ORIGINAL RESEARCH

PHLPP Sensitizes Multiple Myeloma Cells to Bortezomib Through Regulating LAMP2

Xiao Liu
Chengyuan Li
Yunfeng Fu
Jing Liu

Department of Hematology, The Third Xiangya Hospital of Central South University, Changsha 410013, People's Republic of China

Introduction: Treatment of bortezomib (BTZ) improves the clinical outcomes of patients with multiple myeloma (MM). However, primary resistance and acquired resistance to BTZ frequently develop in patients with MM. PH domain leucine-rich repeat protein phosphatase (PHLPP) plays an important role in chemoresistance in a number of cancers. However, the role of PHLPP on MM remains unclear. In this study, we investigated the role of PHLPP in BTZ-resistant MM cells.

Methods: BrdU assays, immunoprecipitation, flow cytometry analyses, and immunofluorescence assays were performed.

Results: PHLPP and lysosome-associated membrane protein 2 (LAMP2) levels were downregulated in BTZ-resistant MM cells compared with BTZ-sensitive MM cells, accompanied by inactivation of autophagy pathway evaluated by a reduction in Beclin1, Atg5 and LC3B and increase in p62. Gain- and loss-of-function experiments revealed that PHLPP partially re-sensitized MM cells to BTZ. In addition, PHLPP overexpression increased whereas PHLPP knockdown reduced LAMP2 expression, subsequently regulating the autophagy pathway in MM cells. Further findings demonstrated that LAMP2 knockdown reversed PHLPP-mediated cell apoptosis and autophagy activation in MM cells.

Conclusion: This study demonstrated that PHLPP is a potential strategy for overcoming BTZ resistance in patients with MM.

Keywords: multiple myeloma, PHLPP, bortezomib, resistance, chaperone-mediated autophagy, LAMP2

Introduction

Multiple myeloma (MM) is a common hematologic malignancy of plasma cells (accounting for ~1% of hematologic malignancy).[1] Recently, treatments with immunomodulatory drugs, monoclonal antibodies, and second-generation proteasome inhibitors, such as bortezomib (BTZ), have increased recurrence-free survival and overall survival rates for patients with MM.[2,3] However, most patients who have primary or acquired resistance to BTZ will suffer a relapse.[4]

PH domain leucine-rich repeat protein phosphatase (PHLPP) is a Ser/Thr protein phosphatase that plays a critical role in maintaining cellular homeostasis by directly regulating Akt, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK).[5] Loss of PHLPP expression is associated with tumor progression, and increasing evidence indicates that PHLPP is a tumor suppressor and a potential therapeutic target.[6] Patients with lung adenocarcinoma that have low PHLPP levels have poorer survival rates than those with high PHLPP.[7] In addition, hypoxia-induced chemoresistance is associated with the downregulation of PHLPP in colon

Correspondence: Jing Liu; Yunfeng Fu
The Third Xiangya Hospital of Central South University, Tongzipo Road 138, Changsha 410013, People's Republic of China
Tel/Fax + 86-073188618511
Email liujing3571@163.com;
fuyunfeng@csu.edu.cn

cancer cells,[8] suggesting that PHLPP plays an important role in chemoresistance. However, the role of PHLPP on MM remains unclear.

Lysosomal associated membrane proteins (LAMPs) family is characterized by an evolutionary-conserved membrane-proximal LAMP domain and composed of five known members: LAMP1, LAMP2, LAMP3, LAMP4 and LAMP5.[9] LAMP2 has been well documented in a variety of cellular processes including autophagy, specifically chaperone-mediated autophagy (CMA), a process that targets specific proteins to degradation by lysosomes.[10] LAMP2 can affect the lysosomes localization and the autophagic flux, and LAMP2 deficiency was responsible for Azacytidine resistance in MDS/AML cells.[11] However, the expression and roles of LAMP2 in BTZ-resistant MM cells are unknown.

In this study, we found that PHLPP levels were decreased in bortezomib-resistant cells and bone marrow samples of patients with MM, and overexpression of PHLPP partially sensitized MM cells to BTZ by increasing LAMP2. Our study indicates that the rescue of PHLPP is a potential strategy for the management of acquired BTZ resistance in multiple myeloma.

Materials and Methods
Clinical Samples Collection
Three newly diagnosed with MM and three BTZ-resistant MM patients were enrolled in this study. This study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University. Written informed consent was obtained from each subject in accordance with the Declaration of Helsinki. Bone marrow tissues were obtained from these patients. Cell flow cytometry was used to sort CD138^{+} cells.

Cell Culture
The MM cell line U266 was purchased from Cell Bank (Chinese Academy of Sciences, Beijing, China). Cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (GlutaMAX™-I, cat no. 72400120, Gibco). 10% fetal bovine serum (Gibco, CA, USA) was added in the medium. Cells were cultured under the condition in a 37°C humidified atmosphere of 5% CO_2.

Generation of BTZ-Resistant U266 Cells
The bortezomib-resistant U266 cells (U266-R) were established as follows: U266 cells were treated with the initial concentration of bortezomib (2 nM). The medium was

refreshed once every 2 days. After cultured for 2 weeks under the same concentration, the dose was increased for two folds, and continuously maintained for 2 weeks. The cycles were repeated 10 times, so that the U266-R cells can be resistant to bortezomib up to 20 nM. The cells were kept under 10 nM bortezomib pressure before experimental usage.

Cell Transfection and Treatment
Lentivirus mediated PHLPP (shRNA) and LAMP2 (shRNA) downregulation, and PHLPP and LAMP2 overexpression were obtained from Genefullen (Guangzhou, China). The lentivirus-containing scramble sequences were used as control. Lentivirus infection was performed according to the manufacture's protocol. The cells were then co-treated with rapamycin (RAP) or hydroxychloroquine (HCQ), or BTZ (5 nM or 40 nM) for 48 hrs and then used for further analysis.

BrdU Assay
BrdU Cell Proliferation ELISA kit (Abcam, Shanghai, China) was used in this experiment. After indicated treatment, the cells were added BrdU and incubate for 12 hrs to incorporate BrdU into their DNA. The cells then were fixed with Fixing Solution and incubate at room temperature. Add the TMB Solution to each well for 5 mins and then add the Stop Solution to stop the reaction. The color development was recorded immediately.

