Preferential cytotoxicity of bortezomib toward highly malignant human liposarcoma cells via suppression of MDR1 expression and function

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Liposarcoma is the most common soft tissue sarcoma with a high risk of relapse. Few therapeutic options are available for the aggressive local or metastatic disease. Here, we report that the clinically used proteasome inhibitor bortezomib exhibits significantly stronger cytotoxicity toward highly malignant human liposarcoma SW872-S cells compared with its parental SW872 cells, which is accompanied by enhanced activation of apoptotic signaling both in vitro and in vivo. Treatment of cells with Jun-N-terminal kinase (JNK) inhibitor SP60015 or the translation inhibitor cycloheximide ameliorated this enhanced apoptosis. Bortezomib inhibited MDR1 expression and function more effectively in SW872-S cells than in SW872 cells, indicating that the increased cytotoxicity relies on the degree of proteasome inhibition. Furthermore, the pharmacological or genetic inhibition of sarco/endoplasmic reticulum calcium-ATPase (SERCA) 2, which is highly expressed in SW872-S cells, resulted in partial reversal of cell growth inhibition and increase of MDR1 expression in bortezomib-treated SW872-S cells. These results show that bortezomib exhibits preferential cytotoxicity toward SW872-S cells possibly via highly expressed SERCA2-associated MDR1 suppression and suggest that bortezomib may serve as a potent agent for treating advanced liposarcoma.

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Introduction

Soft tissue sarcomas are rare tumors of mesenchymal origin primarily affecting adults, and liposarcoma is the most frequent histopathological subtype (Conyers et al., 2011). These tumors have substantial morbidity and mortality with retroperitoneal sarcomas having particularly poor prognosis (Lewis et al., 1998; Singer et al., 2003; Jemal et al., 2006). The 2 major locations of liposarcoma are the extremities and the retroperitoneum, and the surgical excision represents the corner-stone of treatment, which is often followed by radiation and/or adjuvant chemotherapy (Skubitz and D’Adamo, 2007). However, despite the best locoregional control, disease relapse is common and only the small subgroup of liposarcoma is chemotherapy sensitive (Jones et al., 2005). Few therapeutic options are available for aggressive local or metastatic liposarcoma. Therefore, there is an urgent need for efficient liposarcoma therapies.

The proteasome is responsible for the degradation of most cellular proteins and involved in the regulation of many processes, including cell cycle progression and apoptosis. The role of the proteasome in regulating the growth and survival of tumor cells makes it an attractive therapeutic target for anticancer drugs (Adams, 2004). Bortezomib, a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome, is the first defined therapeutic proteasome inhibitor approved by FDA for treating refractory, advanced or rapidly relapsed multiple myeloma (Roccaro et al., 2006; Chhrial et al., 2007). It is in clinical trials as a single agent or in combination with chemotherapeutics against other solid tumor malignancies (Richardson et al., 2006). The mechanisms of bortezomib against tumor cells involve induction of apoptosis via a Jun-N-terminal kinase (JNK)- or NF-κB-dependent pathway, as well as augmentation of endoplasmic reticulum (ER) stress or autophagy (Lauricella et al., 2006; Moriya et al., 2013). In the present study, we investigated the therapeutic potency of bortezomib against human liposarcoma, particularly aggressive malignant liposarcoma by using a pair of human liposarcoma cell lines, SW872 and its new sub-cell line SW872-S that was established by us previously through repeated in vivo inoculation approach (Wang et al., 2011a). The SW872-S cells show much higher capabilities of tumorigenesis and proliferation, and lower differential capabilities than its parental SW872 cells (Wang et al., 2014). Interestingly, we found that SW872-S cells showed the opposite characteristics toward different anticancer drugs, which were more resistant to most cytotoxic agents or tyrosine kinase inhibitor sunitinib but more sensitive to bortezomib, than SW872 cells did.
The greater sensitivity of SW872-S cells to bortezomib may be due to more effectively downregulated MDR1 mRNA, protein expression and transport activity. The pharmacological or genetic inhibition of sarco/endoplasmic reticulum calcium-ATPase (SERCA) 2, which is highly expressed in SW872-S cells, resulted in partial reversal of cell growth inhibition and increase of MDR1 expression in bortezomib-treated SW872-S cells. These findings implicate SERCA2-associated MDR1 suppression and provide new insights into the regulatory mechanism for preferential sensitivity of SW872-S cells to bortezomib and suggest that bortezomib may serve as a potent agent for treating advanced liposarcoma.

