Proteasome Subunit Beta Type 1 P11A Polymorphism Is a New Prognostic Marker in Multiple Myeloma

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Abstract

We retrospectively analyzed the prognostic impact of proteasome subunit beta type 1 rs12717 polymorphism in 211 consecutively diagnosed multiple myeloma cases. Patients carrying the variant G allele showed significantly shorter progression-free survival. In proteasomes of individuals with G/G genotype, we found significantly reduced protease activity and lower inhibitory capacity of bortezomib on the caspase- and trypsin-like activity.

Background: Proteasome subunit beta type 1 (*PSMB1*) rs12717 polymorphism, a single nucleotide polymorphism with unknown functional effect, was recently reported to influence response to bortezomib-based therapy in follicular lymphoma. **Patients and Methods:** We retrospectively analyzed the prognostic impact of this polymorphism in 211 consecutively diagnosed multiple myeloma cases, and performed in vitro experiments to look into its functional consequences. **Results:** On univariate analysis, patients carrying the variant G allele showed significantly shorter progression-free survival (PFS) with a pattern suggestive of a gene-dose effect (PFS 26.4, 22.3, and 16.4 months in C/C, C/G, and G/G patients, respectively, $P = .002$). On multivariate analysis, carrying the G/G genotype was a significant independent risk factor for relapse (hazard ratio [HR] 2.29, $P < .001$) with a similar trend in C/G carriers (HR 1.33, $P = .097$) when compared with the major allele carrier C/C cohort. Our subsequent in vitro analyses demonstrated significantly reduced protease activity in proteasomes of individuals with G/G genotype compared with that of C/C carriers, despite that PSMB1 expression and proteasome assembly remained unaltered. Bortezomib exhibited a lower inhibitory capacity on the caspase- and trypsin-like activity of proteasomes from G/G individuals. **Conclusion:** Our results show that carriership of *PSMB1* rs12717 minor allele is predictive for suboptimal response with bortezomib treatment, which could be explained by less active proteasomes that are less sensitive to bortezomib, and myeloma cells consequently relying on other escape mechanisms to cope with the abundance of misfolded proteins.

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Introduction

Proteasome inhibitors (PIs) have a fundamental role in the treatment of multiple myeloma (MM). Bortezomib, the first in class PI received accelerated approval from the U.S. Food and Drug Administration (FDA) in relapsed refractory MM in 2003, and entered into phase 3 trials in first-line setting.[1-3] Bortezomib-based combinations are currently standard upfront treatments for MM in most countries, and second-generation PIs are increasingly used following the FDA and subsequent European Medicines Agency approval of carfilzomib and ixazomib.

Proteasomes are multienzyme complexes providing a pathway for the degradation of poly-ubiquitinated intracellular proteins, thereby

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eliminating misfolded or unfolded proteins, as well as key regulators of important cellular processes, including cell-cycle progression, DNA repair, apoptosis, immune response, signal transduction, transcription, metabolism, and developmental programs.[4,5] The 26S proteasome, a large 2.4-MDa ATP-dependent proteolytic complex located in both the cytoplasm and the nucleus, consists of a 20S core catalytic cylindrical complex capped at both ends by 19S regulatory subunits.[6] The core structure is composed of 4 axially stacked rings of nonidentical subunits: 2 alpha rings each composed of 7 nonidentical alpha subunits (α1-7, encoded by *PSMA1-7*) and 2 beta rings each formed by 7 nonidentical beta subunits (β1-7, encoded by *PSMB1-7*).[7] Three β subunits (β5, β2, and β1) are located on the inner surface of the proteolytic chamber and are responsible for chymotrypsin-like, trypsin-like, and post-glutamyl peptide hydrolyzing (caspase-like) activities, respectively.[8] Other β subunits are essential for the formation and stability of the proteolytic environment on the inner surface of the heptameric rings.[4] The exact role of these subunits, however, is yet to be characterized. It has also been reported that they have potential roles in regulating the assembly and stability of the ring, and in addition *PSMB1* (coding for β6 subunit) has a potential role as a transcriptional activator.[9,10]

High proteasome activity has been reported in different malignancies.[11] In malignant plasma cells that exhibit extremely active protein synthesis, proteasomes along with chaperones, such as heat shock proteins, play a critical role in cellular homeostasis. This might explain the therapeutic window of PI treatment in MM and the additional effect of heat shock protein inhibitors combined with PIs.[12] Bortezomib binds to the β5 subunit, but also interacts with other subunits of proteasome, such as the β1 subunit.[13] Some of the new-generation PIs, such as marizomib, inhibit all 3 proteolytic activities. Although *PSMB5* variants have been identified previously in preclinical models of bortezomib resistance, they were not detected in relapsed/refractory patients' tumor samples, suggesting alternative mechanisms behind bortezomib resistance.[14]