Apoptosis Analysis
MEBCYTO Apoptosis Kit (MBL Beijing Biotech Co., Ltd, Beijing, China) was used in this experiment. After treatment, cells were collected and then resuspended in binding buffer. Annexin V-FITC (5 μL) and Propidium Iodide (5 μL) were added and mixed well. The cells were incubated with the mixed solution for 15 mins. Finally, flow cytometric analysis was performed in Attune NxT (Thermofisher, Shanghai, China).

Western Blot
Protein was extracted using RIPA (Beyotime, Hangzhou, China) and their concentrations were determined by Enhanced BCA Protein Assay Kit (Beyotime, Hangzhou, China). Sixty microgram proteins were separated in 10% SDS/PAG and transferred on PVDF membrane. The membranes were incubated with primary antibody: anti-LAMP2 antibody (1:1000, cat no. 49067, Cell Signaling Technology), anti-LAMP1 antibody (1:1000, cat no. ab13523, Abcam), anti-LAMP2A antibody (1:1000, cat no. ab125068, Abcam), anti-LAMP2B antibody (1:1000,

cat no. ab18529, Abcam), anti-AKT antibody (1:1000, cat no. ab18785, Abcam), anti-phospho-Akt (Ser473) antibody (1:1000, cat no. 4058, Cell Signaling Technology), anti-PHLPP antibody (1:1000, cat no. ab62830, Abcam); anti-Beclin1 antibody (1:1000, cat no. ab210498, Abcam); anti-Atg5 antibody (1:1000, cat no. ab228668, Abcam); anti-p62 antibody (1:1000, cat no. ab56416, Abcam); anti-LC3B antibody (1:1000, cat no. ab51520, Abcam). GAPDH (1:1000, cat no. ab181602, Abcam) was used as control. After being washed, the membranes were reacted with peroxidase-conjugated secondary antibody. The signals were visualized by enhanced chemiluminescence reagents (GE, Fairfield, CT, USA).

Immunoprecipitation was performed to determine the interaction between PHLPP and LAMP2. Ice-cold cell lysis buffer (500 μL) was added to each plate and incubate for 5 mins. The cells were collected to microcentrifuge tubes. The samples were sonicated on ice three times for 5 s each and micro-centrifuged for 10 mins at 14000 g, 4°C. 200 μL cell lysate was incubated with PHLPP or LAMP2 primary antibodies with rocking overnight at 4°C. Protein A agarose beads (Beyotime, Hangzhou, China) were added and incubated with for 3 hrs at 4°C. After microcentrifuge, the pellets were washed with cell lysis buffer and were resuspended with SDS sample buffer and heated to 98°C for 5 mins. 30 μL of the sample was loaded on SDS-PAGE gel (12%) for Western blotting analysis.

Immunofluorescence Assay
After blocked with 5% BSA, the cells were incubated with primary antibodies (anti-LAMP2 antibody, 1:200 and anti-PHLPP, 1:500) at 4°C overnight. The cells were incubated with appropriate secondary antibodies at 37°C for 1 hr. The cells were stained with 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) (1:1000, Beyotime, Hangzhou, China) for 5 mins and mounted on slides. Immunofluorescence images were captured by a fluorescence microscope (BX51, Olympus, Japan).

Lysotracker-Red Staining
Lysosome was stained with Lysotracker-Red DND-99 (Molecular Probes, Eugene, USA). After infected with PHLPP lentivirus (labelled with EGFP), U266 cells were incubated in the presence of 50 nM Lysotracker-Red for 30 mins at 37°C. Immunofluorescence images were captured by a fluorescence microscope (BX51, Olympus, Japan).

Animal Experiment
Animal experiments were approved by the Ethics Committee for Animal Research of the Third Xiangya Hospital of Central South University following the National Research Council's Guide for the Care and Use of Laboratory Animals (Eight Edition). The male, 2-month-old nude mice were purchased from Animal Center of Central South University. U266 were transfected with PHLPP or negative control for 48 hrs. Transfected U266 cells (1×10^6) were then subcutaneously injected into nude mice (N = 5/each group). Tumor volume was calculated as $0.5 \times L \times W,^2$ where L and W are long and short diameters of the tumor, respectively.

Statistical Analysis
GraphPad Prism software (GraphPad Software Inc., La Jolla, CA) was used for statistical analyses. Student's *t*-test for two-group and one-way ANOVA with post hoc Bonferroni test for three or more groups were used to assess the statistical significance. P < 0.05 was considered statistically significant.

