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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Role of proteasome-ubiquitination degradation pathway in animal cancer - an insight

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Abstract

Most intracellular proteins are degraded by the proteasome, a multicatalytic enzyme complex containing a 20S catalytic core and two 19S regulatory complexes. The proteasome is responsible for the degradation of intracellular proteins that are particularly involved in cell cycle control and the regulation of apoptosis. Preclinical studies have in the past used various forms of proteosome inhibitors such as chemical (Bortezomib), natural resources (marine microbial metabolites, green tea polyphenols, flavonoids etc.) and metal complexes. Such proteosome inhibitors have been observed to decrease proliferation, induce apoptosis, and enhance the activity of chemotherapy and radiation, and reverses chemoresistance in a variety of hematologic and solid malignancies. However, their clinical significance and relevance of targeting the tumor survival-associated proteasome pathway for cancer treatment, intervention and prevention needs to be discussed. We therefore, reviewed here how the structure and function of the proteasome deals with various forms of proteasome inhibitors that counteract cancer propagation in animals.

Keywords: Proteasome, p53; Ubiquitin; neoplasm; cancer

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Introduction

The proteasome is a multicatalytic enzyme complex that degrades most intracellular proteins by a targeted and controlled mechanism (Adams, 2002). Proteins that are first targeted for degradation are tagged with a polyubiquitin chain and then gets rapidly and irreversibly degraded to oligopeptides by the proteasome and thereafter the used ubiquitins in the process gets recycled (Glickman and Ceichanover, 2002). Proteasomes perform a critical role in the degradation of key signaling molecules that promote cell cycle progression, cellular adhesion, and proliferation and induce anti-apoptotic pathways (Almond and Cohen, 2002). However, with the disruption or inhibition of proteasome- various critical signaling cascades are dys-regulated and lead to apoptosis. Therefore, due to these modes of action and functions the proteasome has emerged as an attractive target for cancer therapy (Adams, 2003).

The p53 plays a crucial role in coordinating the cellular response to genotoxic stress (Hartwell and Kastan, 1994; Levine, 1997). p53 regulates the expression of many downstream effectors involved in cell growth, cell cycle arrest and apoptosis (El-Deiry, 1998). The cellular levels of p53 protein are tightly regulated through ubiquitin-proteosomal system (UPS) (Haupt et al., 1997). Early studies have suggested that oncoprotein MDM2 is a primary E3 Ub ligase for p53 degradation (Kubbutat et al., 1997). MDM2 itself is transcriptionally activated by p53 and it regulates p53 in a negative feedback loop (Baugh et al., 2009). Moreover, regulation of p53 stability is a central process in controlling p53 function and the mechanism of p53 proteasomal degradation through polyubiquitination has already been characterized previously. The basic assumption behind this mechanism is that p53 is

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inherently stable unless sensitized to degradation by polyubiquitination. However, a number of studies provide evidence for p53 to be naturally unstable. Consistent with this attribute is the fact that both p53 N- and C-termini are intrinsically unstructured. Recent findings have provided evidence for p53 to be degraded by the 20S proteasome by default unless it escapes this process.

Proteosome structural and functional properties

The proteasome is a multi-subunit, cylindrical complex consisting of a 20S core catalytic component and 19S regulatory particles or an 11S REG (PA28) activator capping the ends of the core (Glickman and Ceichanover, 2002) was initially recognized or identified as proteolytic pathways that requires ATP hydrolysis and causes degradation of protein (Almonds and Cohen, 2002). (Fig. 1). The 19S particle contains polyubiquitin-binding sites and isopeptidase activity that was later observed to be necessary for the cleavage and release of ubiquitin from the protein substrate (Delcros et al., 2003). It also contains six different ATPases that unfold the protein substrate and open a channel in the α-ring that allows the denatured protein to enter the catalytic chamber formed by the two inner β-rings. Each β-ring contains three active enzyme sites with trypsin-like, chymotrypsin-like, and post-glutamyl peptide hydrolase-like activities (Delcros et al., 2003). The protein substrate progressively degrades and results in the release of short peptides. The PA28 activator enhances the activity of the proteasome and plays a role in immune presentation of major histocompatibility complex class I antigens (Kloetzel, 2004). The proteasome degrades a wide range of protein substrates involved in cell cycle. The proteasome is responsible for the degradation of intracellular proteins particularly involved in cell cycle controls and regulate the process of apoptosis. Preclinical studies have in the past used various forms of proteosome inhibitors such as chemical (Bortezomib), natural resources (marine microbial metabolites, green tea polyphenols, flavonoids etc.) and metal complexes that have shown to decrease proliferation, induce apoptosis, enhance the activity of chemotherapy and radiation, and reverse the chemo-resistance in various forms to malignancies. However, their clinical significance and relevance of targeting the tumor survival-associated with proteasome pathway for cancer treatment, intervention and prevention.

