PTPROt Enhances the Sensitivity of Multiple Myeloma to BP-1-102 and Bortezomib

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Abstract

Background

Multiple myeloma (MM) is an incurable plasma cell malignancy for which novel treatment options are urgently required. STAT3 overexpression is associated with poor prognosis of MM and could enhance chemotherapy resistance of MM cells. Therefore, targeting the expression and/or activity of STAT3 may represent a potential treatment strategy for MM.

Methods and results

This study aimed to examine the effects of protein tyrosine phosphatase receptor type O (PTPROt) overexpression and BP-1-102, an inhibitor of STAT3, on MM cell growth and survival. The results showed that PTPROt overexpression suppressed MM cell progression by inhibiting STAT3 phosphorylation, and that BP-1-102 inhibited the proliferation, migration, and invasion of MM cell lines in a dose-dependent manner. We also found that BP-1-102 hampered STAT3 phosphorylation and induced apoptosis in vitro, and inhibited the growth of H929 and MM1S xenografts in vivo. Furthermore, the combination of BP-1-102 and bortezomib (BTZ) was more efficacious at killing MM cells than BP-1-102 or BTZ alone in vitro and in vivo. Notably, PTPROt also enhanced the sensitivities of multiple myeloma cell lines, primary myeloma cells, and H929-PTPROt xenograft tumors to BP-1-102 and BTZ treatments.

Conclusions

The findings of the current study strongly indicated that STAT3 could be a promising therapeutic target for the treatment of MM.

Introduction

Multiple myeloma (MM) is a clonal malignant disease characterized by abnormal plasma cell proliferation, and the second most common hematologic malignancy (1). Although the curative effects of proteasome inhibitors, such as bortezomib (BTZ), have demonstrated considerable progress, MM is still characterized by a high relapse rate in the terminal stage, with gradual acquisition of resistance to existing treatment options (2–4). With improved treatment options and combined myeloablative chemotherapy and autologous stem cell transplantation, the median survival time now exceeds 6 years. Despite drastic changes in treatment strategy, myeloma remains largely incurable (5). Therefore, effective therapies that either cure MM or provide persistent disease control with symptomatic relief are urgently needed.

The mammalian Signal Transducer and Activator of Transcription (STAT) family mediates various intracellular signaling pathways (6). STAT3 regulates cellular immunity as well as cell proliferation, apoptosis and differentiation (7–8). Persistent STAT3 hyperactivation (predominantly by phosphorylation) is a common event in MM cells. The activation of this protein has attracted increasing
attention in MM research regardless of whether it directly leads to the occurrence of MM or is a physiological phenomenon without any biological significance (9). Therefore, this finding strongly indicated that STAT3 activation was closely linked to the occurrence of MM, and that STAT3 may be a primary therapeutic target for MM treatment.

Protein tyrosine phosphatase receptor type O (PTPROt) is a transmembrane protein that is mainly expressed in B lymphocytes and macrophages (10). PTPROt affects the occurrence and progression of liver cancer by regulating STAT3 phosphorylation (11). However, it remains unclear whether PTPROt affects MM cell proliferation, differentiation, apoptosis, and chemotherapy resistance by regulating the STAT3 pathway. Prior evidence showed that STAT3 may be involved in the development of resistance to conventional chemotherapy drugs (12–13); therefore, blocking STAT3 hyperactivation may reverse drug resistance during chemotherapy. BP-1-102, an inhibitor of STAT3, has been evaluated for antitumor effects in humans in vitro and in vivo (14–16); however, its role in MM has not been reported. Here, we show that PTPROt enhances the sensitivity of MM to BP-1-102, alone or in combination with bortezomib, by downregulating STAT3 activity.

Methods

Reagents and antibodies

BP-1-102 and BTZ were obtained from Selleck Chemical and stored at −20°C (Selleckchem, Houston, TX). STAT3 (1:1000), phosphorylated STAT3 (p-STAT3) (Y705; 1:1000), C-MYC (1:1000), BCL-2 (1:1000), cleaved caspase3 (1:1000), cleaved caspase8 (1:1000), cleaved caspase9 (1:1000), and MCL-1 (1:1000) were purchased from Cell Signaling Technology (Cell Signaling Technology, Inc., Danvers, MA). β-Actin (1:1000) was purchased from ProteinTech Group, Inc. Rabbit IgG HRP-conjugated antibody (1:1000) was purchased from Solarbio (China). Goat anti-mouse IgG/HRP (1:5000) was purchased from R&D Systems (Minneapolis, MN).