Results
PHLPP and LAMP2 Levels are Increased in BTZ-Resistant MM Cells
To investigate the role of PHLPP and LAMP2 in BTZ-resistant MM, we measured their expression levels in plasma cell from patients with MM and BTZ-resistant MM cell lines. We found that LAMP2 and PHLPP expression levels in BTZ-resistant plasma cells of human samples were down-regulated compared with BTZ-sensitive samples (Figure 1A). Their expressions were also lower in BTZ-resistant U266 cells compared with parental U266 cells (Figure 1B). However, the expression of another member of LAMP family, LAMP1 was comparable between BTZ-sensitive and BTZ-resistant patients with MM. The expression of LAMP2 isoform LAMP2A, not LAMP2B, was significantly downregulated in BTZ-resistant patients with MM compared the BTZ-sensitive patients with MM (supplementary Figure 1A). In addition, downregulation of PHLPP and LAMP2 was accompanied by inactivation of autophagy, as determined by decreased Beclin1 and Atg5 levels, and ratio of LC3B-II/LC3B-I, and increased p62 expression in plasma cells from BTZ-resistant MM and U266-R cells (Figure 1). These results suggest that PHLPP downregulation is associated with LAMP2 and chaperone-mediated autophagy and involved in BTZ resistance.
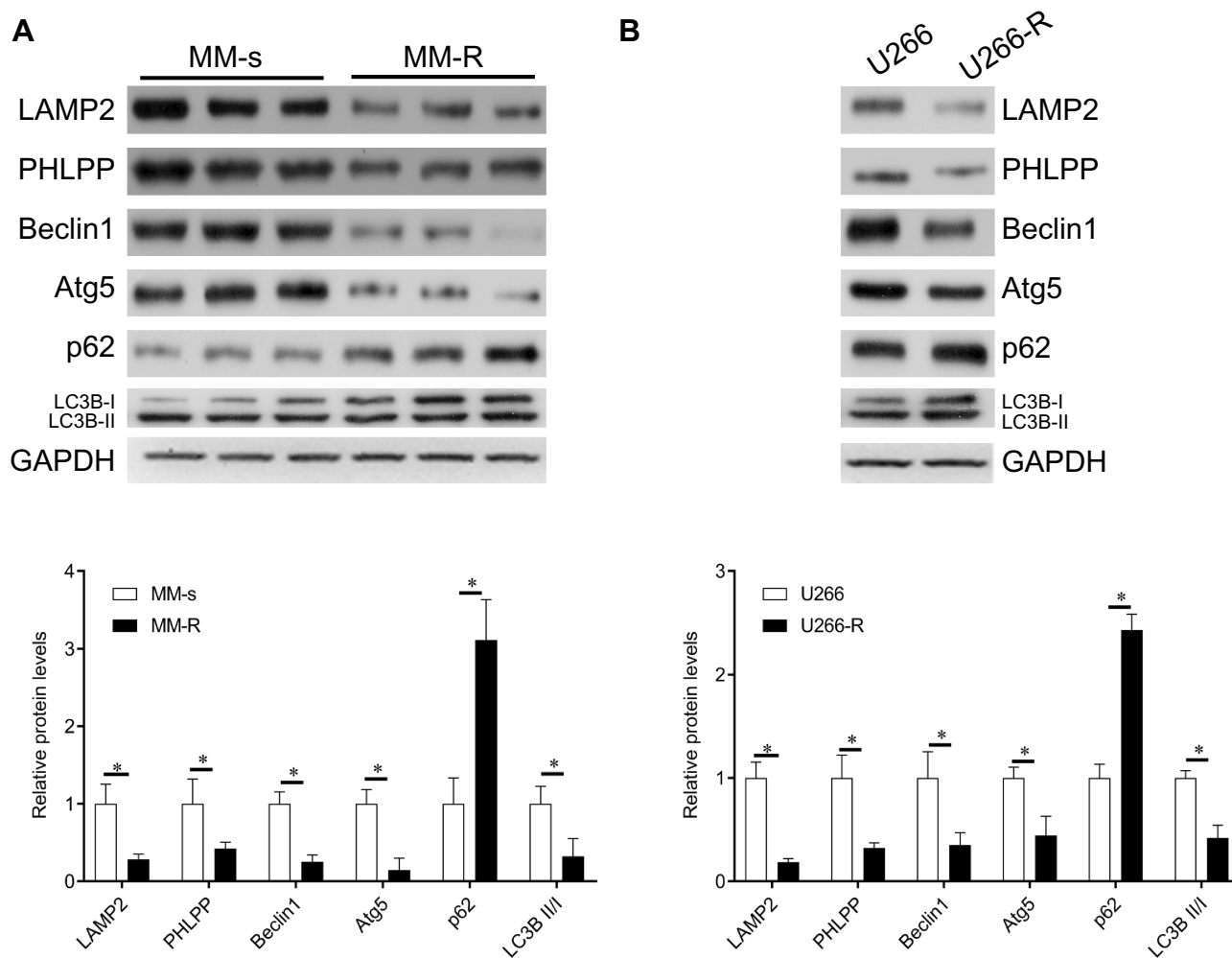
Figure 1 The expression of PHLPP in bortezomib-resistant MM cells. (**A**) Western blot analyses of the expression of PHLPP, LAMP2, and key autophagy signaling molecules in bone marrow samples from patients with MM (upper), and quantification of the bands (lower). (**B**) Western blot analyses of the expression of PHLPP, LAMP2, and key autophagy signaling molecules in U266 cells and bortezomib-resistant U266 cells (upper), and quantification of the bands (lower). MM-S, BTZ-sensitive MM; MM-R, BTZ-resistant MM; U266-R, BTZ-resistant U266 cells. *P < 0.05.

PHLPP Sensitizes MM Cells to BTZ

PHLPP was knocked-down in U266 cells and was overexpressed in U266-R cells (Figure 2A). PHLPP knockdown significantly promoted U266 cell proliferation, and inhibited cell apoptosis following BTZ treatment (Figure 2B and C). However, PHLPP overexpression significantly inhibited U266-R cell proliferation, and induced cell apoptosis following BTZ treatment (Figure 2B and C). These results suggest that PHLPP sensitizes MM cells to BTZ treatment.

PHLPP Suppresses MM Cells Growth in vivo

Furthermore, we performed xenografted tumor experiments in nude mice using PHLPP-expressing U266 cells to examine the effects of PHLPP on tumor growth in vivo. PHLPP overexpression slowed down tumor growth in vivo (Figure 2D). Immunohistochemical staining showed that

PHLPP and LAMP2 expression were upregulated in tumor tissues (Figure 2E).