Among various substrates for proteosome p53 (tumor suppressor protein) is an important substrate for proteasomal degradation (Salvat et al., 1999). Activated p53 arrests cells in the G1-phase and may inhibit progression into S-phase (Waldman et al., 1995) and the kind of cell cycle arrest happens here could be attributed to a p53-mediated increase in p21Cip1 expression has been demonstrated (Waldman et al., 1995). p53 promotes apoptosis to allow elimination of damaged cells through induction of the proapoptotic protein Bax. Proapoptotic protein "Bax" in fact inhibits the antiapoptotic proteins Bcl-2 and Bcl-xL in mitochondria and thus triggers cytochrome c release. Cytochrome c causes the activation of the caspase cascade and ultimately the cell death (Voorhees et al., 2003). Bax itself is also a proteasomal substrate and therefore proteasome inhibition brings in the stabilization of p53, p21, p27, and Bax, resulting in dysregulation of cell cycle progression (Almond and Cohen, 2002). Finally, the proteasome modulates the activity of the transcription factor nuclear factor- kB (NF-kB). In quiescent cells, the regulatory protein inhibitor of NF-kB (IkB) generally binds to NF-kB in the cytoplasm and prevents its translocation into the nucleus. The NF-kB pathway is activated by a variety of cellular stress signals, including tumor necrosis factor (TNF-α), chemotherapy, and radiation therapy, which lead to phosphorylation of a serine residue on it and finally such cells gets targeted for ubiquitination and subsequently leading to proteasomal degradation. This process allows the activated NF-kB subunit to translocate into the nucleus, where it induces expression of genes encoding proinflammatory cytokines (TNF-α, interleukin [IL]-1, IL-6), eicosanoid products (cyclooxygenase- 2, 5-lipoxygenase), cell adhesion molecules (ICAM-1, VCAM-1, E-selectin), and anti-apoptotic factors (Bcl-2, inhibitor of apoptosis protein). NF-kB also up-regulates its own transcription and thereby amplifies and sustains its own effects (Karin et al., 2002) (Fig. 3.).

p53 Proteasomal Degradation Mechanism (Ubiquitin-Independent)

Lately, it was noticed that there are certain proteins exist which can stabilize p53 and with this information the ubiquitin-independent process that was given least importance in the past became validated. NADH quinone oxidoreductase 1 (NQO1), or DT-diaphorase, is a flavin-containing quinine reductase with broad substrate specificity (Loidl et al., 1999) has been a widely studied protein and that has been recently recognized to stabilize p53. NQO1 catalyzes the reduction in various quinones through a two-electron reduction mechanism using either with NADH or NADPH as a reducing cofactor, and it is inhibited by the competitive inhibitor dicoumarol (Hanada et al., 1992). The two-electron reduction prevents the formation of free radicals (semiquinones) and highly reactive oxygen species (ROS) (Fig. 4), thus protecting cells against quinones and their derivatives (Ross et al., 2000). NQO1 can be induced by various stimuli including phenolic antioxidants, azo dyes, and oxidative stress. Induction of NQO1 occurs through both ARE and XRE elements in the NQO1 promoter (Jaiswal, 2000) and have been demonstrated to be regulated mainly by the transcription factor Nrf2 (Nioi et al., 2004). In addition to this detoxifying role, NQO1 regulates p53 stability in vitro and in living cells. Human colon carcinoma cells that over express NQO1 accumulate elevated level of p53