Cell culture

U266, H929, and MM1S cell lines were cultured with RPMI-1640 medium with 10% fetal bovine serum and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, MA) in an incubator at 37°C in a 5% CO₂ environment.

Cell viability assay

The cell lines and primary MM cells (1×10⁴ cells) were seeded in 96-well plates and exposed to different concentrations of BP-1-102 (2, 4, 6, and 10 µM) or BTZ (4 or 5nM). BP-1-102 and BTZ were dissolved in DMSO and the maximum final concentration of DMSO in the cell-culture medium was ≤0.1%. After a 24-hour incubation period at 37°C, the cell viability was determined using the Cell Counting Kit-8 (CCK8; Dojindo Molecular Technologies, China).

Flow cytometry
The cells, at a density of $1 \times 10^5$, were seeded into 24-well plates and incubated overnight. Thereafter, the cells were treated with BP-1-102 (2, 4, 6, and 10 µM) or BTZ (4 or 5nM) for 24 h. The maximum final concentration of DMSO in the cell-culture medium was $\leq 0.1\%$. Annexin V-Fluorescein Isothiocyanate (APC)/7-AAD Detection Kit (MULTI SCIENCES, China) was used to detect cell apoptosis within one hour by a Beckman Coulter (Gallios, Beckman).

Cell proliferation was detected using CSFE (Invitrogen-Life Technologies). CFSE was stored at 10 mM in 90 µL of anhydrous DMSO. The cells were incubated with CFSE (1:1,000) for 30 min at 37°C in the dark. The cells (U266, H929, and MM1S) were seeded in 24-well plates and incubated overnight. Thereafter, the cells were exposed to different concentrations of BP-1-102 (2, 4, 6, and 10 µM) or BTZ (4 or 5nM) for 24 h, and the samples were analyzed by flow cytometry (Gallios, Beckman).

**Migration and Invasion assay**

Cells, at a density of $1 \times 10^5$, were exposed to BTZ (5nM), BP-1-102 (6 µM), or DMSO in 300µL serum-free RPMI-1640 medium and seeded into the upper chambers of a transwell chamber (Corning Incorporated, USA). The chambers were either coated with Matrigel or left uncoated. Thereafter, 600 µL RPMI-1640 medium with 10% fetal bovine serum was added to the lower chambers. After a 24-hour incubation period, the cells migrated through the membrane to the lower chamber. After removing dead cells, the cells in the lower chamber were counted using a cell counter.

**Western blotting analysis**

The cells were harvested, rinsed twice with ice-cold phosphate-buffered saline, and homogenized using NE-PERTM Nuclear and Cytoplasmic Extraction Reagent (Thermo Fisher Scientific). Protein concentrations were determined using a BCA kit (Beyotime Biotechnology). Total proteins from each sample were separated by 10% SDS-PAGE at a constant current. The proteins were transferred to nitrocellulose membranes and incubated with Tris-buffered saline containing 0.1% Tween-20 (TBST) and 5% nonfat dry milk at 4°C overnight in the presence of antibodies against STAT3, p-STAT3, MCL-1, BCL-2, C-MYC, cleaved caspase3/8/9, and β-actin. The membranes were washed and incubated with HRP-conjugated secondary antibody in TBST containing 5% nonfat dry milk for one hour at room temperature. Immunoreactivity was detected using Enhanced Chemiluminescent Substrate (PerkinEliner, inc. USA).