PHLPP Interacts with LAMP2

Given that PHLPP expression was associated with LAMP2 expression, we investigated whether PHLPP interacts physically with LAMP2. Immunofluorescence assays showed that PHLPP and LAMP2 were co-localized in U266 cells (Figure 3A). Co-immunoprecipitation (co-IP) experiments further confirmed that PHLPP interacts with LAMP2 (Figure 3B), and they were co-expressed in the lysosome (Figure 3C). In addition, we found that knockdown of PHLPP decreased LAMP2 expression (Figure 3D). Knockdown of PHLPP also reduced Beclin1 and Atg5 levels and ratio of LC3B-II/LC3B-I, and increased p-AKT (ser473) and p62 expression, suggesting autophagy
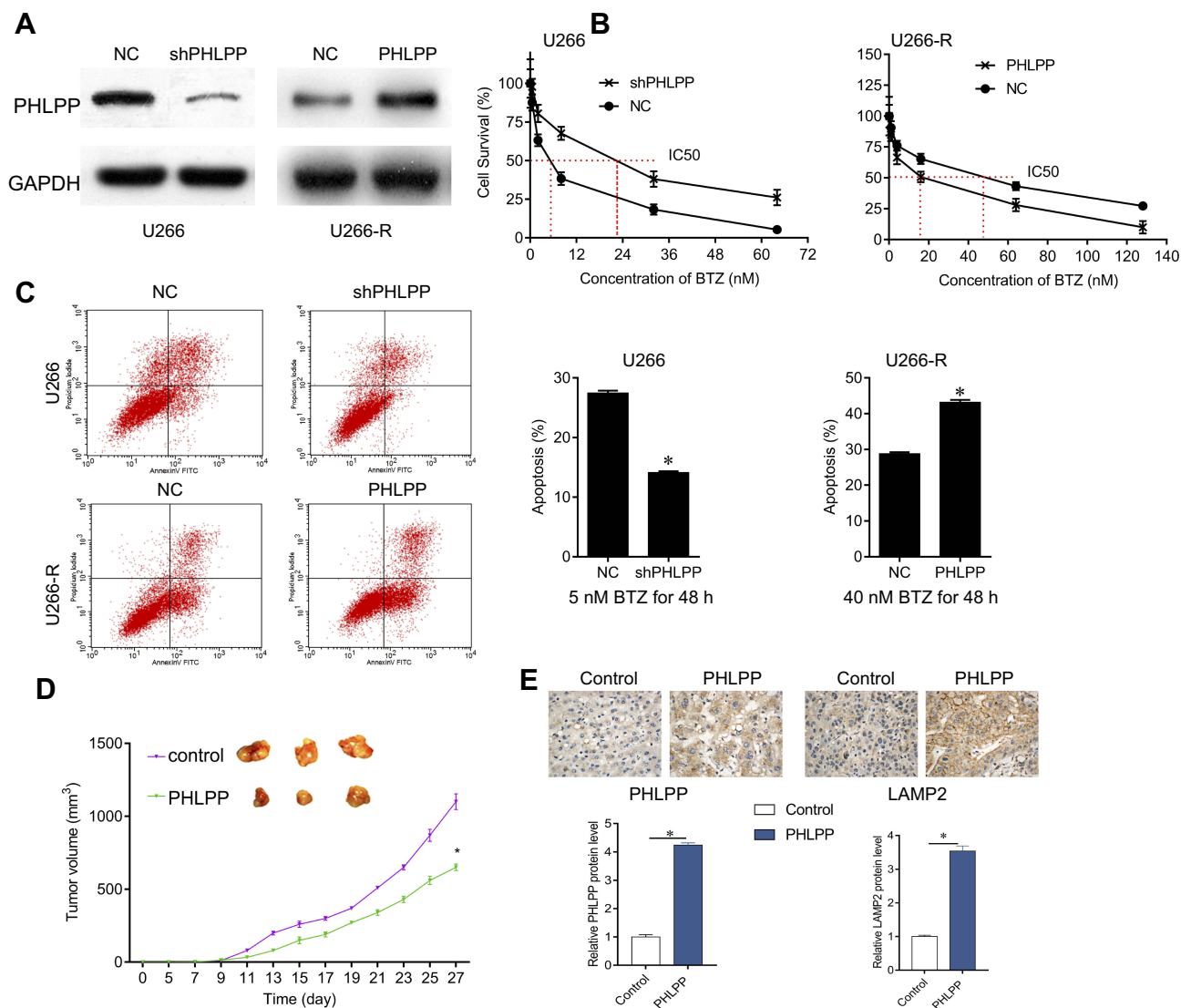
Figure 2 Overexpression of PHLPP sensitizes MM cells to BTZ. (**A**) Western blot analyses of PHLPP expression in U266 cells and BTZ-resistant U266 cells after lentivirus infection. (**B**) BrdU assays were used to determine cell viability after sh-PHLPP or PHLPP lentivirus infection in U266 and U266-R cells, respectively. (**C**) Flow cytometry was used to determine apoptosis after knockdown or overexpression of PHLPP under BTZ treatment. (**D**) U266 cells were infected with PHLPP lentivirus and were then injected into nude mice. Tumor volumes were measured weekly. (**E**) PHLPP and LAMP2 expression in tumor sections were evaluated using immunohistochemistry (IHC); Magnification, 100X; *$P < 0.05$.

signaling inactivation in U266 cells, whereas overexpression of PHLPP increased the expression of LAMP2 and LAMP2A, but did not alter the expression of LAMP1 and LAMP2B (supplementary Figure 1B) and inhibited phosphorylation of AKT, activating autophagy signaling in U266-R cells (Figure 3D).

PHLPP Partially Sensitizes MM Cells to BTZ Through LAMP2 and Autophagy

We next tested the role of LAMP2 in BTZ-induced cell apoptosis. We found that LAMP2 overexpression enhanced while LAMP2 knockdown attenuated BTZ-induced growth inhibition and cell apoptosis (supplementary Figure 2). To