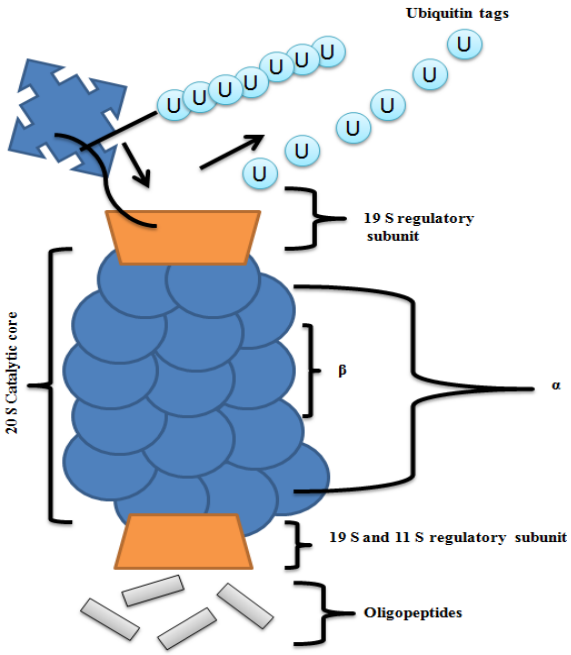

Fig. 1: Schematic presentation of proteasome showing 20 S catalytic core and 19 S or 11 S REG (PA28) regulatory particles.

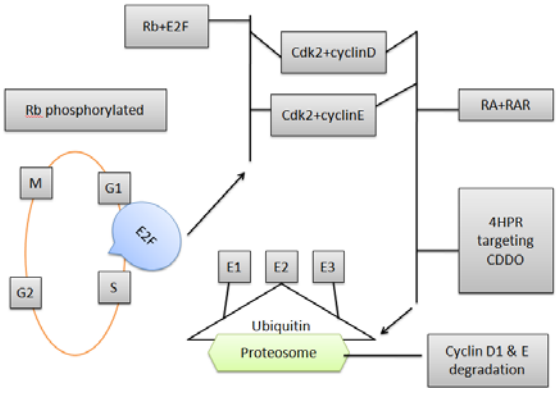

Fig. 2: Induction of cyclin degradation by chemopreventive agents.

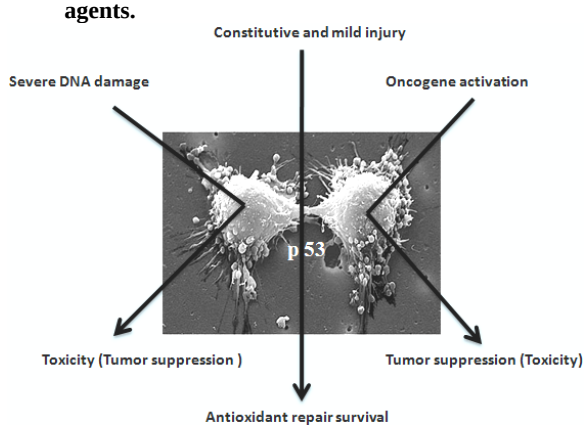

Fig. 3: Activation and response of p53.

(Fig. 5) (Asher et al., 2002). Furthermore, NQO1 null mice in one experiment has been shown to exhibit reduced p53 protein levels and decreased apoptosis in the bone marrow (Long et al., 2002) and therefore it has become apparent now that NQO1 binds to p53 in an NADH-dependent manner.

Interestingly, dicoumarol and other competitive inhibitors of NQO1 compete with NADH for the binding to NQO1, resulting in the dissociation of the NQO1-p53 complex. Remarkably, p53 becomes highly unstable and prone to proteasomal degradation in the presence of inhibitors (Asher et al., 2005). Furthermore, the degradation takes place even under conditions whereby the pathway of protein ubiquitination is completely inhibited (Asher et al., 2005). Another inhibitor of NQO1, which was recently identified as curcumin has been reported when normal T cells are exposed to DNA damage in the presence of curcumin, p53 accumulation is not induced, and T-cell apoptosis is significantly decreased (Tsvetkov et al., 2005). Recently, it was suggested that as much as 20% of all cellular proteins can be degraded or cleaved by the 20S proteasome specifically at unstructured domains (Baugh et al., 2009). In fact, the susceptibility to the 20S proteasome may be used as an operational definition approach to determine whether a given protein is unstructured (Tsvetkov et al., 2008). Consistently, p53 that is unstructured at both N- and C-termini undergoes 20S proteasomal degradation in vitro (Asher et al., 2005). Previous finding associates NQO1 with the 20S proteasome, and that it prevents the degradation of proteins with unstructured regions, such as p53, p73,17 and ODC 29 has been found to be consistent with a model in which NQO1 plays the role of 'gatekeeper' of the 20S proteasome . NADH regulates the association of NQO1 with the potential 20S proteasome substrates, but does not control NQO1association (Zwicki et al., 1999).

