteasome, proteasome inhibitors, cancer.

Introduction

Cellular proteins could be degraded in two ways. Extracellular proteins undergo proteolysis in lysosomes and the ubiquitin-proteasome pathway is important for degradation of intracellular proteins.

Proteasomes are catalytic complexes omnipresent in cytoplasm and nucleus of all mammalian cells [1], representing the key constituents of ubiquitin-proteasome pathway by which proteins that are marked by covalent attachment of ubiquitin are destroyed [2].

Description of the ubiquitin-proteasome pathway

Ubiquitin (Ub) is a small protein and its better understood biological role is to label intracellular proteins preparing them for degradation in proteasome. This molecule can be attached as single entity or as

polyubiquitin chains. Besides its role in proteins degradation, ubiquitination has been shown to be involved in regulation of a variety of cellular processes such as endocytosis, DNA repair or cell division [3].

Attachment of ubiquitin to substrate protein is assisted by three enzymes with distinct roles in ubiquitination. Ubiquitin is activated by E1, named ubiquitin-activating enzyme, followed by conjugation to an ubiquitin-conjugating enzyme (E2). After that activated ubiquitin is linked to the lysine residues of target protein by the ubiquitin ligases (E3) [4]. E3 are enzymes that determine the specificity of protein substrates, representing a novel class of therapeutic targets for pharmaceutical intervention [5]. Ubiquitination comes in many ways - monoubiquitination, multi-monoubiquitination or polyubiquitination - depending on the number of ubiquitin entities conjugated to the target protein. Regularly, multiple ubiquitin molecules are conjugated to the initial ubiquitin to form polyubiquitin chains. Monoubiquitination or multimonoubiquitination, attachment of a single ubiquitin molecule of multiple sites of a protein, are processes generally involved in cellular signaling or endocytosis [3, 6]. Ub has seven internal lysine residues which can undergo ubiquitination to form distinct polyubiquitin chains, each of these residues could be involved in regulation of a wide range of cellular processes: DNA repair, degradation in proteasome, etc. [6]. For substrate recognition by the 26S proteasome is necessary a chain of four or more ubiquitin molecules [7]. Synthesis of a polyubiquitin chain is realized by progressive transfer of activated Ub to an internal lysine residue or NH₂-terminus of previous conjugated ubiquitin [8].

The 26S proteasome is a multicatalytic complex that degrades polyubiquitinated proteins to small peptides.

Structurally, it contains a 20S core and one or two 19S outer regulatory particles. The 20S core particle has a barrel-shaped structure composed of four stacked rings identical two by two [2].

The outer region of core particle is constituted by two α rings and inner region is constituted by two β rings. In eukaryotes, α and β rings are composed of seven distinct subunits which gives the general structure $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ [9]. Only three of the seven β -subunits have functional proteolytic sites that include two site of each of the following types: *chymotrypsin-like* activity that cut hydrophobic residues, *trypsin-like* sites that cleave basic amino acids and *caspase-like* sites (or *post-glutamyl peptide hydrolase-like* –PGPH – sites) that cleave acidic residues [10]. These sites named also $\beta 5$ (*chymotrypsin-like*), $\beta 2$ (*trypsin-like*) and $\beta 1$ (*caspase-like*) contain catalytically active threonine residues at their NH₂-terminal end and present

N-terminal nucleophile hydrolase activity that cleaves peptide bonds [11] being targeted by diverse inhibitors [12].

The extremities of the 20S core particle can be capped by one or two 19S regulatory particles. These regulatory particles are constituted of at least 18 subunits, six of which are ATPases, which bind the substrate to be degraded, being involved in unfolding and translocation of proteins into 20S particle [2]. 19S particle contains subunits that bind the polyubiquitin chains and two deubiquitinating enzymes (also called isopeptidases) that disassemble the ubiquitin chains so that the ubiquitin can be reused in the degradation of other proteins [1]. Well known roles of 19S regulatory particles are to recognize ubiquitinated proteins for proteasomal degradation and to open an orifice in the α -ring allowing the entrance of the substrate into the proteolytic "chamber" [13].