**Murine models**

NSG female mice were purchased from BIOCYTOGEN (China). Myeloma xenograft tumors were generated by subcutaneous injection of $1 \times 10^6$ H929, $2 \times 10^6$ MM1S, or $3 \times 10^6$ H929-PTPROt cells resuspended in Matrigel (BD Biosciences) into the right flank of the NSG female mice. The mice were intraperitoneally administered BTZ (0.5mg/kg), in 10mg/mL mannitol in saline twice weekly, and BP-1-102 (6mg/kg) orally every day for 2 weeks. Control group: the group who received PBS-only. BP-1-102-treated group: the group who received BP-1-102-only. BTZ-treated group: the group who received BTZ-only. BP-1-102+BTZ group: the group who received the combination of BP-1-102 and BTZ. The tumor volume was measured every three days and estimated using the formula $(\text{length} \times \text{width}^2)/2$. 
Quantitative real-time PCR

Total RNA was extracted using an RNA isolation reagent kit (Omega Bio-tek, Inc). cDNA was generated by reverse transcription of total RNA (0.1–0.5µg) using random primers and a Reverse Transcription kit (Thermo Fisher Scientific) in a total reaction volume of 20µL.

Quantitative PCR was performed using assays specific for PTPRo (ID, HS00243097_m1) or GAPDH (ID, Hs99999905_m1, endogenous control), TaqMan Universal PCR Master Mix (Applied Biosystems), and a SYBR PCR Array kit (Thermo Fisher Scientific). The primers used were as follows:

| Gene     | Forward Primer    | Reverse Primer    |
|----------|-------------------|-------------------|
| β-actin  | TTCTACAATGAGCTGCGTGTG | GGGGTGTTGAAGGTCTCAA |
| STAT3    | GGAGGAGTTGCAGCAAAAAG | TGTGTTTGTGCCAGAATGT |
| MCL-1    | CTTACGACGGTTGGG   | GGTTCGATGCAGCTTTCTTGG |
| C-MYC    | AAACACAACATTGAACAGCTAC | ATTTGAGGCAGTTTACATTATGG |
| cyclin D1| CCGTCCATGCAGGAAGATC | GAAGACCTCCTCCTCGCAGCT |
| BCL-XL   | CAGAGCTTTGAACAGGTAG | GCTCTCGGGTGCTGTATTG |
| BCL-2    | GATTGTGGCCTTCTTTGAG | CAAACTGAGCAGATGTCCTT |
| PTPRo    | AGTTCGAGGAGCTGAAGTTAATCGG | GCCAGGATGTTGAGTATCTGTTCT |

Reactions were performed in triplicate using the Q6 Sequence Detection System (Applied Biosystems). Quantification was determined by delta-delta Ct method, and transcript levels of target genes were normalized with β-actin.

Primary patient samples

Primary myeloma cells were obtained from the bone marrow of patients diagnosed with MM. Bone marrow plasma cells of patients were sorted using StraightFrom Whole Blood CD138 MicroBeads (Miltenyi Biotec).

Statistical analysis

Statistical analyses were performed using Student's t-test (GraphPad Prism 6.01, GraphPad, Inc., La Jolla, CA, USA). A P value<0.05 was considered statistically significant.

Results

PTPRo suppressed MM cell proliferation by inhibiting STAT3 phosphorylation
First, we sorted myeloma cells from newly diagnosed multiple myeloma (NDMM) and relapsed drug-resistant multiple myeloma (RRMM) patients and detected the expression of PTPROt using qPCR. The results showed that PTPROt expression was higher in NDMM patients than in RRMM patients (Fig. 1A). This finding indicated that patients with low PTPROt expression had a higher tumor burden and vice versa. Previous studies reported that PTPROt inhibits liver cancer cell growth through the STAT3 signaling pathway (17–18). We wanted to investigate if PTPROt affected the proliferation of myeloma cells by regulating the STAT3 signaling pathway. Therefore, we constructed U266-PTPROt, H929-PTPROt, and MM1S-PTPROt cell lines, that overexpressed PTPROt (Fig. 1B), and used qPCR to analyze STAT3, MCL-1, BCL-XL, BCL-2, C-MYC, and D-cyclin mRNA levels in MM cell lines (Fig. 1C). Compared with U266, H929, MM1S, U266-GFP, H929-GFP and MM1S-GFP cells; U266-PTPROt, H929-PTPROt and MM1S-PTPROt cells had lower expression levels of BCL-2, BCL-XL, D-cyclin, and C-MYC. However, the expression of STAT3 did not change significantly. Western blot results also showed that the expression levels of cleaved caspase9, cleaved caspase8 and cleaved caspase3 increased while the expression levels of C-MYC, MCL-1, p-STAT3 (Y705), BCL-2 and SOCS3 decreased; however, the expression of STAT3 did not change in U266-PTPROt, H929-PTPROt and MM1S-PTPROt cells as compared to U266, H929, MM1S, U266-GFP, H929-GFP and MM1S-GFP cells (Fig. 1D). These findings indicated that PTPROt inhibited cell proliferation and promotes cell death by hampering STAT3 phosphorylation.