Proteasome degradation leading to tumor suppression and control

Mostly the tumorigenesis has been associated with the over-expressions of Cyclin D1 and other cyclins activate cyclin-dependent kinases as that has been linked with the cell growth promotion and cell transformation (Swanton, 2004). Therefore, it was thought that promoting proteasomal degradation of cyclin D1 and cyclin E would do something towards the control and prevention of cancer through their cell cycle arrest ability. Cell cycle arrest through proteosomal degradation is mainly accomplished by all-*trans*-retinoic acid (RA) in combination and with some other structurally unrelated agents (Dragnev et al., 2004). Dragnev et al. (2004) has shown previously that RA prevents carcinogenic transformation of immortalized human bronchial epithelial cell line BEAS-2B by causing G1 cell cycle arrest and triggering cyclin D1 degradation via the

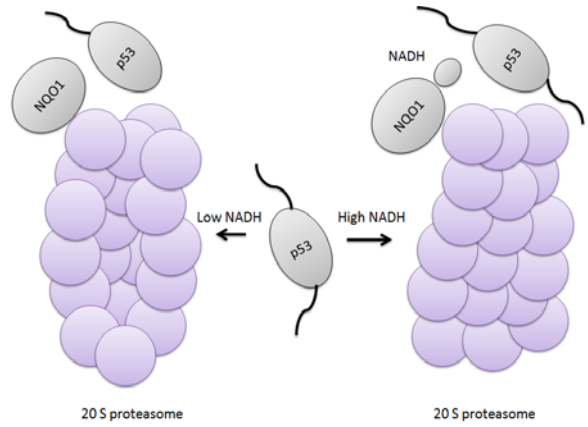

Fig. 4: Metabolic regulation of the degradation by default of p53.

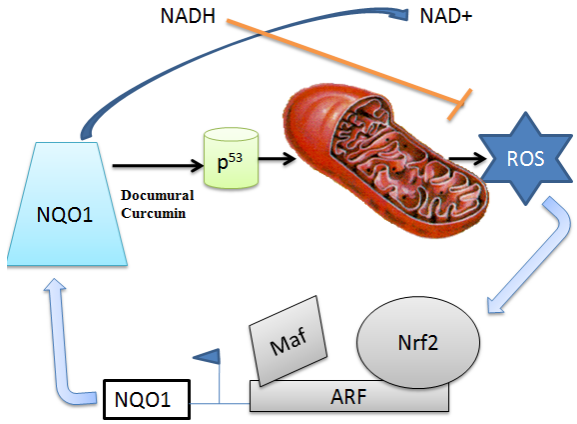

Fig. 5: NQO1 regulation under oxidative stress. P53 plays an important role in the process of aerobic respiration by up-regulating mito- chondrial activity.

ubiquitin proteasome pathway (Langenfeld et al., 1997). Some of the researchers have further demonstrated that cyclin E is also targeted for degradation by RA treatment (Boyle et al., 1999). Moreover, the treatment of BEAS-2B cells with N-(4-hydroxyphenyl) retinamide (4HPR), a nonclassical retinoid) and 2- cyano-3,12-dioxooleana-1,9-dien-28-oic acid [CDDO (a synthetic trierpenoid)] have also been shown to increase proteasomal degradation of the G1 cyclins and cell growth suppression. This type of degradation results mainly due to the inhibition by a proteasomal inhibitor, Nacetyl- leucyl-leucyl-norleucinal (ALLN). However, RO-24-5531 (a vitamin D analog), resiglitazone (a peroxisome proliferators activated receptor γ agonist), and indomethacin (a cyclooxygenase inhibitor) have been found to have no effect on cyclin levels and inspite of that they bring about the inhibition of cellular propagation.