Materials and methods

Cells and reagents

Human liposarcoma cell line SW872 cells were purchased from the American Type Culture Collection (Rockville, MD). SW872-S cells with high tumorigenicity were established by in vivo re-inoculation approach as described previously (Wang et al., 2011a). Cells were maintained in DMEM (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Life Technologies), 100 U/mL penicillin, 100 μg/mL streptomycin. Bortezomib (>98% purity) was purchased from Selleck Chemicals (Houston, TX), Edopocide, doxorubicin, methotrexate, 5-fluorouracil (5-FU) and cisplatin were purchased from Sigma-Aldrich (St. Louis, MO). Sunitinib was from Pfizer. The JNK inhibitor SP600125 and cycloheximide were from EMD Biosciences (San Diego, CA).

Mice and growth of human liposarcoma in nude mice

Six-week-old female NCR-nu/nu (nude) mice were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. Cultured SW872 or SW872-S cells were washed with and resuspended in ice-cold PBS. Portions of the suspension (1.5 × 10⁶ cells in 0.1 mL) were injected into the right flank of nude mice. When the tumors grew to 50 mm³, the tumor-bearing mice were distributed into two groups. Bortezomib, dissolved in PBS, was given daily for 30 days by intraperitoneal injection at the dose of 100 mg/kg. Tumor volumes were measured every 3 days and calculated by the following formula: 0.5236 × L1 × (L2)², where L1 and L2 are the long and short diameters of the tumor mass, respectively. Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals (The Ministry of Science and Technology of China, 2006). All the animal experiments were approved by Nanjing University Animal Care and Use Committee and made to minimize suffering and to reduce the number of animals used.

MTT assay

Cells were plated into the 96-well plates and incubated with or without various concentrations of drugs. At the end of the incubation, MTT solution (4 mg/ml in PBS, Sigma-Aldrich) was added (10 μl/well), and the plates were incubated for a further 4 h at 37 °C. The purple formazan crystals were dissolved in 100 μL of DMSO. Then the plates were read on an automated microplate spectrophotometer at 570 nm. Assays were performed in triplicate on three independent experiments.

Western blot

Western blot was performed as described (Wang et al., 2011b). Antibodies to ubiquitin, caspase 9, cleaved caspase 3 and PARP, cleaved PARP, Bcl-xl, cytochrome c, Bax, JNK, phosphorylated JNK (Cell Signal Technology, Beverly, MA), antibodies to MDR1 (Sigma) and antibodies to Bcl-2, GAPDH, a-tublin, COX IV (Santa Cruz Biotechnology, Santa Cruz, CA) were used for blotting. Final detection was performed using a LumiGLO chemiluminescent substrate system (KPL, Guildford, UK). Protein bands were visualized using western blotting detection system according to the manufacturer’s instructions.

Histology and TUNEL assay

H&E staining in tumor tissues were done following the manufacturer’s protocol (Beyotime, Nanjing). TUNEL assay was performed to detect apoptotic cells using the TUNEL BrightGreen Apoptosis Detection Kit from Vazyme (Piscataway, NJ) according to the manufacturer’s instructions.

Real-time PCR

Quantitative PCR was performed with the ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA) using SYBR Green I dye (Biotium, Inc.). Condition for amplification was one cycle of 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 35 s and 72 °C for 45 s. The primer sequences used in this study were as follows: MDR1 forward, 5′-GGGATGTCACTGTGTGATGGA-3′; MDR1 reverse, 5′-CTATCCTGCTGGACAAAAATATA-3′; GAPDH forward, 5′-CATGCCTTCCGTGTCTCCTA-3′; GAPDH reverse, 5′-GCGGACGTCAAGATCCA-3′.