It is also assumed that the 19S particles are involved in unfolding substrate proteins that otherwise cannot be able to fit through the narrow proteasomal channel and inserts them into the 20S core particle [14].

The cleaved peptides leave the 20S core particle and can be further broken down into individual smaller peptides, for incorporation into major histocompatibility complex class I molecules for antigen presentation, or into amino acids for protein synthesis [15].

Ubiquitin-proteasome pathway inhibitors

Inhibition of the ubiquitin-proteasome pathway was limited in the past being considered harmful to life because this pathway is essential for normal cellular homeostasis. Many drugs already used for treatment of various diseases have proven an influence to this pathway [16]. For example, cyclosporine, a selective immunosuppressant agent that binds to cyclophylline to inhibit calcineurin [17] acts also as an uncompetitive inhibitor of the 26S proteasome [18].

Beside drugs with primary effects on other targets, two decade ago was initiated the synthesis of specific proteasome targeted inhibitors [16]. Initially proteasome inhibitors were developed to investigate the catalytic activity of the proteasome. With the elucidation of the proteasome functions in the cell was taken into account the possibility that proteasome inhibitors could have therapeutic potential.

Proteasome inhibitors may act either at α -subunits, thus influencing the pore opening that allow the entry of substrate into the proteolytic chamber, or at the catalytic sites of β -subunits thereby directly influencing substrate binding to the catalytic N-terminal threonine of these subunits [19].

Proteasome inhibitors are compounds with different chemical structures, acting through the formation of covalent or non-covalent bonds with the active threonine residues.

Inhibitors that form covalent bonds with the active sites of the proteasome include natural or synthetic compounds that could be further divided into different structural classes such as *peptide aldehydes*, *peptide boronates*, *peptide α,β -epoxyketones*, *peptide ketoaldehydes*, *β -lactones*, *peptide vinyl sulfones*, *oxathiazole-2-ones* [12].

Covalent inhibitors have an electrophilic group that can react with the active threonine residues, this group being usually attached to the C-terminal end of a peptide, or within a non-peptide molecule [20]. These inhibitors bind irreversibly or, some of them, reversibly at the catalytic sites of the proteasome.

Non-covalent inhibitors interact with the proteasome active sites forming reversible weak bonds providing an alternative mechanism for proteasome inhibition [20]. They may offer advantages by enabling more widespread tissue distribution through their more rapid binding and dissociation kinetics [21]. In this category there may be included natural products such as argyirin A, TMC-95 or scytonemides [22].

Inhibition of the proteasome leads to cellular apoptosis by modulation of several proteins (oncogenes, tumor suppressors, transcription factors) resulting in inhibition of nuclear factor- κ B (NF- κ B) activity, increased activity of p53 and Bax proteins, and accumulation of cyclin-dependent kinase inhibitors p27 and p21 [23]. Thus, the control of cellular proliferation could be the reason for using proteasome inhibitors in cancer treatment.

Bortezomib was the first inhibitor approved in 2003 by the US Food and Drug Administration (FDA) to treat patients with refractory multiple myeloma and from 2004 was approved by the Committee for Proprietary Medicinal Products for use in the European Union [24].

This compound is a dipeptide boronate that contains boronic acids instead of a carboxylic acid at C-terminal end which binds covalently and reversibly at chymotrypsin-like subunits of proteasome [25]. Bortezomib binds and inhibits to a lesser extent the caspase-like activity at the β 1-subunit and other serin proteases, in this way contributing to the neurotoxicity [26]. Despite bortezomib is an effective agent in multiple myeloma therapy, it was observed that some patients do not respond to therapy or briefly respond than relapse, developing resistance to this drug.

Mechanisms of acquired bortezomib resistance include point mutations of chymotrypsin-like subunits resulting in up-regulation of

proteasome, alterations of gene expression in cell survival and antiapoptotic pathways [27].

Bortezomib possesses a significant anti-tumor activity but it is mainly used in combined therapy to overcome chemoresistance and induce sensitivity of other drugs such as doxorubicin, melphalan, dexamethasone without increasing toxicity in treated patients [28]. Recent *in vitro* studies have provided evidence of enhanced efficacy of bortezomib when administered in combination with hyperthermia [29]. Although they have shown promising results, further investigation is needed in order to transfer them to the clinic.