Moreover, it has been propagated lately that phosphorylation of the retinoblastoma tumor suppressor protein (Rb) is crucial for the progression of the cell cycle from G1 into S phase (Fig. 2). This phosphorylation causes Rb-mediated inhibition of E2F transcription activity that is necessary for the transcription of genes essential for entry into S phase. The phosphorylated state of Rb dependant on cyclin D and E and with their respective cyclin-dependent kinases critically controls the repression of E2F by Rb. RA (Retinoic acids) induces degradation of cyclins D1 and E however, the mode of mechanism is still not known (Kitareewan et al., 2002).

It is now known that CDDO activates peroxisome proliferators-activated receptor and cause mammary cancer cell growth arrest (Lapillonne et al., 2003). It also induces proteasomal degradation of cyclin D1 protein and enhanced p21WAF1 expression (Lapillonne et al., 2003). Both these events have been shown to have direct consequences in leading CDDO-mediated growth inhibition. It is possible that RA, 4HPR, and CDDO also inhibit cell growth or transformation by mechanisms other than promotion of the proteasomal degradation of G1 cyclins. In order to demonstrate that proteasomal degradation as the major mechanism for the chemo-preventive activity of RA, 4HPR, and CDDO, it would be interesting to know whether the suppression of cell growth and transformation by these agents could be abolished by specific inhibitors that block the degradation. Ubiquitin ligation is a multistep process accomplished by three enzymes (Schwartz et al., 2003). A ubiquitin-activating enzyme (E1) activates ubiquitin in an ATP-dependent manner, and the ubiquitin is then transferred to a ubiquitin-conjugating protein (E2), which works in concert with a ubiquitin-protein ligase (E3) to conjugate the target protein at lysine residues. Here, the targeting process is the multistep process where E3 enzymes play the dominant role in target binding. This recently proposed mechanism depends on the degradation of specific groups of proteins, and the specificity is likely to come from E2 and E3 of the ubiquitination complex (Boyle et al., 1999). A general activation of the proteasomal activity may not be a useful chemo-prevention mechanism. There is ample evidence that activation of the proteasome may contribute to the carcinogenic process. The bile acid deoxycholic acid is reported to stimulate proteasome-mediated degradation of p53 resulting in impaired p53 transactivation and response to DNA damage (Qiao et al., 2001). Many publications deal with the inhibition of the proteasome as a cancer chemotherapeutic mechanism. For example, bertezomib (Velcade) has recently been approved for the treatment of multiple myeloma (Adams, 2003). Inhibition of the proteasome as a cancer chemotherapeutic mechanism has given rise to many proteasome inhibitors such as “velcade”. These inhibitors are active against many tumor types, both as a single agent and in combination with standard chemotherapy drugs such as gemcytabine and irinotecan. Several potential mechanisms

Table 1: Adverse effects or events associated with Bortezomib in clinical presentations of patients with multiple myelomas or Hematological malignancies.

Adverse events	Percentage of occurrence
Peripheral neuropathy	12-15% in the patients with multiple myeloma and 5-8% in other malignancies
Thrombocytopenia	23-31% in the patients with multiple myeloma and 29-49% in other malignancies
Fatigue	12% in the patients with multiple myeloma and 4-15% in other malignancies
Rash	< 1 % in the patients with multiple myeloma and 0-3% in other malignancies
Gastrointestinal effects	25% in the patients with multiple myeloma and 17-25% in other malignancies
Tumor lysis syndrome	Rare event, typically seen in early cycles with high tumor load

have also been proposed for the antitumor activity of this proteasome inhibitor.

