The Proteasome, a New Target for Cancer Therapy

S. Gillessen\textsuperscript{a,b} M. Groettrup\textsuperscript{b,c} T. Cerny\textsuperscript{a}

\textsuperscript{a} Department of Oncology/Hematology, \textsuperscript{b} Research Department, Kantonsspital St. Gallen \textsuperscript{c} Department of Biology, Universität Konstanz

**Key Words**

Ubiquitin-proteasome pathway · Proteasome inhibitor · Combination treatment

**Summary**

The proteasome is a multicatalytic protease and the principal non-lysosomal proteolytic system in all eukaryotic cells. It plays a central role in virtually all regulatory pathways as for instance cell-cycle regulation, differentiation, and apoptosis. The proteasome degrades regulatory proteins and their inhibitors and, thus, is an interesting target for therapeutic drugs. Inhibitors of the proteasome are small molecules that function by stabilizing various proteins, including cell-cycle regulators, tumor suppressors, and growth factors. Because proteasome inhibition blocks cellular proliferation and induces apoptosis, these agents have been tested as anticancer drugs in tumor models and have shown impressive potential. In addition, treatment with proteasome inhibitors can sensitize cells to other cancer treatments like radio- or chemotherapy. This review introduces the ubiquitin-proteasome pathway and outlines the recent progress in the development of proteasome inhibition as a treatment option for clinical cancer therapy.

Dr. Silke Gillessen
Onkologie/Hämatologie, Kantonsspital St. Gallen
Rorschacherstrasse
CH-9007 St. Gallen (Switzerland)
Tel. +41 71 494-1067, Fax -6325
E-mail silke.gillessen@kssg.ch

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The Proteasome

Proteasomes are found in the nucleus and cytosol of all eukaryotic cells. The functional 26S proteasome is a large, multisubunit protease complex consisting of a 20S catalytic core complex and a 19S regulatory subunit. The 20S proteasome is a barrel-shaped complex consisting of 4 rings with 7 subunits each. The subunits of the inner 2 rings are of the beta-type and bear the proteolytically active centers oriented towards the lumen of the barrel in which proteins are degraded. Each of the beta-rings contains 3 proteolytic sites that function together to hydrolyse proteins into small peptides. One preferentially cleaves after hydrophobic residues (i.e. the chymotrypsin-like activity), one after basic residues (i.e. the trypsin-like activity), and one after acidic residues (i.e. the caspase-like activity). The outer 2 rings are of the alpha-type and control access to the inner catalytic chamber and associate with regulatory complexes [1–3]. The 19S complex consists of a 6-membered proximal ring of ATPases called ‘the base’ and a distal subcomplex of 11 non-ATPase subunits called ‘the lid’ which together can associate to one or both ends of the 20S proteasome (fig. 1).

Proteins that have to be degraded are marked by covalent linkage to ubiquitin, a small protein tag, which becomes isopeptide-linked to protein substrates through an ATP-dependent process which is tightly regulated by an enzymatic cascade. Ubiquitinated proteins bind to at least 2 receptor subunits on the 19S complex. After binding to the regulatory complex, the ubiquitin molecules are removed by isopeptidases and the protein is unfolded under hydrolysis of ATP. The unfolded protein is then inserted into the lumen of the 20S complex and cleaved by the proteolytic activity of the proteasome into short polypeptides (fig. 2). The proteolytic mechanism of the proteasome relies on a threonine as the catalytic end of the 20S proteasome (fig. 1).

This ubiquitin-proteasome pathway is the principal non-lysosomal pathway for protein turnover in all eukaryotic cells. Key biological processes like cell-cycle regulation, apoptosis, differentiation, metabolic control and antigen presentation are regulated by this pathway.

Proteasome Inhibitors

Synthetic and naturally occurring inhibitors of the ubiquitin-proteasome pathway have been identified. Lactacystin is a natural Streptomyces metabolite that is selective for proteasomes when applied at low concentrations. Since proteasome function is essential for survival, a proteasome inhibitor would be expected to lead straight away to cell death. But, surprisingly, lactacystin is an agent that induces cell differentiation as shown in a mouse neuroblastoma cell line. Only at higher concentrations lactacystin was toxic to these cells. Also depending on the model system and the concentration used, lactacystin was able to induce or prevent apoptosis [5–13]. In addition, lactacystin inhibited MHC class I restricted antigen presentation of most of the T-cell epitopes tested so far [8, 14].