**BP-1-102 alone, or in combination with BTZ, promoted myeloma cell apoptosis by inhibiting STAT3 phosphorylation**

PTPROt inhibits the growth of myeloma cells by inhibiting STAT3 phosphorylation. Therefore, we investigated whether the inhibition of STAT3 signaling could also inhibit the growth of myeloma cells using BP-1-102 in combination with BTZ to treat MM cells. BP-1-102 alone or combined with BTZ upregulated cleaved caspase3 expression, downregulated BCL-2 and MCL-1 expression, and inhibited p-STAT3 (Y705) in a dose dependent manner (Fig. 2A). BP-1-102 alone (10µM) caused apoptosis of 15% of U266 cells (Fig. 2B), 90% of H929 cells (Fig. 2C), and 55% of MM1S cells (Fig. 2D); however, in combination with BTZ, these apoptosis percentages increased to 18%, 95%, and 70%, respectively (Fig. 2B-D). These findings demonstrated that BP-1-102 alone or in combination with BTZ induced myeloma cell apoptosis by inhibiting the phosphorylation of STAT3, which indicates that expression and/or activity blockade of STAT3 may induce myeloma cell apoptosis.

**BP-1-102 alone or in combination with BTZ inhibited the growth of myeloma cells**

The U266, H929, and MM1S cells were then exposed to different concentrations of BP-1-102 and BTZ to detect their effects on the proliferation of the myeloma cells by the CCK8 assay. Compared with the rate of proliferation in the control group, BP-1-102 dose-dependently inhibited the proliferation of U266 (left panel), H929 (middle panel), and MM1S cells (right panel) (Fig. 3A). Furthermore, we used CFSE to detect the proliferation of U266, H929, and MM1S cells and found that cell proliferation was dependent on the
concentration of BP-1-102. Compared with BP-1-102 alone, the combination of BP-1-102 and BTZ better inhibited cell proliferation (Fig. 3B-D). Transwell assays were performed to assess the effects of BTZ and BP-1-102 on the migration and invasion of myeloma cells. The number of penetrating cells was remarkably lower in the BP-1-102-treated group (6µM) than in the control group (untreated) (Fig. 3E-F), and the number of migratory and invasive cells in the BP-1-102 and BTZ treated groups was the lowest. These data indicated that BP-1-102 alone or in combination with BTZ inhibited the proliferation, migration, and invasion of myeloma cells. The results suggest that expression and/or activity blockade of STAT3 may inhibit the growth of myeloma cells.

**Combined treatment with BP-1-102 and BTZ inhibited tumor growth**

To determine whether BP-1-102 alone, or in combination with BTZ, inhibits the growth of myeloma cells *in vivo*, we selected H929 cells in the logarithmic growth phase and inoculated, at a density of 1×10⁶, subcutaneously into the outer right limb of mice. After six days, BP-1-102 was orally administered daily, and BTZ was intravenously injected into the tail vein twice per week (Fig. 4A). After 15 days of treatment, the mice in the treatment and control groups were sacrificed, and the grossly visible tumors were removed (Fig. 4B). The tumor weights and volumes in each group are presented in Fig. 4C-D. The results revealed that the tumor weight and volume were smaller in the BTZ and BP-1-102 groups than in the PBS group. Furthermore, the tumor volume and weight were smaller in the combined treatment group than in the BP-1-102 group or BTZ group. To confirm that BP-1-102 alone, or in combination with BTZ, inhibits tumor cell growth, we constructed a mouse xenograft model using MM1S cells. MM1S cells, at a density of 2×10⁶ cells, were inoculated subcutaneously into the NSG mice. BP-1-102 was orally administered daily, and BTZ was intravenously injected twice weekly (Fig. 3E). The tumor weight and volume were significantly larger in the PBS group than in the BP-1-102, BTZ, and BP-1-102+BTZ groups. Importantly, there was only one tumor in the BP-1-102+BTZ group (Fig. 3F-H). These results showed that BP-1-102, alone or in combination with BTZ, exerted a strong inhibitory effect on MM1S cells *in vivo* and inhibited the growth of MM cells *in vivo* and *in vitro*.