p21 and p27 (inhibitors of cell cycle progression), Rb and p53 (tumor suppressors), and inhibitor kB are all substrates for the proteasome. Inhibition of the degradation of these substrates would result in the accumulation of proteins that are negative regulators of the cell cycle and cell proliferation and would lead to induction of apoptosis (Qiao et al., 2001). Inhibition of the proteasome by green tea polyphenol (-)epigallocate-chin-3-gallate could also result in increased levels of the tumor suppressor genes p53, pRB, p21, and Bax, any of which may inhibit cell proliferation and/or induce apoptosis and all of these has been proposed as the cancer prevention mechanism (Nam et al., 2001). Dragnev et al. (2004) have suggested that these compounds might be useful in overcoming clinical resistance to classical retinoids by virtue of their ability to activate pathways downstream of retinoic acid receptor β. Dragnev et al. (2004) also advocate the use of combination chemo- prevention with agents activating non-cross-resistant pathways. The concept of using combinations of agents to increase efficacy and decrease toxicity in cancer therapy and chemoprevention has been proposed to imbibe for future.

Proteasome Inhibitors

Currently available inhibitors of the ubiquitin-proteasome pathway directly target and inhibit the 20S proteasome, the core of the proteolysis machinery, rather than the upstream ubiquitination and recognition of ubiquitinated protein substrates. These proteasome inhibitors are broadly categorized into two groups: synthetic and natural.

Synthetic inhibitors are peptide-based compounds with diverse pharmacophores. These include peptide benzamides, peptide a-ketoamides, peptide aldehydes, peptide a-ketoaldehydes, peptide vinyl sulfones, and peptide boronic acids. In contrast, natural product proteasome inhibitors display a variety of scaffolds of core structures and pharmacophores. Known natural product proteasome inhibitors include linear peptide epoxyketones, peptide macrocycles, g-lactam thiol ester, and epipolythiodioxopiperazine toxin.

Synthetic proteasome inhibitors

Peptide aldehydes have long been used as inhibitors for both serine and cysteine proteases. Aldehyde functional group is commonly subjected to a nucleophilic attack by hydroxyl or thiol groups and as the proteosome uses hydroxyl group as nucleophile, it is not surprising to know that the commercially available peptide aldehydes were probably the first proteasome inhibitor family which might have been identified. Leupeptin, a standard serine (trypsin, plasmin) and cysteine (papain, cathepsin B) protease inhibitor, was recognized in early 1980's as a T-L activity blocking agent of the 20S proteasome. Later, calpain inhibitors I and II were also shown to inhibit the CT-L activity of the 20S proteasome (Figueiredo-Pereira et al., 1994). Upon binding to the active site of the 20S proteasome, a peptide aldehyde forms a covalent hemi-acetal adduct that is reversible under physiological conditions. Since peptide aldehydes can be easily prepared and optimized, researchers have developed myriad of aldehyde inhibitors with higher potency as well as increased selectivity toward the CT-L activity of the 20S proteasome. Such examples include MG115, MG132, 175 and PSI which are all potent and CT-L selective inhibitors that are widely used in studying the role of the proteasome in various cellular processes. Similarly, other peptide aldehyde inhibitors have been synthesized to study other proteasomal activities, such as BrAAP activity. Meanwhile, researchers have also attempting to improve peptide aldehydes' potency by adding an additional ketone moiety at a position, yielding a glyoxal (Vinitsky et al., 1994).

Although, these peptide aldehydes, in general, are cell-permeable, potent inhibitors of the 20S proteasome, and are still widely used in biological studies but the lack of specificity due in part to the presence of highly reactive aldehyde functional group is a major limitation for their use as potential therapeutic agents or as molecular probes in dissecting complex signaling pathways. To overcome such limitations, researchers have attempted to develop non-reactive peptide inhibitors, such as a-ketocarbonyl and boronic ester derivatives, indanone dipeptide benzamides, and P0- extended alpha-ketoamides. Non-aldehyde irreversible proteasome inhibitors have also been developed, such as tripeptides alpha and beta-epoxyketones and vinyl sulfones (Chatterjee et al., 1999).

Vinyl sulfones were also the first to get introduced as cysteine protease inhibitors. Although vinyl sulfones are sufficiently inert in the absence of a target, it is well characterized that they are capable of forming a hydrogen bond in the active site of enzymes, thereby becoming

more electrophilic and have also been developed as potent inhibitors toward the 20S proteasome and successfully employed for many biological studies. Peptide vinyl sulfones have also been shown to be irreversible and active site-directed proteasome inhibitors produced by modification of the hydroxyl group of the amino terminal threonine. Inspite of this, the lack of specificity is a major concern for this class of inhibitors, since the peptide vinyl sulfones inhibit both the 20S proteasome and other cysteine proteases (Adams et al., 2002).