Clinical trials mainly investigated bortezomib in combinatory therapy for hematological malignancies, but there have been conducted also studies regarding solid tumors (non-small cell lung carcinomas, renal cell carcinoma, and breast cancer), and their results disappointed to date [30].

Although bortezomib is an effective treatment in malignancies it is still needed to design proteasome inhibitors with reduced toxicity, improved efficacy and oral bioavailability [31].

In 2012, FDA approved the treatment with *carfilzomib* for multiple myeloma patients who have failed prior therapies, including bortezomib and an immunomodulatory drug, and have demonstrated disease progression within 60 days after the end of the last therapy [32].

Carfilzomib is an epoxomicin-based proteasome inhibitor with improved pharmaceutical profile. It binds irreversibly to the catalytic site of the proteasome and inhibits primarily the chymotrypsin-like subunits [33]. Carfilzomib, at high doses, has inhibitory effects on trypsin-like and caspase-like sites and in contrast to bortezomib it does not affect the activity of other proteases [26]. Carfilzomib demonstrated its efficacy in combined therapy. Clinical studies realized with combination between carfilzomib, lenalidomide and low-dose dexamethasone administrated to patients with relapsed or progressive multiple myeloma have demonstrated robust, rapid, and durable clinical activity with an acceptable tolerability profile in these patients, including patients who were refractory to lenalidomide and bortezomib [34].

Different pharmaceutical companies have designed a series of new proteasome inhibitors to be more manageable and to have fewer side effects than bortezomib or carfilzomib, such as *ixazomib* (MLN9708), *oprozomib* (ONX0912) or *marizomib* (NPI0052) [12, 31].

Ixazomib citrate (MLN9074), a boronate dipeptide, is a reversible oral/intravenous proteasome inhibitor hydrolyzed *in vivo* to its active biological form, MLN2238, which acts by activating some proapoptotic

enzymes and inhibiting NF- κ B [35]. This proteasome inhibitor was administrated in weekly doses like single agent in phase I of clinical trial for treatment of patients with relapsed or refractory light-chain amyloidosis [36] or in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma [37].

Oprozomib (ONX 0912) is a peptide epoxyketone proteasome inhibitor, a structural analog of carfilzomib, which selective by and irreversibly binds to the chymotrypsin-like subunit of the proteasome [38]. It is orally bioavailable and preclinical studies demonstrated its anti-multiple myeloma activity and reduction of tumor progression in animal tumor model studies [39].

In phase I of an open-label dose-escalation study, realized on patients with advanced refractory or recurrent solid tumors, oprozomib was orally administrated like single agent. The results indicated that oprozomib achieves levels of proteasome inhibition more than 80% and has an acceptable safety profile [40].

Marizomib (NPI0052 or salinosporamide A) is a β -lactone- γ -lactam natural compound derived from *Salinospora tropica* which inhibits all three proteolytic sites of proteasome [41]. It is a potent inhibitor of proteasome that induces apoptosis *via* caspase-8 dependent mechanism in different cell types [42], demonstrating anti-tumor activity in hematologic malignancies or solid tumor [41]. Marizomib is the subject of several clinical trials as single agent or in combination with other drugs (for example, vorinostat) for treatment of different malignancies such as multiple myeloma, non-small cell lung cancer, pancreatic cancer, melanoma, lymphoma [43-46]. Despite bortezomib, this drug doesn't induce peripheral toxicity, neutropenia and thrombocytopenia [47]. At effective doses, marizomib was well tolerated by patients, with manageable toxicity, administrated twice weekly with longer infusions. It produces rapid, broad and prolonged inhibition on catalytic sites of proteasome and markedly different safety and efficacy profiles [48].

Conclusions

As a conclusion, results obtained in various clinical trials regarding the use of proteasome inhibitors have proved that ubiquitin-proteasome pathway is a viable therapeutic target in cancer, opening new horizons in the treatment of this disease.

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