Synthetic inhibitors that target the proteasome are virtually all chemically modified oligopeptides equipped with a chemical warhead which covalently binds to and inactivates the active centers. The first generation of proteasome inhibitors were peptide aldehydes, such as N-acetyl-leucyl-leucyl-norleucinal, that react with the threonine of the active site. Synthetic and naturally occurring inhibitors of the ubiquitin-proteasome complex consisting of a 20S catalytic core particle and of the 19S regulator (also named PA700), which can bind to one (as shown) or both endplates of the 20S proteasome. The 20S core particle is constituted from 4 stacked rings with the outer 2 rings consisting of 7 different α-type subunits and the inner 2 rings consisting of 7 different β-type subunits. The 19S regulator is composed from a proximal hexameric ‘base’ of ATPase subunits in (violet) and a ‘lid’ (11 subunits, a linker of 3 subunits in dark blue and at least further 8 subunits in light blue). The linker subunit S5a and the ATPase subunit S6’ have been shown to bind polyubiquitin chains and are hence likely to act as receptors for polyubiquitylated substrates.

The HIV-1 protease inhibitor Ritonavir is also a weak proteasome inhibitor and may at least partially act via attenuation of the cytotoxic immune response [20]. Interestingly this compound is given on a regular basis to patients and is generally well tolerated, suggesting that at least partial inhibition of the proteasome over long periods of time is possible in humans.
Fig. 2. Scheme of ubiquitin conjugation. The small protein ubiquitin is activated at its C-terminus by thioester linkage to a ubiquitin activating enzyme (E1) under consumption of ATP. It is then transferred onto a ubiquitin conjugating enzyme (E2). The E2 enzyme and a target protein is bound by a specific ubiquitin ligase (E3) which catalyzes the transfer of the activated ubiquitin onto a lysine residue in the target protein. An isopeptide bond between ubiquitin and the target protein is formed. The polyubiquitylated protein is bound by the 26S proteasome and unfolded under the expense of ATP. The protein is degraded into peptides and the polyubiquitin chain is removed by ubiquitin-specific proteases (UBPs) and disintegrated into free ubiquitin which is reused in further degradative cycles.

The Role of Proteosome Inhibitors in Cell-Cycle Regulation, Apoptosis and Angiogenesis

It has been shown by many groups that proteosome inhibition has an effect on the stability of many cell cycle regulatory proteins. The cell cycle, for instance, is directly regulated by cyclins (A, B, D, E) and some of them are overexpressed in human tumors. The activity of cyclins can be regulated by cyclin-dependent kinase (CDK) inhibitors like p21 or p27. Cyclins and CDK inhibitors are degraded by the ubiquitin-proteosome pathway and inhibition of this pathway has been shown to sensitize cells to apoptosis. The same applies to tumor suppressors like p53 or pRB [21–26]. Additionally, it has been shown in a pancreatic cancer cell line that proteosome inhibition not only led to accumulation of the CDK inhibitors p27 and p21 but also to augmented protein levels of the proliferation-associated nuclear antigen Ki-67. The elevation of the Ki-67 protein and enhanced levels of the 2 CDK inhibitors were most likely responsible for suppression of tumor growth through proteosome inhibition in that cell line [27].

Moreover, the NF-κB signalling pathway seems to be an important target for proteosome inhibitors. Constitutive activation of NF-κB has been implicated in the development of many human malignancies. NF-κB is a critical transcription factor involved in immune responses and cellular growth. It is regulated mainly by interaction with an inhibitor protein called IκB. This inhibitor is degraded by the ubiquitin-proteosome pathway. After degradation of IκB, NF-κB translocates to the nucleus and regulates genes encoding cytokines like tumor necrosis factor (TNF), interleukin-1, interleukin-2 or interleukin-6, proinflammatory enzymes like nitric-oxide synthase (NOS) or cyclooxygenase-2 (COX-2), chemotactic factors like interleukin-8 or monocyte chemoattractant protein-1 and cell adhesion molecules like intracellular cell adhesion molecule (ICAM-1), vascular cell-adhesion molecule (VCAM-1) or E-selectin. Importantly, NF-κB also regulates genes involved in the expression of antiapoptotic proteins like members of the Bcl-2 family or members of the inhibitor of apoptosis (IAP) family [21, 23, 28]. Proteosome inhibition can inhibit NF-κB activation indirectly via stabilization of IκB. By this mechanism the sensitivity of cancer cells to apoptosis is increased and angiogenesis as well as metastasis are impeded. In fact, it has been shown recently in vivo that treatment with a proteosome inhibitor (PS-341) inhibited growth of murine and human squamous cell carcinoma cell lines in mice and that this growth inhibition was associated with a marked decrease in vessel density [29].