**PTPROt enhanced the sensitivity of MM to BP-1-102 and BTZ**

To examine whether PTPROt mediates the therapeutic effect of BP-1-102 alone, or in combination with bortezomib, on MM, these two drugs were used to treat myeloma cell lines (U266, U266-GFP, U266-PTPROt; H929, H929-GFP, H929-PTPROt; MM1S, MM1S-GFP, MM1S-PTPROt). Treatment with BP-1-102 alone (10µm) or in combination with bortezomib reduced the number of myeloma cells overexpressing PTPROt (Fig. 5A). PTPROt expression was upregulated in myeloma cells of NDMM patients, whereas it was downregulated in myeloma cells of RRMM patients. Therefore, we used CSFE to detect the proliferation of primary myeloma cells. When exposed to BP-1-102 (6 µM) for 24 h, the average fluorescence intensity of CSFE of myeloma cells from RRMM patients and NDMM patients decreased by 4000 and 7000 MFI, respectively (Fig. 5B-C). This finding indicated that PTPROt enhanced the sensitivity
of myeloma cells to BP-1-102 and BTZ. Next, to determine whether PTPROt augments the sensitivity of myeloma cells to BP-1-102 and BTZ \textit{in vivo}, we constructed an H929-PTPROt mouse model. Briefly, H929-PTPROt cells, at a density of $3 \times 10^6$, were injected subcutaneously into NSG mice. After 21 days, BP-1-102 was orally administered daily, whereas BTZ was injected twice a week via the tail vein (Fig. 5D). The tumor volume was measured every three days (Fig. 5E). After 15 days, the tumor volume and weight were greater in the PBS group than in the BP-1-102 and BTZ groups. The tumor volume and weight were smaller in the BP-1-102+BTZ group than in the BP-1-102 group or BTZ group (Fig. 5E-G). Further, the tumor volume, weight, and growth rate were lower in the H929-PTPROt group than in the H929 xenograft tumors group (Fig. 4E-H). These results proved that PTPROt enhanced the sensitivity of multiple myeloma cells to BP-1-102 and BTZ \textit{in vivo} and \textit{in vitro}. Together, our findings indicated that STAT3 could be a promising therapeutic target for MM.

\textbf{Discussion}

The STAT3 signaling pathway plays an important role in cell proliferation, apoptosis, and invasion (7–8, 19), and Constitutive STAT3 activation leads to the occurrence of myeloma (9, 19). The genetic suppression of dominant chromosome function and RNA interference showed that a decrease in STAT3 activity was associated with a decrease in the activity of MM cells. This phenomenon strongly implies that there is a close relationship between STAT3 activation and the occurrence of MM (20). Consequently, expression and/or activity blockade of STAT3 may be a potential treatment strategy for MM (14). Our experiments proved that PTPROt with BP-1-102 alone, or in combination with BTZ, inhibited the proliferation of myeloma cells by inhibiting the phosphorylation of STAT3.

Previous studies demonstrated that PTPROt suppressed the growth of liver cancer cells by inhibiting STAT3 phosphorylation (17–18). In our present study, the STAT3 phosphorylation decreased in myeloma cells overexpressing PTPROt, causing a decreased expression of BCL-1, MCL-1, and C-MYC. There was also a concurrent increase in the expression levels of cleaved caspases3, 8, and 9. This indicated that PTPROt inhibited the growth of myeloma cells by inhibiting the phosphorylation of STAT3. BP-1-102, which acts by direct interaction with relatively low concentrations of STAT3, has been proven to be effective in various tumors (14–16, 21). However, to the best of our knowledge, its role in MM has not been adequately investigated. In the present study, we found, for the first time, that BP-1-102 alone or in combination with BTZ had superior suppressive effects on cell proliferation, migration, and invasion in U266, H929, and MM1S cells. In addition, STAT3 activation and apoptosis of MM were inhibited by BP-1-102 alone in a dose-dependent manner in combination with BTZ. Furthermore, BP-1-102 alone, or in combination with BTZ, inhibited the growth of H929 and MM1S tumor cells \textit{in vivo}. While BP-1-102 alone inhibited MM cell growth, and the combined use of BP-1-102 and BTZ further enhanced these inhibitory effects on MM cells \textit{in vitro} and \textit{in vivo}.