investigate the role of LAMP2 in PHLPP-mediated BTZ sensitization, LAMP2 was knocked down by shRNA in U266-R cells (Figure 4A) and overexpressed in U266 cells (Figure 4B). Under BTZ treatment, LAMP2 knockdown reversed PHLPP-mediated autophagy activation as determined by downregulation of Beclin1 and Atg5 levels and the ratio of LC3B-II/LC3B-I and upregulation of p-AKT (ser473) and p62 (Figure 4A), proliferation inhibition (Figure 4C), and apoptosis (Figure 4D) in U266-R cells. LAMP2 overexpression rescued the effects of shPHLPP treatment on autophagy (Figure 4B), cell proliferation (Figure 4E), and cell apoptosis (Figure 4F) in U266 cells, suggesting that LAMP2 is required for PHLPP in re-sensitizing MM cells to
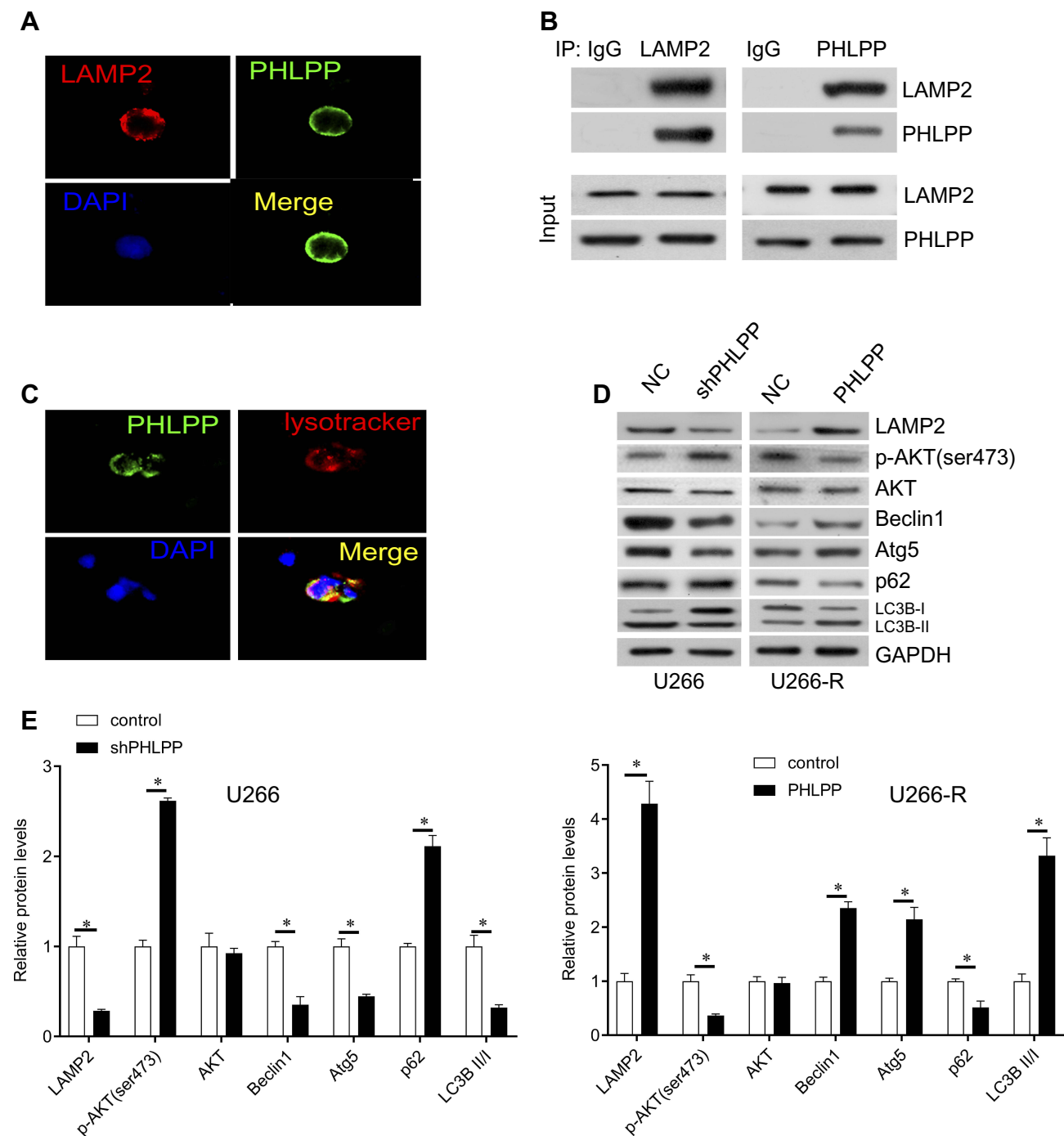
Figure 3 PHLPP positively regulates LAMP2 expression. (**A**) Immunofluorescence assays were performed to investigate the interactions between PHLPP and LAMP2 in U266 cells. (**B**) Immunoprecipitation confirmed the interactions between PHLPP and LAMP2 in U266 cells; (**C**) EGFP-PHLPP was expressed in U266 cells for 48 hrs and loaded with lysotracker-Red DND-99 for 30 mins at 37°C. Cells were fixed and analyzed by confocal microscopy. (**D**) Western blot analyses of the expression of PHLPP, LAMP2, and key autophagy signaling molecules in U266 and U266-R cells after infection with sh-PHLPP or PHLPP lentivirus. (**E**) Quantification of the bands in (**D**). *$P < 0.05$.

