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Rapid Communication

Parathyroid hormone receptor mediates the anti-myeloma effect of proteasome inhibitors

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ABSTRACT

Clinically significant serum parathyroid hormone (PTH) variations have been reported in multiple myeloma (MM) patients treated with proteasome inhibitors. To elucidate the association between serum PTH variations and proteasome inhibition in MM, the effect of PTH and PTHR1 ligands on the proteasome inhibitors bortezomib and carfilzomib *in vitro* and *in vivo* was determined. The MM cell lines ARP1, OC1 and 5TGM1 expressed mRNA and protein encoding PTH receptor 1 (PTHR1). Treatment of 5TGM1 cells with either PTH(1–34), bortezomib or carfilzomib alone dose-dependently inhibited 5TGM1 cell proliferation. However, treatment with the potent PTHR1 antagonist [TYR34]PTH(7–34) (PTH(7–34)) had no significant effect on myeloma cell proliferation and cell viability. In contrast, when used in combination with bortezomib or carfilzomib, PTH(7–34) treatment significantly reduced the bortezomib or carfilzomib-associated decrease in cell proliferation. Treatment of the C57BL/KaLwRij mouse myeloma model with either bortezomib or carfilzomib provided a significantly prolonged survival benefit compared to controls ($p = 0.04$; $p = 0.01$ respectfully). This potent anti-myeloma effect was completely abrogated by concomitant treatment with PTH(7–34). These results suggest an important role of the PTHR1 in the anti-myeloma effect of proteosome inhibition.

Introduction

The pathogenesis of disease progression in multiple myeloma (MM) is a complex phenomenon and numerous processes and pathways have been implicated in the regulation of osteoblast-induced bone formation and bone resorption by osteoclasts [1,2]. In fact, bone disease is one of the most debilitating manifestations of the disease. The ubiquitin proteasome pathway is an essential cellular degradative system that has been shown to play an important regulatory role in multiple myeloma [3]. A number of clinical studies have demonstrated that proteasome inhibition increases osteoblast precursor differentiation through interference with canonical Wnt signaling and increased DKK1 [4–6]. The effect of proteosome inhibition on MM bone disease is believed to be direct and not merely a consequence of the anti-myeloma properties [7]. In addition, retrospective and prospective clinical studies examining variations in serum bone alkaline phosphatase in response to proteasome inhibition with bortezomib in myeloma patients have demonstrated a close correlation between drug activity and increased bone anabolic activity [8,9]. As such, proteosome inhibitor treatment is an attractive therapeutic option that may combine potent anti-myeloma activity with perceived beneficial effects on the skeleton.

Parathyroid hormone (PTH) is an 84 amino acid peptide synthesized by the parathyroid glands that is stored in secretory vesicles and dense core granules [10]. Extracellular calcium levels sensed by the calcium receptor on parathyroid chief cells regulate PTH release, thereby controlling serum calcium levels systemically [10]. The clinical use of intermittent PTH(1–34) therapy in the postmenopausal osteoporosis setting elicits a rapid increase in bone-formation markers, followed by increases in bone-resorption markers [11]. PTH(1–34) is able to reverse the structural damage seen in postmenopausal osteoporosis and restores the structure and strength of trabecular bone [12]. In myeloma patients, during the first bortezomib treatment cycle, serial serum PTH measurements demonstrated a significant difference in the first treatment cycle in responders *versus* non-responders patients [13]. The cyclic PTH variations associated with the potent anti-myeloma effect of proteosome

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inhibition suggested an unknown mechanism of action, therefore, we tested the role of the PTH axis in MM cell lines and in a myeloma mouse model during proteasome inhibitor treatment.

Materials and methods

Tissue culture, peptides and proteosome inhibitors

All tissue culture and laboratory plasticware were purchased from (Fisher Scientific Inc.), and medium was purchased from (Invitrogen NY, USA). The human PTH(1–34) and the antagonist [TYR^{34}] PTH(7–34) (PTH(7–34)) were purchased (Bachem Pharmaceutical Company, Torrance, CA). The proteosome inhibitors bortezomib and carfilzomib were provided by the manufacturers (Millennium The TAKEDA Oncology Company, Cambridge MA; Onyx Pharmaceutical, San Francisco, CA). 5TGM1, ARP1 and OC1 myeloma cells were grown in Hyclone classical liquid media RPMI 1640 (Thermo Scientific Hyclone, Logan, UT) supplemented with heat-inactivated fetal bovine serum (Thermo Scientific Hyclone, Logan, UT) and 1× penicillin–streptomycin solution (GIBCO, Grand Island, NY) at 37 °C in 5% CO_2 atmosphere.

Evaluation of PTHR1 expression

Briefly ARP1, OC1 and 5TGM1 cell lysates were prepared as previously described [14]. Samples (30 μg/lane) were then subjected to 8% SDS-PAGE and immunoblotted onto nitrocellulose. Blots were incubated with 0.1 mg/ml anti-PTHR1 antibody (5G1.3) specific for PTHR1 [14]. Bound primary antibody was detected by incubation with fluorescently-labeled secondary antibody (goat-anti mouse) in Odyssey Blocking Buffer 1:40,000 dilution in 1/10 in 5% Evaporated milk (stock is 20%) and PBS followed by Odyssey infrared detection (Li-Cor Biosciences, Lincoln, NE USA).

5TGM1 Cell-based experiments

5TGM1 myeloma cells were plated in 24 well plates at 10^5 cells per milliliter in RPMI-1640 then supplemented with 2% FBS in the presence or absence of bortezomib, PTH(1–34) or PTH(7–34). Cell number and viability were measured by Trypan Blue exclusion at various time intervals every day (from day 0 to 5) and the total number of cells and the number of Trypan blue-positive cells counted using a Neubauer chamber under bright field illumination.

