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Review

The proteasome: A worthwhile target for the treatment of solid tumours?

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ABSTRACT

Proteasomes have a fundamental function since they degrade numerous different proteins, including those involved in the regulation of the cell cycle. Proteasome inhibition is a novel approach to the treatment of solid tumours. PS-341 (bortezomib) is a small, cell-permeable molecule that selectively inhibits the proteasome binding it in a reversible manner. The proteasome has been established as an important target in haematologic malignancies and has been approved for the treatment of multiple myeloma. Bortezomib induces apoptosis of malignant cells through the inhibition of NF-κB and stabilisation of proapoptotic proteins. In preclinical studies, bortezomib also promoted chemo and radiosensitisation of malignant cells *in vitro* and inhibited tumour growth in murine xenografts models. The single-agent and combination studies of bortezomib in solid tumours are detailed.

1. Introduction

The balance between protein synthesis and degradation is essential for normal cellular functioning. The proteasome is a multicatalytic enzyme complex that degrades several intracellular proteins by a targeted and controlled mechanism.[1,2] The ubiquitin-proteasome pathway degrades intracellular proteins which mediate various cellular functions such as transcription, stress response, cell cycle regulation, oncogenesis, ribosome biogenesis, cellular differentiation, and DNA repair.[3] The capacity of proteasome for degradation of tumour-suppressing and proapoptotic protein targets known to be dysregulated in many human malignancies provides the rationale for its selection as a target for cancer therapy. Moreover, preclinical studies have shown that proteasome inhibition decreases proliferation, induces apoptosis, enhances the activity of chemotherapy and radiation, and reverses chemoresistance in a variety of haematologic and solid malignancy models *in vitro* and *in vivo*.

PS-341 (bortezomib) is the first proteasome inhibitor investigated in clinical trials. It is approved in the United States and Europe for treating multiple myeloma patients who have received at least one prior therapy. Two phase II trials have shown that treatment with bortezomib, alone or in combination with dexamethasone, produced durable responses with meaningful survival benefits in patients with recurrent and/

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or refractory multiple myeloma.[4,5] In the APEX phase III study, comparing bortezomib and dexamethasone in patients with multiple myeloma who had had a relapse after one to three previous therapies, the proteasome inhibitor yielded a rate of 6% complete and 32% partial responses versus 1% and 17%, respectively, for dexamethasone. The median time to progression was significantly increased from 106 days with dexamethasone to 189 days with bortezomib and the 1-year overall survival was also higher in the bortezomib arm (80% versus 66%).[6]

Bortezomib has also shown activity in preclinical studies of a variety of solid tumours, such as breast, gastric, colon, non-small lung cancer (NSCLC), pancreas, and this has prompted several phase I/II clinical studies. Moreover, additional understanding of the mechanisms of action of proteasome inhibitors has led to their incorporation into combination regimens with both standard chemotherapeutics and novel agents. Taken together, these studies demonstrate the power of rational drug design and development to provide novel effective therapies for patients with haematological and solid malignancies.

In this paper we mainly focus on the way the proteasome works, and on the anticancer effects of bortezomib, with particular emphasis on preclinical and clinical studies in solid tumours.