BTZ. Moreover, pharmacological activator of autophagy RAP treatment significantly decreased cell proliferation and enhanced cell apoptosis compared with shPHLPP transfection alone in U266 cells (Figure 5A and B); the autophagy inhibitor hydroxychloroquine (HCQ) treatment dramatically promoted cell proliferation and reduced cell apoptosis compared with PHLPP transfection alone in U266-R cells (Figure 5C and D). These results suggest that PHLPP sensitizes MM cells to BTZ through LAMP2 and the autophagy pathway.
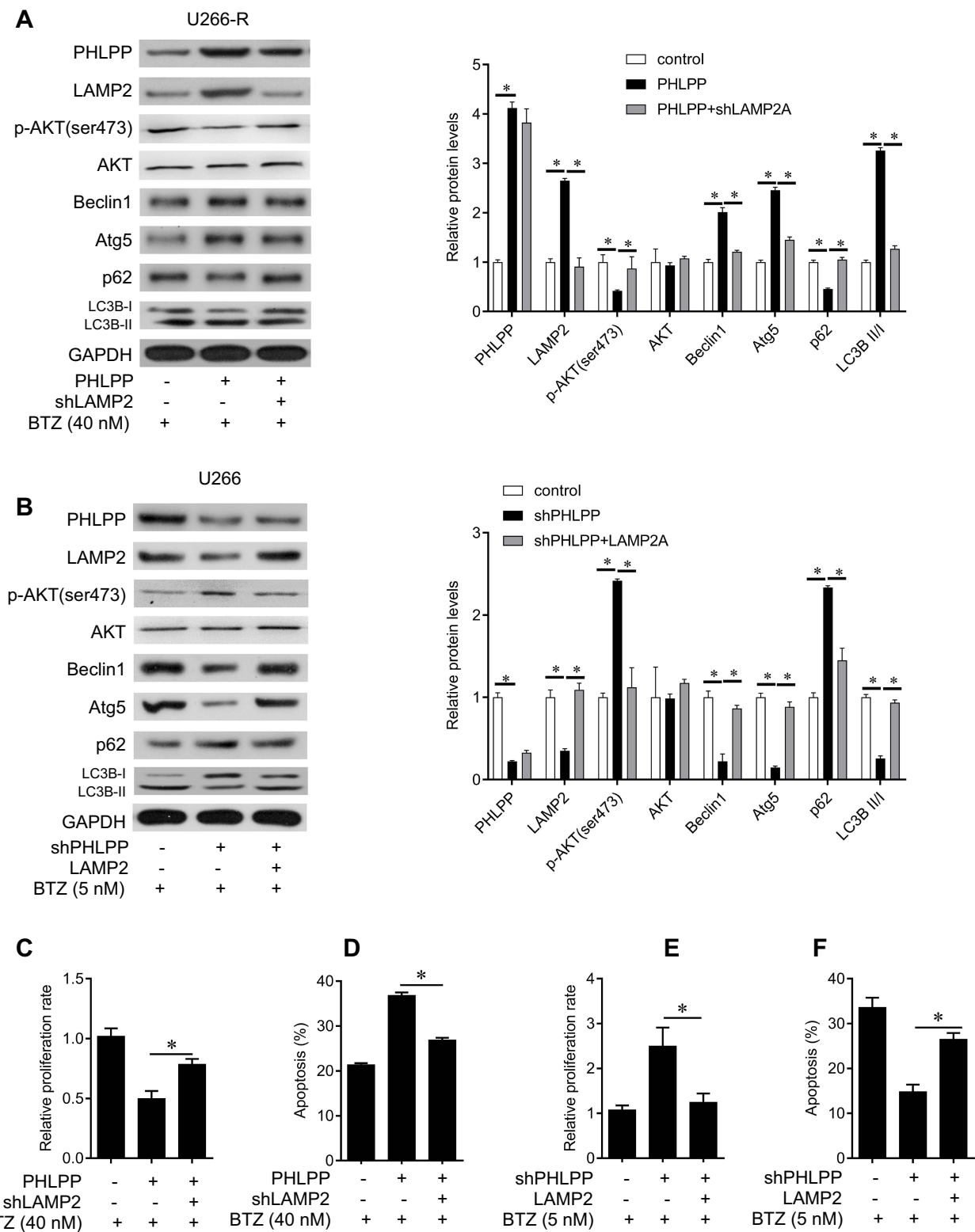
Figure 4 LAMP2 knockdown reverses the inhibitory effects of PHLPP in U266-R cells. (**A**) Western blot analyses of the expression of PHLPP, LAMP2, and key autophagy signaling molecules in U266-R cells infected with sh-LAMP2 or PHLPP lentivirus under 40 nM BTZ treatment (left), and quantification of the bands (right). (**B**) Western blot analyses of the expression of PHLPP, LAMP2, and key autophagy signaling molecules in U266 cells that were infected with LAMP2 or shPHLPP lentivirus under 5 nM BTZ treatment (left), and quantification of the bands (right). (**C**) Cell proliferation was evaluated using BrdU assay in U266-R cells that were infected with sh-LAMP2 or PHLPP lentivirus under 40 nM BTZ treatment. (**D**) Apoptosis was evaluated using flow cytometry in U266-R cells that were infected with sh-LAMP2 or PHLPP lentivirus under 40 nM BTZ treatment. (**E**) Cell proliferation was evaluated using BrdU assay in U266 cells that were infected with LAMP2 or shPHLPP lentivirus under 5 nM BTZ treatment. (**F**) Apoptosis was evaluated using flow cytometry in U266 cells that were infected with LAMP2 or shPHLPP lentivirus under 5 nM BTZ treatment. *$P < 0.05$.
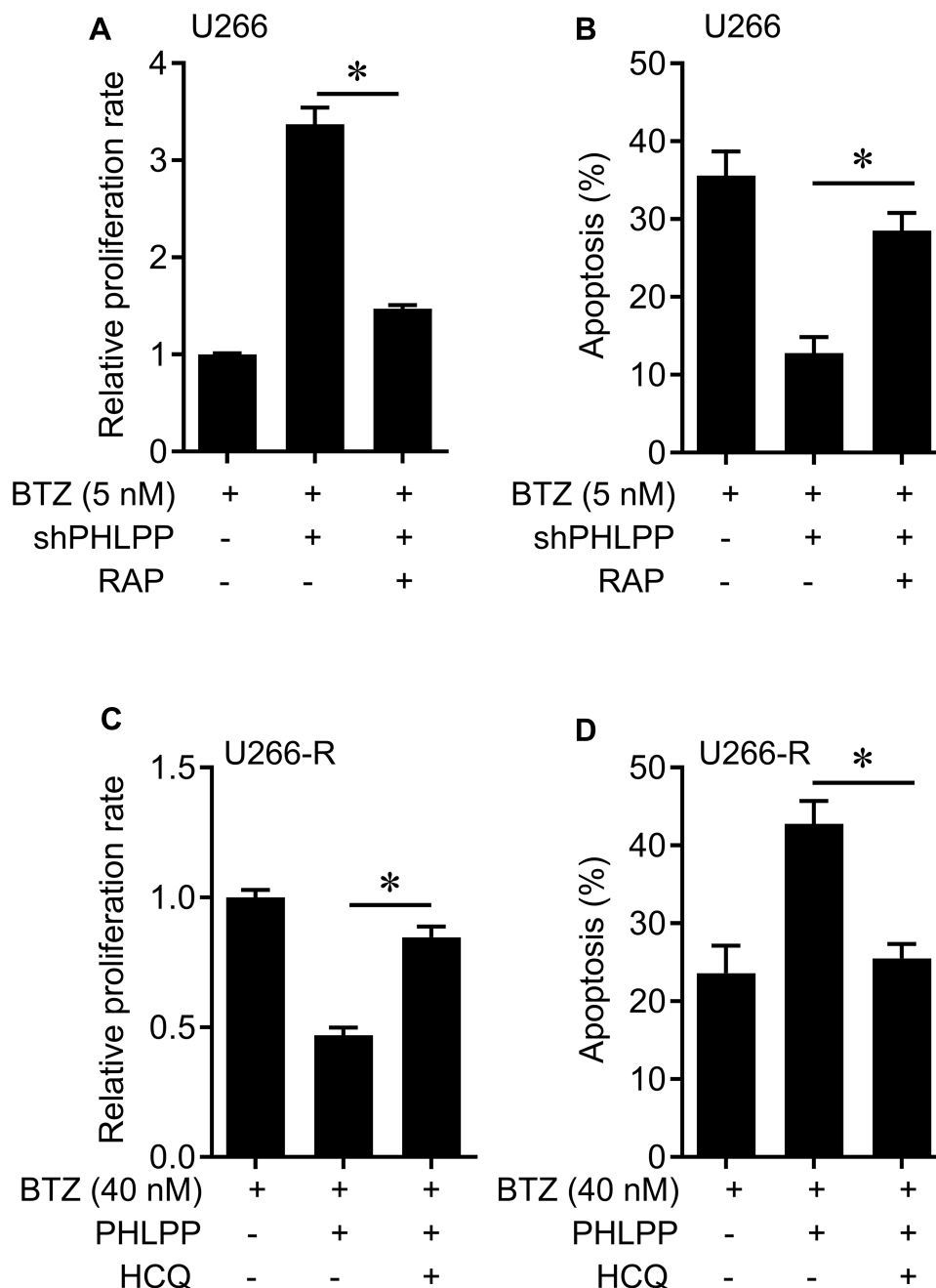
Figure 5 The effects of pharmacological inhibitor of autophagy on PHLPP-mediated cell apoptosis. (**A**) Cell proliferation was evaluated using BrdU assay in U266 cells that were infected with shPHLPP lentivirus and treated with rapamycin (RAP) under 5 nM BTZ treatment. (**B**) Apoptosis was evaluated using flow cytometry in U266 cells that were infected with shPHLPP lentivirus and treated with rapamycin (RAP) under 5 nM BTZ treatment. (**C**) Cell proliferation was evaluated using BrdU assay in U266-R cells that were infected with PHLPP lentivirus and treated with hydroxychloroquine (HCQ, 25 μM) under 40 nM BTZ treatment. (**D**) Apoptosis was evaluated using flow cytometry in U266-R cells that were infected with PHLPP lentivirus and treated with hydroxychloroquine (HCQ, 25 μM) under 40 nM BTZ treatment. *$P < 0.05$.