The 5TGM1 cell line was exposed to bortezomib, carfilzomib, PTH(1–34) and PTH(7–34) at various concentrations and combinations. Bortezomib was added as single agent at 5 nM and 10 nM to the medium of 5TGM1 cells and replenished every other day when the medium was changed. Carfilzomib was tested at 2.5 nM and 5 nM concentration following the same treatment regimen. PTH(1–34) was added at 10 nM, 50 nM and 100 nM concentrations daily from day 0. The PTHR1 antagonist PTH(7–34) was added at 100 nM, 500 nM and 1 μM on days −3 to −1 before the addition of each proteasome inhibitor. Bortezomib and carfilzomib were tested (at the above concentrations) in combination with PTH(1–34) and with PTH(7–34).

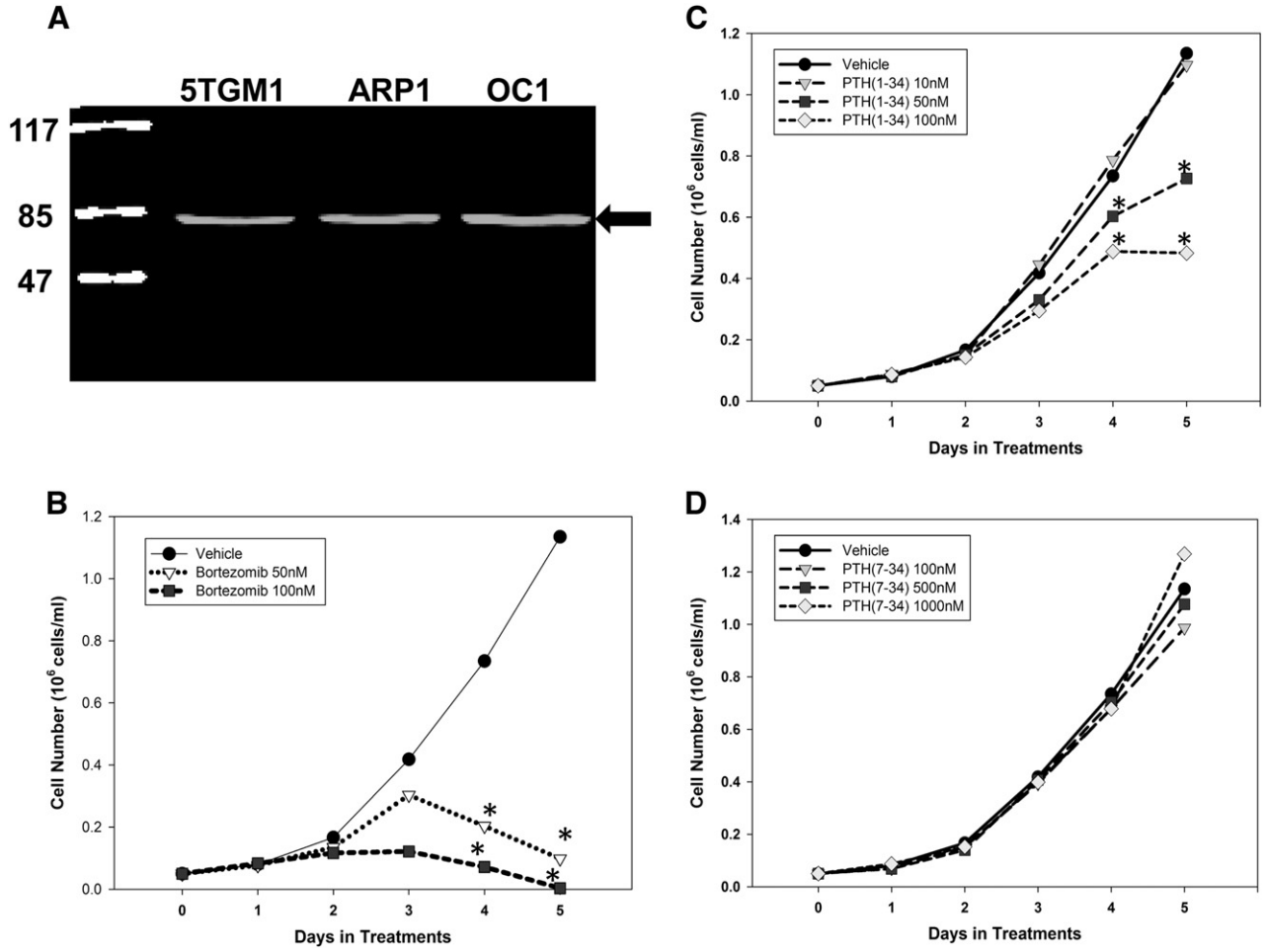

Fig. 1. PTHR1 expression and effect of PTH and proteasome inhibitors on myeloma cell proliferation (A) Western Blot Analysis for PTHR1 expression in ARP1, OC1 and 5TGM1 myeloma cell lines. Arrow shows the position of PTHR1 at ~80 kDa. (B) Concentration dependent inhibitory effect of bortezomib (50 nM and 100 nM) on the 5TGM1 cell proliferation. (C) Concentration dependent inhibitory effect of PTH (10, 50, 100 nM) on 5TGM1 cell. (D) No significant effect of PTH(7–34) (100 nM, 500 nM, 1 μM) on 5TGM1 cell proliferation. (* denotes $p < 0.05$).