Proteins of oncogenes like c-fos or c-myc are also substrates for the ubiquitin-proteosome pathway [21, 23, 30]. It would seem that stabilization of these growth factors by proteosome inhibitors would increase tumor growth. But this is not the case as seen in many experiments. This may be due to the fact that augmented oncogene levels in tumor cells maintain maximal proliferation and that a further increase after proteosome inhibition may not add to the proliferative activation.

Preclinical Results of Proteosome Inhibitors

In general, proteosome inhibitors have demonstrated activity in a wide range of malignancies in cancer models for solid and hematological tumors. They are also effective in some cell lines that are resistant to other standard therapies [30, 31]. In addition, proteosome inhibitors seem to be as effective in killing tumor cells grown as multicell spheroids as in killing tumor cells grown in monolayer cell culture – in contrast to most other anticancer drugs that are much more effective in killing cancer cells grown in monolayers [32]. Dividing cells are more sensitive to proteosome-induced apoptosis than are resting cells [33]. Moreover, transformed cells seem to be more responsive to proteosome inhibition than normal dividing cells, as shown in a study by Masdehors et al. [34]. They demonstrated that the pro-apoptotic effect of a proteosome inhibitor (lactacystin) was markedly enhanced in lymphocytes...
from patients with chronic lymphatic leukemia (CLL) compared to lymphocytes from healthy volunteers. The reason for this effect is not fully understood, but tumor cells may be more susceptible to proteasome inhibition because some of the changes that suppress apoptosis in malignant cells can be reversed by proteasome inhibition.

As mentioned above, NF-κB is constitutively activated in myeloma cells, as well as in certain leukemias and some solid tumors. NF-κB is responsible for production of factors that promote growth of cells and has anti-apoptotic effects that can be responsible for protection against chemotherapy and radiotherapy. Wang et al. [35] have shown that tumor cells which are genetically modified to express a form of IκB that is not degraded by the proteasome show a significant augmentation of chemosensitivity and an enhanced induction of apoptosis in a xenograft model. An alternative to this strategy is inhibition of the NF-κB function. Cusack et al. [36] have shown in a human colorectal cancer cell line that pretreatment with a proteasome inhibitor, PS-341 (bortezomib), significantly increased the sensitivity of these cancer cells to SN-38, the active metabolite of the topoisomerase I inhibitor irinotecan. There is another mechanism that could at least partially account for this effect: topoisomerase I inhibitors like irinotecan stabilize an intermediate – the cleavable complex – in the topoisomerase-mediated cleavage-religation reaction. This structure is degraded by the proteasome. By inhibition of the proteasome, the removal of this complex from the chromosomal is prevented and the probability of double-strand breaks is increased [30].

Another proteasome inhibitor (MG-132) could induce apoptosis without changing the levels of NF-κB in a cell line defective in NF-κB regulation (derived from patients with Hodgkin’s lymphoma) and rendered them sensitive to radiation, which suggests that inhibition of activation of NF-κB is not the only mechanism through which proteasome inhibitors are working [37]. Also in other tumor models significant tumor responses have been observed with proteasome inhibitors. For example, pancreatic tumor xenografts were responsive to PS-341 used in combination with gemcitabine or in combination with irinotecan [38, 39]. Recently has it been shown in a model of adult T-cell leukemia that therapy with PS-341 in combination with a humanized antibody against interleukin-2 receptor alpha (HAT) was associated with a complete remission in a proportion of treated mice, whereas only a partial response was observed in mice treated with HAT alone [40]. Interestingly, proteasome inhibitors have also been shown to be active in cell lines resistant to cytotoxic drugs or radiation. Delic et al. [41] tested radiation-resistant isolates from a chronic lymphocytic leukemia cell line. These cells became apoptotic after treatment with a proteasome inhibitor (lactacystin). In addition, Russo et al. [42] demonstrated that radiosensitivity in a colorectal cancer cell line could be enhanced by proteasome inhibition.

