Brief report

Sequence analysis of β-subunit genes of the 20S proteasome in patients with relapsed multiple myeloma treated with bortezomib or dexamethasone

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Variations within proteasome β (PSMB) genes, which encode the β subunits of the 20S proteasome, may affect proteasome function, assembly, and/or binding of proteasome inhibitors. To investigate the potential association between PSMB gene variants and treatment-emergent resistance to bortezomib and/or long-term outcomes, in the present study, PSMB gene sequence variation was characterized in tumor DNA samples from patients who participated in the phase 3 Assessment of Proteasome Inhibition for Extending Remissions (APEX) study of bortezomib versus high-dose dexamethasone for treatment of relapsed multiple myeloma. Twelve new PSMB variants were identified. No associations were found between PSMB single nucleotide polymorphism genotype frequency and clinical response to bortezomib or dexamethasone treatment or between PSMB single nucleotide polymorphism allelic frequency and pooled overall survival or time to progression. Although specific PSMB5 variants have been identified previously in preclinical models of bortezomib resistance, these variants were not detected in patient tumor samples collected after clinical relapse from bortezomib, which suggests that alternative mechanisms underlie bortezomib insensitivity. This study is registered at www.clinicaltrials.gov as NCT00048230. (Blood. 2012;120(23):4513-4516)

Introduction

The 20S core of the 26S proteasome degrades polyubiquitinated intracellular proteins and is composed of 4 stacked rings, each with 7 α and 7 β subunits. Three constitutive proteasome β (PSMB) subunits, β5, β2, and β1 (encoded by the PSMB5, PSMB7, and PSMB6 genes, respectively), are responsible for chymotryptic catalytic site, is in close proximity to the β1 subunit and, when bound to the β5 subunit in the chymotryptic catalytic site, is in close proximity to the β2 subunit. Several studies have shown that PSMB5 variants can arise in vitro when tumor cell lines are cultured with bortezomib; it remains unclear whether this mechanism is relevant for bortezomib resistance in the clinical setting.

The present study addressed whether variations in PSMB genes affect treatment-emergent resistance to bortezomib-treated MM patients or long-term outcome in MM patients. Sequence variation was characterized in coding regions of PSMB genes in pre- and posttreatment samples from patients who participated in the phase 3 Assessment of Proteasome Inhibition for Extending Remissions (APEX) trial of single-agent bortezomib versus high-dose dexamethasone (Dex) for the treatment of relapsed MM.

Study design

Review boards at all participating institutions approved the (APEX) study, and BM aspirates were obtained from consenting patients in accordance with the Declaration of Helsinki during the APEX trial. Tumor cells were purified and frozen for nucleic acid isolation as described previously. Matching germline DNA samples were not collected. DNA samples were amplified using the QIAGEN REPLI-g whole genome amplification kit and the online version of this article contains a data supplement. The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

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population frequency data (European, whenever available) from the National Center for Biotechnology Information (NCBI) SNP database.

Results and discussion

SNP frequency analysis

A total of 76 DNA samples were of adequate yield and quality for sequencing, including 47 (25 bortezomib-treated and 22 Dex-treated) pretreatment samples and 29 (16 bortezomib-treated and 13 Dex-treated) posttreatment samples. Paired pre- and posttreatment samples were available from 6 patients (3 bortezomib-treated and 3 Dex-treated). The dataset size limits formal statistical analyses of SNP associations with MM incidence or baseline characteristics; however, it is unique with respect to data regarding sensitivity to single-agent bortezomib and sampling before and after therapy. Allelic and genotype frequency of nonsynonymous SNPs in pre- and posttreatment MM samples did not differ significantly from population frequency data in dbSNP (Table 1 and supplemental Table 2), suggesting that nonsynonymous variants in PSMB are not specifically selected in MM. No unique nonsynonymous substitutions were observed in posttreatment samples. Further, recurrent variants (eg, in PSMB5) occurred at similar frequencies in pre- and posttreatment samples, suggesting that PSMB variation does not arise from selective pressure exerted during treatment and that these variants are more likely to represent naturally occurring germline SNPs. Supporting this, 3 SNPs detected in this study were also detected in a previous study of gene variants in MM, and 2 (rs2304974, rs2304975) were detected at similar frequencies in pre- and posttreatment MM samples. Further, recurrent variants (eg, in PSMB5) occurred at similar frequencies in pre- and posttreatment samples, suggesting that PSMB variation does not arise from selective pressure exerted during treatment and that these variants are more likely to represent naturally occurring germline SNPs. Supporting this, 3 SNPs detected in this study were also detected in a previous study of gene variants in MM, and 2 (rs2304974, rs2304975) were detected at similar frequencies in both studies.