Discussion

In this study, we found that PHLPP and LAPM2 were down-regulated in BTZ-resistant MM cells. Induced PHLPP expression partially re-sensitized MM cells to BTZ through activating the autophagy pathway.

MM is a common hematologic malignancy, constituting 1% of all malignancies. Although treatment regimens have greatly improved, MM remains incurable because of resistance to drugs such as dexamethasone and bortezomib.[12] Drug resistance involves the dysregulation of various signaling pathways, including the JAK/STAT, MEK/MAPK, and PI3K/AKT.[13] AKT phosphorylation activates AKT signaling, which is associated with anti-apoptotic effects of plasma cell-derived growth factor against the effects of dexamethasone.[14]

MM cells produce the growth factor IGF-1, and high levels of IGF1 and its receptor (IGF-1R) are associated with poor survival and BTZ resistance in patients with MM.[15] The IGF-1R inhibitor OSI-906 can increase the cytotoxicity of BTZ in MM cells, and PI3K and AKT are the downstream targets of IGF1.[16] AKT inhibition also causes BTZ-resistant cell death.[17] Therefore, combination bortezomib and OSI-906 therapy could be a useful treatment for patients with BTZ resistance.

PHLPP negatively regulates AKT activity, and FK506-binding protein 51 functions as a scaffolding protein for Akt and PHLPP. PHLPP can promote dephosphorylation of AKT and enhances the efficacy of chemotherapy.[18] PHLPP over-expression inactivates Akt, whereas PHLPP knockdown increases phosphorylation and activation of Akt.[19] In addition, hypoxia-induced chemoresistance is associated with the downregulation of PHLPP in colon cancer cells.[8] In this study, we found that PHLPP levels were reduced in BTZ-resistant MM cells, and induced PHLPP expression re-sensitized MM cells to BTZ. Interestingly, we found that PHLPP inhibited proliferation and promoted apoptosis in MM cells by enhancing CMA. CMA contributes to the maintenance of proteostasis through the degradation of cyto-solic proteins in lysosomes.[20] Cytosolic chaperones delivered CMA substrates to the lysosomal surface, and are internalized through a membrane translocation complex.[21] It was pre-viously found that PHLPP positively regulated CMA through inhibition of lysosomal Akt.[22] However, in addition to AKT and autophagy pathways, PHLPP is also associated with activation of NF-κB signaling.[23] Tang et al reported that PHLPP upregulation was mediated by NF-κB transcriptional activity.[24] The NF-κB pathway regulates numerous genes, which influence the development and pathogenesis of multiple myeloma. IGF-1 was reported to be important for cell survival by AKT signaling pathways, which further mediate NF-κB activation in MM cells.[25] Thus, there may be a negative feed-back loop among NF-κB, PHLPP and Akt, which may lead to a mild effect on cell apoptosis. However, the precise mechan-ism needs more evidences to illustrate.

Low expression of LAMP2 was associated with poor over-all survival in a cohort of 150 AML patients. Moreover, in Azacytidine (Aza)-treated MDS cell lines and cells from MDS/AML patients, a progressive reduction in LAMP2 expression was correlated with a loss of sensitivity to Aza.[11] Bao et al found that the downregulation of LAMP2 in the lung cancer cells increased their resistance to Temozolomide.[26] In LAMP1/LAMP2 double-knockout mice, autophagic vacuoles accumu-lated in many tissues suggesting impaired autophagosome maturation.[27] In this study, we found that LAMP2 but not LAMP1 was downregulated in BTZ-resistant patients with MM and the expression of LAMP2A, not LAMP2B, was significantly downregulated in BTZ-resistant patients with MM compared the BTZ-sensitive patients with MM. LAMP2A was encoded by transcript variant A of Lamp2 gene, which is the longest and predominant form of this gene. These results indicated that LAMP2A is the executor in the regulation of CMA in MM cells. Re-expression of LAMP2 in LAMP2-deficient BTZ-resistant MM cells restored chaper-one-mediated autophagy (CMA), highlighting the key role of LAMP2 in this process. CMA is a highly selective form of autophagy that allows the elimination of substrates endowed with a KFERQ motif through direct transport to the lysosome. Lysosomes can sensitize cancer cells to lysosomal membrane permeabilization and cell death.[28,29] In this study, we found that LAMP2 levels were reduced in BTZ-resistant MM cells, and knockdown of LAMP2 reversed PHLPP-mediated cell apoptosis in BTZ-resistant U266 cells. Therefore, LAMP2 contributes to BTZ resistance and cell apoptosis may be via CMA activation. Our results showed that PHLPP interacted with LAMP2, and LAMP2 is required for PHLPP-mediated autophagy. It would be reasonable that PHLPP acts primarily to interact with and stabilize LAMP2 on the lysosomes to promote the fusion between autophagosome and lysosome (ie, formation of autolysosome) and thereby removal of autophagosome.

In summary, our findings reveal for the first time that interaction of PHLPP and LAMP2 partially sensitizes MM cells to BTZ by enhancing autophagy. Rescue of PHLPP is a potential strategy for the management of BTZ resistance in patients with MM.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of The Third Xiangya Hospital, Central South University. Informed consent was obtained from each subject in accordance with the Declaration of Helsinki. Animal experiments were approved by the Ethics Committee for Animal Research of the Third Xiangya Hospital of Central South University following the National Research Council's Guide for the Care and Use of Laboratory Animals (Eight Edition).

Acknowledgment

Author Contributions

Funding

Disclosure

The authors declare that they have no conflicts of interest in this work.

References

1. Hu J, Hu WX. Targeting signaling pathways in multiple myeloma: pathogenesis and implication for treatments. *Cancer Lett*. 2018;414:214–221.

2. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2005;352(24):2487–2498. doi:10.1056/NEJMoa043445

3. Palumbo A, Chanan-Khan A, Weisel K, et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016;375(8):754–766. doi:10.1056/NEJMoa1606038

4. Farrell ML, Reagan MR. Soluble and cell-cell-mediated drivers of proteasome inhibitor resistance in multiple myeloma. *Front Endocrinol (Lausanne)*. 2018;9:218.

5. Grzechnik AT, Newton AC. PHLPPing through history: a decade in the life of PHLPP phosphatases. *Biochem Soc Trans*. 2016;44 (6):1675–1682. doi:10.1042/BST20160170

6. Newton AC, Trotman LC. Turning off AKT: PHLPP as a drug target. *Annu Rev Pharmacol Toxicol*. 2014;54:537–558.

7. Lv D, Yang H, Wang W, et al. High PHLPP expression is associated with better prognosis in patients with resected lung adenocarcinoma. *BMC Cancer*. 2015;15:687.

8. Wen YA, Stevens PD, Gasser ML, Andrei R, Gao T. Downregulation of PHLPP expression contributes to hypoxia-induced resistance to chemotherapy in colon cancer cells. *Mol Cell Biol*. 2013;33 (22):4594–4605. doi:10.1128/MCB.00695-13

9. Alessandrini F, Pezze L, Ciribilli Y. LAMPs: shedding light on cancer biology. *Semin Oncol*. 2017;44(4):239–253. doi:10.1053/j.seminoncol. 2017.10.013

10. Pajares M, Rojo AI, Arias E, et al. Transcription factor NFE2L2/ NRF2 modulates chaperone-mediated autophagy through the regulation of LAMP2A. *Autophagy*. 2018;14(8):1310–1322. doi:10.1080/ 15548627.2018.1474992

11. Dubois A, Furstoss N, Calleja A, et al. LAMP2 expression dictates azacytidine response and prognosis in MDS/AML. *Leukemia*. 2019;33:1501–1513. doi:10.1038/s41375-018-0336-1

12. Gentile M, Recchia AG, Mazzone C, Morabito F. Emerging biological insights and novel treatment strategies in multiple myeloma. *Expert Opin Emerg Drugs*. 2012;17(3):407–438. doi:10.1517/ 14728214.2012.713345

13. Fuchs O. Targeting of NF-kappaB signaling pathway, other signaling pathways and epigenetics in therapy of multiple myeloma. *Cardiovasc Hematol Disord Drug Targets*. 2013;13(1):16–34. doi:10.2174/1871529X11313010003

14. Wang W, Hayashi J, Serrero G. PC cell-derived growth factor confers resistance to dexamethasone and promotes tumorigenesis in human multiple myeloma. *Clin Cancer Res*. 2006;12(1):49–56. doi:10.1158/ 1078-0432.CCR-05-0929

15. Tsuyama N, Ishikawa H, Abroun S, et al. The regulatory mechanism of IL-6-dependent proliferation of human myeloma cells. *Hematology*. 2003;8(6):409–411. doi:10.1080/102453303100016213 05

16. Kuhn DJ, Berkova Z, Jones RJ, et al. Targeting the insulin-like growth factor-1 receptor to overcome bortezomib resistance in preclinical models of multiple myeloma. *Blood*. 2012;120(16):3260–3270. doi:10.1182/ blood-2011-10-386789

17. Xiang RF, Wang Y, Zhang N, et al. MK2206 enhances the cytocidal effects of bufalin in multiple myeloma by inhibiting the AKT/mTOR pathway. *Cell Death Dis*. 2017;8(5):e2776. doi:10.1038/cddis. 2017.188

18. Pei H, Li L, Fridley BL, et al. FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt. *Cancer Cell*. 2009;16 (3):259–266. doi:10.1016/j.ccr.2009.07.016

19. Nitsche C, Edderkaoui M, Moore RM, et al. The phosphatase PHLPP1 regulates Akt2, promotes pancreatic cancer cell death, and inhibits tumor formation. *Gastroenterology*. 2012;142(2):377–387. doi:10.1053/j.gastro.2011.10.026

20. Qu YQ, Gordillo-Martinez F, Law B, et al. 2-Aminoethoxydiphenylborane sensitizes anti-tumor effect of bortezomib via suppression of calcium-mediated autophagy. *Cell Death Dis*. 2018;9(3):361. doi:10.1038/s41419-018-0397-0

21. Arias E. Lysosomal mTORC2/PHLPP1/Akt axis: a new point of control of chaperone-mediated autophagy. *Oncotarget*. 2015;6 (34):35147–35148. doi:10.18632/oncotarget.v6i34

22. Arias E, Koga H, Diaz A, et al. Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy. *Mol Cell*. 2015;59 (2):270–284. doi:10.1016/j.molcel.2015.05.030

23. Shang Z, Yu J, Sun L, et al. LncRNA PCAT1 activates AKT and NF-kappaB signaling in castration-resistant prostate cancer by regulating the PHLPP/FKBP51/IKKalpha complex. *Nucleic Acids Res*. 2019;47(8):4211–4225. doi:10.1093/nar/gkz108

24. Tang B, Ma J, Ha X, Zhang Y, Xing Y. Tumor necrosis factor-alpha upregulated PHLPP1 through activating nuclear factor-kappa B during myocardial ischemia/reperfusion. *Life Sci*. 2018;207:355–363.

25. Mitsiades CS, Mitsiades N, Poulaki V, et al. Activation of NF-kappaB and upregulation of intracellular anti-apoptotic proteins via the IGF-1/Akt signaling in human multiple myeloma cells: therapeutic implications. *Oncogene*. 2002;21(37):5673–5683. doi:10.103 8/sj.onc.1205664

26. Bao L, Lv L, Feng J, et al. miR-487b-5p regulates temozolomide resistance of lung cancer cells through LAMP2-medicated autophagy. *DNA Cell Biol*. 2016;35(8):385–392. doi:10.1089/dna.2016.3259

27. Rowland TJ, Sweet ME, Mestroni L, Taylor MR. Danon disease - dysregulation of autophagy in a multisystem disorder with cardiomyopathy. *J Cell Sci*. 2016;129(11):2135–2143. doi:10.1242/ jcs.184770

28. Kallunki T, Olsen OD, Jaattela M. Cancer-associated lysosomal changes: friends or foes? *Oncogene*. 2013;32(16):1995–2004. doi:10.1038/onc.2012.292

29. Piao S, Amaravadi RK. Targeting the lysosome in cancer. *Ann N Y Acad Sci*. 2016;1371(1):45–54. doi:10.1111/nyas.12953