Proteasome subunit beta type 1 (*PSMB1*) rs12717 single nucleotide polymorphism (c.31C>G substitution resulting in p.Pro11Ala amino acid change) was recently reported to be associated with greater clinical benefit in patients with relapsed follicular lymphoma treated with a bortezomib-containing combination.[15] Our hypothesis was that *PSMB1* P11A polymorphism could affect the function of proteasomes and therefore have an impact on the prognosis of myeloma, especially in patients treated with PI-based regimens. To the best of our knowledge, the functional consequence of *PSMB1* P11A polymorphism has not been tested in MM, apart from a single study with a limited number of patients showing no statistically significant association between genotype and survival.[14]

Patients and Methods

Patients, Clinical Data, Treatment, and Response Criteria

We analyzed the association of *PSMB1* P11A polymorphism and treatment outcome of 211 patients consecutively diagnosed with myeloma having had first-line chemotherapy at the St László Hospital, Budapest, Hungary, between January 2007 and November 2013. International scoring system (ISS) and fluorescence in situ hybridization (FISH) status were established at diagnosis; FISH testing was performed on bone marrow slides using probes for chromosome 13q and 17p deletion, translocation (11;14), (4;14), (14;16), and 1q amplification. FISH results were available in 194 of the 211 patients. For the purpose of this study, patients with t(4;14), t(14;16), 1q amplification, and del(17p) were grouped together as high risk. Previous studies have shown that del(13q) in patients lacking t(4;14) and del(17p) was no longer of prognostic significance.[16,17]

The treatment decision was the discretion of the treating physician; treatment continued until best response and then the patients who were eligible for transplantation received a high-dose cyclophosphamide-primed stem cell mobilization, followed by high-dose melphalan-conditioned autologous stem cell transplantation (ASCT). Response (complete response [CR], very good partial response [VGPR], partial response [PR], no response [NR], and progressive disease [PD]), survival measures (progression-free survival [PFS] and overall survival [OS]), and relapse criteria were defined according to published guidelines.[18] Response was formally assessed at the end of the treatment, or following transplantation in the case of patients receiving ASCT. During their follow-up, patients were reviewed regularly every 2 to 3 months until disease progression or death. The median follow-up was 40 months.

The study was approved by the Hungarian National Ethics Committee, and participants signed informed consents.

Genotyping

Genomic DNA was isolated from bone marrow or peripheral blood. *PSMB1* P11A polymorphism was tested using LightCycler 480II (Roche Diagnostics, Basel, Switzerland) melting curve analysis. Amplification primers (PSMB1-LCF: 5′-GTG AGA CAG CAA GTG TCG-3′ and PSMB1-LCR: 5′-GTG ACT CCT AAA TAG GCT TCA G-3′) and hybridization probes (PSMB1-SENS: 5′-GGC TCC TGG CAG AGA CTT GG-Fluorescein and PSMB1-ANC: 5′-Cy5-ATG GAA CCG CAC AGA GCC G-Phosphate) were designed by LightCycler Probe Design software (Roche Diagnostics). Asymmetric polymerase chain reaction with shifted forward (0.15 μM) and reverse primer (0.5 μM) concentrations was performed with the addition of 25 ng genomic DNA, and labeled oligonucleotides (0.25 μM each) with MyTaq Mix (Bio-25042; Bioline USA, Taunton, MA) according to the manufacturers' instructions. Cycling conditions were as follows: initial denaturation at 95C for 3 minutes, 70 cycles of 95°C denaturation, 50°C annealing, and 72°C extension, melting curve analysis from 40°C to 80°C.

Flow Cytometry Analysis of Proteasomes and PSMB1 Expression

For in vitro characterization of proteasome expression and function, healthy volunteers previously genotyped as either *PSMB1* major variant C/C or homozygous G/G minor variant (3 men and 4 women, mean age: 42 ± 8 years) were investigated. EDTA anticoagulated whole blood samples were fixed with 1% paraformaldehyde (37°C, 5 minutes), spun down (500*g*, 5 minutes), washed with phosphate-buffered saline (PBS), and sonicated (10 seconds, 37 kHz, 50/100W). The cells were resuspended in PBS and labeled with rabbit polyclonal anti-PSMB1 (1:80; Biomol, Hamburg, Germany) or anti-proteasome 20S alpha + beta (1:100; Abcam, Cambridge, UK) antibodies along with cell type-specific markers, such as phycoerythrin (PE)-conjugated anti-CD3 (1:200),

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