2. Mechanism of action of proteasome

The ubiquitin-mediated proteasome pathway regulates a group of intracellular proteins that govern cell cycle, tumour growth, and survival (Fig. 1). This pathway is the principal mechanism of degradation for short-lived cellular regulatory proteins, including p53, cyclins and the cyclin-dependent kinase (CDK) inhibitors p21 and p27, the oestrogen receptor, and the inhibitor (IκB) of nuclear transcription factor kappa B (NF-κB).[7–11] 26S proteasome consists of a multisubunit, cylindrical complex including a 20S core catalytic component and 19S regulatory particles that contain polyubiquitin-binding sites and isopeptidase activity for the cleavage and release of ubiquitin from the protein substrate.[12] The proteasome requires adenosine triphosphate (ATP) hydrolysis and regulates multicatalytic protease that selectively degrades polyubiquinated proteins. These proteins are degraded by a multistep process that involves specific protein ligases. The first step includes protein mark with a chain of small polypeptides named ubiquitin; ubiquitin-activating enzyme (E1) activates ubiquitin molecule to the protein and, consequently, a long polypeptide chain of ubiquitin moieties is formed; finally, the multi-enzyme proteolytic complex 26S proteasome degrades protein into small fragments in an ATP-dependent manner.[3,13] In particular, the proteasome degrades a wide range of protein substrates involved in cell cycle regulation, apoptosis and other cellular functions. Controlled transitions between cell cycle stage depend on the timely activation of cyclins and CDK complexes. CDKs are serine/threonine kinases that are activated upon association with regulatory cyclin subunits at specific phases during cell-cycle progression. Expression of specific cyclins is regulated differentially by proteasome degradation during each phase of the cell cycle. In addition, the activity of CDKs is regulated further by a variety of inhibitor factors, such as $p21^{Cip1}$ $p27^{Kip1}$, that are able to prevent the formation of a variety of CDK-cyclin complexes and to arrest cell-cycle progression; both $p21^{Cip1}$ and $p27^{Kip1}$ are also proteasomal substrates.[14] The tumour suppressor protein p53 is another important substrate for proteasomal degradation. Activated p53 arrests cells in the G1-phase and promotes apoptosis to allow elimination of damaged cells through induction of the proapoptotic protein Bax, which, in turn, is also a proteasomal substrate. Taken together, these findings suggest that proteasome inhibition results in the stabilisation of p53, $p21^{Cip1}$, $p27^{Kip1}$ and Bax, dysregulation of cell-cycle progression and, finally, apoptosis.[15]

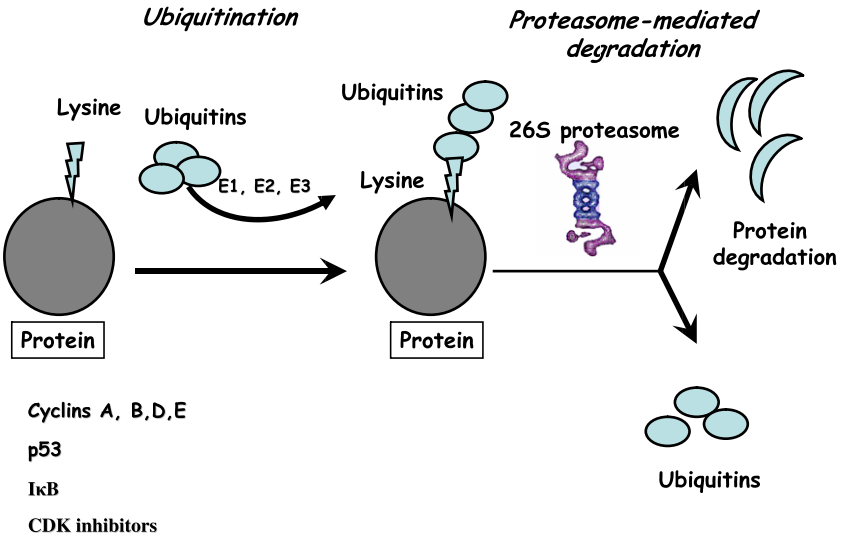

Fig. 1 – The ubiquitin-proteasome pathway is shown. On the left, the ubiquitination mechanism is explained: polyubiquitinated tails are added to specific lysine moieties on the protein. On the right, the proteasome-mediated degradation is shown: ubiquitinated proteins are degraded by the 26S proteasome. I-κB: nuclear factor-kappa B inhibitor; CDK: cyclin-dependant kinase; E1: ubiquitin-activating enzyme; E2: ubiquitin-conjugating enzyme; E3: ubiquitin ligase.