Previous studies also showed that PTPROt may increase sensitivity to chemotherapeutic drugs (22). BP-1-102 treatment exerted a better effect, \textit{in vitro}, on cell expressing high levels of PTPROt than on those with low PTPROt expression levels. BP-1-102 alone, or in combination with BTZ, better inhibited the
growth of H929-PTPROt xenograft tumors than that of H929 xenograft tumor cells in vivo. The limitation of this study was the fact that even when the tumor volume of H929 xenograft cells reached 1000 mm$^3$, the H929-PTPROt tumor remained invisible to the naked eye. Therefore, we could not inoculate H929 and H929-PTPROt cells into the same mouse simultaneously.

Our study is the first to demonstrate that PTPROt and BP-1-102 alone, or in combination with BTZ, inhibit the proliferation of myeloma cells by inhibiting the phosphorylation of STAT3 and that PTPROt enhances the sensitivity of MM to BP-1-102 and bortezomib. These findings indicate that STAT3 may serve as a potential therapeutic target for the development of new MM therapies.

**Declarations**

**Data availability**

All data included in this study are available upon request by contacting the corresponding authors.

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**Author contributions**

Lin Yang, Zhengzheng Fu, and Zixuan Li are responsible for the experimental design, writing, and revision of the manuscript. Yong Liu, Yan Xie, Tian Wang, Shuaiyu Tian, Kailu Zhang, and Gangli An provided help during animal and cell experiments.

**Author statement**

All authors declare that there is no conflict of interest. All authors have read and approved the manuscript.

**Competing interests**

The authors declare no potential conflicts of interest for the research, authorship, and/or publication of the present article.
Ethics approval

Patient samples were collected from the First Affiliated Hospital of Soochow University, after obtaining a written informed consent from each patient and research ethics board approval in accordance with the Declaration of Helsinki (approval no. NSFC201881872431).

Mouse studies were conducted in accordance with the federal and institutional guidelines using protocols approved by the Ethics Committee of Soochow University (approval no. NSFC201881872431).

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PTPROt inhibits myeloma cell proliferation by inhibiting STAT3 phosphorylation (A) Myeloma cells were sorted from newly diagnosed multiple myeloma (NDMM) and relapsed drug-resistant multiple myeloma (RRMM) patients. qPCR was performed to determine the expression of PTPROt in NDMM and RRMM patients using TaqMan Universal PCR Master Mix. (B) U266, H929, and MM1S cells were transfected with GFP plasmid and PTPROT plasmid lentivirus. After 24h, qPCR detected, using a SYBR PCR Array kit,
PTPROt expression in U266 (left panel), H929 (middle panel), and MM1S (right panel) cells. (C) The mRNAs expression of STAT3, C-MYC, BCL-XL, MCL-1, and D-cyclin in U266 (left), H929 (middle), and MM1S (right) cells. (D) The protein expression of STAT3, p-STAT3(Y705), C-MYC, BCL-2, cleaved caspase3, cleaved caspase9, cleaved caspase8, MCL-1, and SOCS3. β-Actin was used as a loading control. BTZ: bortezomib.

(student’s t-tests, *p<0.05, **p<0.01 and ***p<0.001, vs. control).
BP-1-102 alone or in combination with BTZ induces myeloma cell line apoptosis (A) Western blot analysis of STAT3, p-STAT3 (Y705), MCL-1, and cleaved caspase3 in U266, H929, and MM1S cells following exposure to different concentrations of BP-1-102 (5, 10, or 15µM) and BTZ (5nM) for 24 h. β-Actin was used as a loading control.