Novel variant identification

Twelve novel, low-frequency PSMB variants were identified that were not listed in dbSNP (Table 2); 10 were nonsynonymous variants. Two variants were located in PSMB5; one, a C/G substitution resulting in a S112R change in PSMB5 approximately.
7 angstroms away from the bortezomib binding pocket, was observed in 1 pretreatment sample from a patient who achieved a partial response (PR) to bortezomib.

The PSMB5 A108T variant, which has been linked to bortezomib resistance in vitro,13,14,17,18 was not observed in any pre- or posttreatment samples. A previous study reported the absence of this variant in the germline DNA of MM patients, but tumor DNA was not characterized either before or after bortezomib therapy.23 In the present study, tumor samples collected after bortezomib treatment were from 10 patients who were relatively insensitive to bortezomib monotherapy (best response of minimal response, stable disease, or progressive disease) and from 6 patients who achieved a confirmed PR to bortezomib and subsequently relapsed on study before sample collection (supplemental Figure 1). In these cases, treatment-emergent resistance to single-agent bortezomib was independent of variants in the proteasome genes PSMB1, PSMB5, PSMB6, PSMB8, PSMB9, and PSMB10.

The most common new variant detected, a C/A substitution resulting in a Q49K change in PSMB8, was observed in 10 pretreatment and 3 posttreatment samples; 2 other PSMB8 variants were found, 1 each in 1 pre- and 1 posttreatment sample, and 9 other variants (in PSMB1, PSMB5, PSMB6, PSMB8, PSMB9, and PSMB10) were detected in 1 sample each. These variants may be rare SNPs or somatic changes that occurred in the context of MM.

Correlation with clinical outcomes

There were no associations between PSMB SNP genotype frequencies in a pooled dataset of pre- and posttreatment samples and subsequent patient response to bortezomib or Dex treatment (supplemental Table 3). However, 3 significant associations were identified between SNP allelic frequencies and OS or TTP (SNPs PSMB6 rs2304975, PSMB6 rs3169950, and PSMB9 rs241419; supplemental Table 4). As a caveat, 2 SNPs, PSMB9 rs241419 and PSMB6 rs2304975, were of low frequency, being found in only 3 and 5 patients, respectively. For a third SNP (PSMB6 rs3169950), patients with the A allele appeared to have shorter OS than those with the G allele, but not shorter TTP, as may have been expected. Acknowledging the limited dataset size, these findings require confirmation in larger populations.

The PSMB1 SNP rs12717 (C/G substitution resulting in a P11A change), which was reported recently to be associated with a relative progression-free survival benefit in relapsed follicular lymphoma patients treated with bortezomib-rituximab versus rituximab,24 was not associated with OS or TTP in this pooled APEX dataset. There was no statistically significant difference in TTP for rs12717 C/G heterozygotes with bortezomib (n = 12) versus Dex treatment (n = 11; P = .077). Because of the limited size of the current dataset, additional studies are required to further evaluate the PSMB1 rs12717 SNP in MM to enable analyses of SNP combinations that may contribute to drug sensitivity.

In summary, in the present study, no unique PSMB5 variants were detected in patient tumor samples collected after bortezomib treatment, including specimens from patients who were initially sensitive to bortezomib (confirmed PR) and then relapsed after prolonged therapy. These results suggest that the bortezomib insensitivity that develops in some initially responsive MM patients is not because of variants in PSMB5 or other catalytic proteasome subunits that form the bortezomib-binding pocket, and also indicate that alternative mechanisms underlie treatment-emergent bortezomib resistance in the clinical setting.

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Authorship


D.-L.E. has ownership interests in Millennium/Takeda and Johnson & Johnson. P.G.R. has served on advisory boards for Millennium Pharmaceuticals, Celgene, Novartis, Bristol-Myers

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Squibb, and Janssen. J.C. and J.B. have received honoraria from Janssen and Celgene. J.B. has served on advisory boards for Janssen and Celgene and has received research funding from Celgene. T.F. has served on advisory boards/speakers’ bureaus for Janssen. R.N. has served as a consultant and on advisory boards/speakers’ bureaus for and has received research funding from Millennium Pharmaceuticals, Celgene, and Onyx. M.A. has served on advisory boards/speakers’ bureaus and as a consultant for Celgene, Millennium Pharmaceuticals, and Ortho Biotech Products and has received research support from Celgene and Millennium Pharmaceuticals. P.S. has served on advisory boards for Millennium Pharmaceuticals, Janssen, Celgene, Novartis, and Onyx and has received research support from Janssen and Celgene. S.L. has served as a consultant for Bristol-Myers Squibb, Celgene, Merck, Millennium Pharmaceuticals, Novartis, and Onyx. H.v.d.V. and D.R. are employees of and have ownership interest in Janssen Research & Development. K.C.A. has served on advisory boards for and has received research funding from Bristol-Myers Squibb, Celgene, Merck, Millennium Pharmaceuticals, Novartis, and Onyx and has ownership interest in Acetylon. W.D. declares no competing financial interests.

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References


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