Another class of peptide-based inhibitors exploits the boronic acid functional group and it works to bring in proteosome inhibition via a noncovalent complex formation. Taking into account no difference in the potency of the boronic ester derivatives as compared to that of the corresponding aldehyde derivatives, the inhibitory potency and the target selectivity towards the 20S proteasome of boronic acid-based inhibitors are quite remarkable. This is presumably due to the fact that an empty p-orbital on a boron atom is positioned to accept the oxygen lone pair of the amino terminal threonine residue of the 20S proteasome to form a stable tetrahedral intermediate. The stable tetrahedral borane complex allows the peptide chain length of the boronic acid-based inhibitor to be truncated to a dipeptide with good retention of inhibitory activity. This provides practical advantages, such as solubility and membrane permeability, as a potential therapeutic agent (Adams et al., 2005).

The most well characterized compound of this class is a dipeptide boronic acid PS-341. Analogous to many other proteasome inhibitors that block cell cycle progression, PS-341 treatment also results in accumulation of cells in the G2-M phase, eventually leading to apoptosis after prolonged treatments of cells (Chandra et al., 1998. Moreover, PS-341 inhibits the proteasome mediated-IkBα degradation and NFkB-dependent gene expression in vivo has shown to reduce the tumor growth in both murine and human xenograft models. Clinicians are now recently using PS-341 for advanced breast and prostate cancer (Omura et al., 1991).

Natural proteasome inhibitors

Apart from the synthetic inhibitors available, number of naturally produced agents has also shown to be potent in action as proteasome inhibitors. For example, lactacystin is a *Streptomyces lactacystinaeus* metabolite was first discovered as it was identified that it exclusively induces neurite outgrowth in the murine neuroblastoma cell line Neuro-2a (Fenteany et al., 1994). Subsequently, others have shown that lactacystin could also inhibit cell cycle progression. Later, Fenteany et al (1995) showed that lactacystin targets the 20S proteasome by an irreversible modification of the amino terminal threonine of beta subunits. Furthermore, clasto-lactacystin beta lactone was found to be the active component of lactacystin and it reacts with the hydroxyl group of the amino terminal threonine to make an ester adduct. Despite initial reports of high proteasome specificity and its wide use in biological studies, there have been reports that lactacystin also inhibits other cellular proteases (Ostrowska et al., 2000). Moreover, complexity in the synthesis of lactocystin has actually hampered its development as therapeutic agents. Meanwhile, epoxomicin was searched as an antitumor agent has been found to belong to family of linear peptide alpha and beta -epoxyketone natural products. Recently, it has been shown that epoxomicin specifically targets the 20S proteasome (Sin et al., 1999). Unlike other classes of proteasome inhibitors that show non-target specificity, epoxomicin is highly specific for the 20S proteasome. This unique specificity of epoxomicin is a result of a six-membered morpholino ring formation between the amino terminal catalytic Thr-1 of the 20S proteasome and the alpha and beta -epoxyketone pharmacophore of epoxomicin (Sin et al., 1999). In addition, eponemycin, an anti-angiogenic linear peptide alpha and beta -epoxyketone isolated from *Streptomyces Hygroscopicus* has been shown to target the 20S proteasome as well (Kohno et al., 2000) More recently, other linear peptide alpha and beta -epoxyketone natural products have also been isolated directly on the basis of proteasome inhibition screening from microbial metabolites. Examples of which includes TMC-86A and B from Streptomyces sp. TC 1084, TMC-89A and B from Streptomyces sp. TC 1087 and TMC-96 from Saccharothrix sp. TC 1094. The facile syntheses of linear peptide alpha and beta -epoxyketones has prompted the development of more potent peptide alpha and beta -epoxyketones, such as YU101 that was developed by the optimization of the amino acids of the P2-P4 positions to maximize its potency toward the CT-L activity. While lactacystin and epoxomicin are the best known natural product inhibitors of the 20S proteasome, there have been other potent natural product proteasome inhibitors with novel structures. For example, in the course of proteasome inhibitor screening procedure, Kohno et al (2000) found a series of TMC-95 from the fermentation broth of Apiospora montagnei Sacc. TC 1093. Despite their unusual macrocyclic peptide architecture, they possess potent inhibitory activity toward the CT-L activity of the 20S proteasome (Koguchi et al., 2000). Another unexpected class of natural product proteasome inhibitors includes gliotoxin and that is a member of the fungal epipolythiodioxopiperazine toxins that are characterized by a heterobicyclic core containing a disulfide bridge(s). Gliotoxin was originally identified as a potent inhibitor of NF-kB activation in T and B cells. Recently, it was shown and demonstrated that gliotoxin noncompetitively targets the CT-L activity of the 20S proteasome and that the disulfide bridge is responsible for its inhibitory action (Koguchi et al., 2000).