Hideshima et al. [43] showed that proteasome inhibition by PS-341 directly inhibited proliferation and induced apoptosis in human multiple myeloma cell lines but also in freshly isolated multiple myeloma cells, which are resistant to conventional drugs used for myeloma therapy including dexamethasone, doxorubicin and melphalan. In this setting PS-341 can inhibit the synthesis of interleukin-6 by its inhibition of NF-κB activation. By this mechanism PS-341 inhibits p44/42 mitogen-activated protein kinase (MAPK) growth signaling triggered by interleukin-6, thus leading to an induction of apoptosis. Also by inhibition of activation of NF-κB, PS-341 may be able to overcome the interleukin-6 mediated resistance against dexamethasone. Apoptosis induction occurs in p53 wild-type as well as in p53 mutant multiple myeloma cells and also overexpression of bcl-2 seems not to protect from apoptosis induction by PS-341 [30].

Remarkably, it has been shown that the proteasome inhibitor PS-341 is also effective in Bcr-abl positive cell lines that are resistant to the tyrosine kinase inhibitor imatinib (STI571) [44]. PS-341 also seems to be a poor substrate for multidrug resistance transporters (MDR1) [21] and demonstrates growth inhibition and cytotoxic activity in the nanomolar range.

In addition to their antitumor effects, proteasome inhibitors may also have potential in inflammatory diseases by blocking the activation of NF-κB which is responsible for expression of most of the principal components of inflammatory responses as described above. Proteasome inhibitors have been shown to be effective in animal models of asthma, arthritis, autoimmune encephalomyelitis and in an animal model of human psoriasis [45, 46]. Also, proteasome inhibition seems to effectively prevent heart allograft rejection in mice by suppressing T-cell proliferation and cytokine secretion [47].

Clinical Studies with Cancer Patients

Most clinical studies have been performed with the proteasome inhibitor PS-341 (bortezomib), which is a boronic acid dipeptide derivative. We will therefore concentrate on trials conducted with this compound.

Phase I studies have been conducted or are ongoing in patients with advanced solid tumors and hematological malignancies to determine the maximally tolerated dose, the toxicity and the dose-limiting toxicity as well as the in-vivo effects on proteasome activity. In approximately 200 patients treated in this setting, PS-341 was generally well tolerated. Side effects are low-grade fever (grade 1/2) and/or fatigue (mostly grade 1/2, rarely grade 3) or anorexia (grade 1/2) on repeated cycles of therapy. Moderate and transient thrombocytopenia (mostly grade 1/2, rarely grade 3) has been observed, but did not necessitate platelet transfusions. Some patients developed diarrhea (grade 1/2), but this has not been dose-limiting and could be relieved by loperamide or completely avoided by prophylactic loperamide.
treatment. Several patients experienced nausea/vomiting (grade 1/2). Several patients experienced peripheral neuropathy and this toxicity was dose-limiting in single cases. Prior treatment with agents known to be neurotoxic like taxanes or platinum derivatives may increase the susceptibility of patients to peripheral neuropathy. Skin rash is another, but infrequent side effect.

Even though phase I trials are not designed to test for tumor response, responses have been seen in patients with non-small lung cancer, melanoma and multiple myeloma, and PSA (prostate-specific antigen) responses have been found in patients with androgen-independent prostate cancer [21, 23, 30]. These results look promising and new trials are ongoing in order to test PS-341 in different combinations. Early results suggest that combinations with gemcitabine or 5-fluorouracil/folinic acid or doxorubicin are feasible [48, 49, 50].

A phase II trial is ongoing testing PS-341 alone or in combination with dexamethasone in patients with multiple myeloma who have relapsed after first-line therapy and are refractory to their most recent therapy. Preliminary data have been presented at the ASH-meeting (American Society of Hematology) 2001 and looked encouraging [51, 52]. Other studies on patients with refractory chronic lymphocytic leukemia, non-Hodgkin’s lymphoma and various solid tumors are presently being conducted.

Conclusions

Proteasome inhibitors are a new promising class of agents in cancer treatment in a wide variety of solid and hematological malignancies. Furthermore, they may also have applications in non-malignant conditions like inflammatory diseases and transplantation.

The identification of the proteasome inhibitors has not only given us interesting new drugs to test in various diseases, but has also enabled us to make progress in understanding the great importance of the ubiquitin-proteasome pathway. Many phase II studies with PS-341 alone or in combination are now ongoing in the US and in Europe.

References

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