(B-D) Dual annexin V and 7-AAD staining of U266, H929, and MM1S cells was analyzed using flow cytometry to evaluate the apoptosis of U266, H929, and MM1S cells after exposure to different concentrations of BP-1-102 (2, 6, or 10µM) and BTZ (5nM) for 24 h. (student’s t-tests, \( *p<0.05, **p<0.01 \) and \( ***p<0.001 \), vs. control). BTZ: bortezomib.
BP-1-102 alone or in combination with BTZ inhibits the proliferation of myeloma cells (A) Cells were treated with various concentrations of BP-1-102 (2, 6, or 10 µM) and BTZ (5nM) for 24 h. Cell activity was detected with CCK-8. (B-D) The cells were incubated with CFSE (1:1000) for 30 min at 37°C, washed thrice, and then exposed to different concentrations of BP-1-102 (2, 6, or 10 µM) and BTZ (5nM) for 24 h. The average fluorescence intensity of CFSE was detected using flow cytometry. (E-F) Transwell assays
showed that BP-1-102 and BTZ significantly attenuated the migration (without Matrigel) and invasion ability (with Matrigel) of MM cells. The top compartment shows the cells after treatment with BP-1-102 (6 µM) and BTZ (5nM) and 24-h incubation. The numbers of migrated and invading cells were quantified from three independent experiments. (*student's t-tests, *p*<0.05, **p*<0.01 and ***p*<0.001, vs. control). BTZ: bortezomib.

Figure 4

BP-1-102 alone or in combination with BTZ inhibits the growth of H929 and MM1S xenograft tumors (A) H929 cells (1×10^6) were injected subcutaneously into NSG mice. After 6d, BP-1-102 (6mg/kg) was administered orally every day and BTZ (0.5mg/kg) was injected intravenously twice per week via the tail vein for 15 consecutive days (n=5/group). (B) Starting from day 0, the tumor size was measured every three days. The tumor size was calculated using the formula (length×width^2)/2 (n=5/group). (C-D) The size and weight of H929 xenograft tumors in the PBS, BP-1-102, BTZ, and BP-1-102+BTZ groups after treatment for 15 days (n=5/group). (E) MM1S cells were injected subcutaneously into NSG mice. After 20 days, BP-1-102 (6mg/kg) was administered orally daily and BTZ (0.5mg/kg) was intravenously
administered twice per week for 16 days (n=5/group). (F) Volume of MM1S xenograft tumors (n=5/group). (G-H) Tumors were harvested 16 days after treatment. The measured size and weight of the MM1S xenograft tumors in the PBS, BP-1-102, BTZ, and BP-1-102+BTZ groups are shown (n=5/group). (student’s t-tests, *p<0.05, **p<0.01 and ***p<0.001, vs. control). BTZ: bortezomib.

Figure 5

PTPROt increases the sensitivity of multiple myeloma to BP-1-102 and BTZ (A) U266, U266-GFP, U266-PTPROt (left panel), H929, H929-GFP, H929-PTPROt (middle panel), and MM1S, MM1S-GFP, MM1S-PTPROt (right panel) cells were seeded into 24-well plates and exposed to different concentrations of BP-1-102 (5 or 10 µM) and BTZ (5nM). Flow cytometry was used to determine the proliferation and activity levels of the myeloma cell lines. (B) Primary myeloma cells from RRMM patients were exposed to
different concentrations of BP-1-102 (2, 4, 6, 8 or 10 µM). After 24 h, CFSE were used to detect cell proliferation. (C) Primary myeloma cells of NDMM patients were exposed to BP-1-102 (2, 4, or 6 µM) and BTZ (4nM). After 24 h, the average CFSE fluorescence intensity was measured using flow cytometry. (D) H929-PTPROt (3×10^6) cells were injected subcutaneously into NSG mice. After 21 days, BP-1-102 (6 mg/kg) was orally administered every day and BTZ (0.5mg/kg) was intravenously injected twice a week for 16 days (n=5/group). (E-G) Tumors were harvested 16 days after treatment, and the volume, size, and weight of the H929-PTPROt xenograft tumors in the PBS, BP-1-102, BTZ, and BP-1-102+BTZ groups were measured (n=5/group). (student’s t-tests, *p<0.05, **p<0.01 and ***p<0.001, vs. control). NDMM: newly diagnosed multiple myeloma; RRMM: relapsed drug-resistant multiple myeloma; BTZ: bortezomib