Whether the catalytic activity of various proteosome subunits deals with overall biological processes is still a thing of investigation. Most of the synthetic and natural proteasome inhibitors are CT-L activity-directed compounds. Moreover, only a limited number of compounds have been shown to inhibit the PGPH or T-L activities of the 20S proteasome. Therefore, a new generation of compounds that specifically inhibit the PGPH or T-L activities may be required to perform a careful analysis of proteasome subunit function and to gain insights into the possibility for potential therapeutic intervention (Loidl et al., 1999).

Newer proteasome inhibitors

Bortezomib, first proteosome targeted drug, was for the first time used to treat multiple myeloma in 2003 and got validation for its was approved in 2003 and got validation for its 20S proteolytic subunit of the proteasome as an anticancer target. Linder and colleagues recently brought out an article in nature journal where they described this drug as a small-molecule inhibitor of proteasomal deubiquitylases (D'Acry et al., 2011) that induces tumour cell apoptosis which is potentially paving the way for the development of a new class of proteasome inhibitors. Moreover, a small molecule b-AP15 was also identified during screening for inhibitors that induces the lysosomal apoptosis pathway. *In vitro* experiments with various tumour cell lines have shown that b-AP15 induced gene expression signatures that closely matched those of cells treated with well-characterized proteasome inhibitors. Cells treated with b-AP15 rapidly accumulated polyubiquitylated proteins, with similar kinetics to cells treated with bortezomib. However, polyubiquitylated proteins showed higher molecular masses and b-AP15 treatment also led to an upregulation of apoptotic markers and the accumulation of cell-cycle regulatory proteins, leading to a G2/M phase cell cycle arrest. Decreased cell viability was observed at a concentration that induces polyubiquitin accumulation, indicating a link between proteasome inhibition and cytotoxicity.

Upon further investigation on the mode of proteasomal inhibition, D' Acry et al. (2011) observed and claimed that b-AP15 acts as a reversible inhibitor of the deubiquitylases ubiquitin thioesterase L5 (UCHL5) and ubiquitin-specific-processing protease 14 (USP14), which form part of the 19S regulatory subunit of the 26S proteasome. Interestingly, the inhibition of the proteasome via the 19S subunit can induce apoptosis regardless of mutations or deletions in the tumour suppressor p53 or amplifications in the *BCL2* oncogene, which are involved in resistance mechanisms to bortezomib. Testing b-AP15 in the NCI60 panel of cancer cell lines have also been demonstrated to have a different therapeutic range compared with bortezomib with regard to tumor type, with the highest sensitivity observed in colon carcinoma and central nervous system tumors (Table 1).

Later on it was shown and demonstrated that daily subcutaneous injections of b-AP15 significantly slowed tumors growth in severe combined immunodeficient mice with human squamous carcinoma xenografts. Mice colon carcinoma xenograft overexpressing BCL-2 when treated with b-AP15 led to significantly delayed tumor onset, with two of six mice completely disease-free at the end of the study period compared with none in the control group. In another instance an aggressive model of acute myeloid leukaemia showed tumour regression with the period of one week comparatively. These findings show that the deubiquitylating activity of the 19S regulatory particle is a promising target for cancer treatment. As the cellular response to inhibition of proteasomal deubiquitylases is distinct from inhibitors of the catalytic core of the proteasome, 19S inhibitors might also expand the range of cancers that could be treated with proteasome-targeted drugs.

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