Flow cytometry

The MDR1 functional activity was determined by Rhodamine 123 (Rh-123, Sigma) efflux, as this fluorescent dye is a substrate for MDR1 (O’Connor et al., 2013). The 2 × 10⁶ cells were seeded in 6-well plates and treated with bortezomib at the concentrations indicated for 72 h. The cells were then pelleted and incubated with 200 ng/mL of Rh-123 dye in the presence or absence of the MDR inhibitor, verapamil (Sigma) at a concentration of 10 μM for 30 min at 37 °C in a humidified atmosphere of air and 5% CO₂. After washing, cells were incubated in a Rh-123-free medium supplemented with 10% FCS, and aliquots were removed for analysis at 30, 60 and 120 min, respectively. Data acquisition and analysis were performed using a FACSscan (Becton Dickinson) at an excitation wavelength of 488 nm, and data were analyzed by Cell Quest software (Molecular Devices Corporation). The results were reported as the mean of the median Rh-123 fluorescence intensity relative to control at each time point.

siRNA transfection

Chemically synthesized 21-nucleotide sense and antisense RNA oligonucleotides were obtained from Invitrogen. Cells were seeded on six-well plates at 3 × 10⁵ cells per well and transfected with bortezomib at the concentrations indicated for 72 h. The cells were then pelleted and incubated with 100 pmol of Rh-123 dye in the presence or absence of the MDR inhibitor, verapamil (Sigma) at a concentration of 10 μM for 30 min at 37 °C in a humidified atmosphere of air and 5% CO₂. After washing, cells were incubated in a Rh-123-free medium supplemented with 10% FCS, and aliquots were removed for analysis at 30, 60 and 120 min, respectively. Data acquisition and analysis were performed using a FACSscan (Becton Dickinson) at an excitation wavelength of 488 nm, and data were analyzed by Cell Quest software (Molecular Devices Corporation). The results were reported as the mean of the median Rh-123 fluorescence intensity relative to control at each time point.

Statistical analysis

Data were expressed as mean ± SEM of three independent experiments. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Student’s two-tailed t-test. The Kaplan–Meier method was used to evaluate the survival results. P < 0.05 was considered significant.

Results

Malignantly progressed liposarcoma subpopulation of SW872 cells shows an opposite sensitivity to different anticancer drugs

Using SW872-S cells and its parental SW872 cells, we tested the cytotoxic effects of chemotherapeutic agents etoposide, 5-FU, methotrexate, cisplatin, doxorubicin and molecular-targeted drug sunitinib. As shown in Fig. 1A, the highly malignant SW872-S cells exhibited more resistance...
to most of the cytotoxic agents and sunitinib than SW872 cells. By contrast, SW872-S cells were significantly more sensitive in a dose- and time-dependent manner when exposed to the proteasome inhibitor bortezomib with about 7-fold lower IC_{50} compared with SW872 cells (IC_{50} was 29.6 nM for SW872-S and 214 nM for SW872, respectively) (Fig. 1B).

**Apoptotic signaling is robustly enhanced in bortezomib-treated SW872-S cells**

The whole extracts of SW872 and SW872-S cells were assayed by western blot after 24-h incubation with various concentrations of bortezomib. As shown in Fig. 2A, bortezomib treatment induced the accumulation of ubiquitinated proteins both in SW872 and SW872-S cells. When the apoptotic signaling was tested, we found that bortezomib induced the cleavage of caspase 9, caspase 3 and PARP while reducing the expression of anti-apoptotic protein Bcl-2 in SW872 cells. Notably, the apoptotic signaling was further enhanced in bortezomib-treated SW872-S cells. In this case, anti-apoptotic Bcl-xl expression was not affected by bortezomib in either cell line. Because caspase 9 and Bcl-2 are mainly involved in mitochondria-dependent apoptosis, the cytoplasmic and mitochondrial extracts of SW872 and SW872-S cells were separated and analyzed. As shown in Fig. 2B, bortezomib-treated SW872-S cells showed a much increased release of cytochrome c.
from mitochondria to cytosol in a dose-dependent manner compared with the treated SW872 cells. The results of Bax expression were opposite.

In order to test the in vivo effect of bortezomib on SW872 and SW872-S cells, xenograft studies were performed. The cells were implanted subcutaneously into the right flank of nude mice. Five days later, the tumors began to enlarge in SW872-S cell-inoculated mice, while tumor formation was observed in the mice injected with the same amount of SW872 cells after 4 weeks. When the tumors grew to 50 mm³, the tumor-bearing mice were randomized into groups for control PBS or bortezomib. As shown in Fig. 3A, the administration of bortezomib at a dose of 100 mg/kg significantly inhibited the tumor growth in SW872-S-bearing mice compared with PBS controls, while no evident inhibition was observed in SW872-bearing mice until day 28 after treatment. The cleavage of PARP and caspase 3 was more evidently elevated in the tumor samples from the bortezomib-treated SW872-S-bearing mice than that from the treated SW872-bearing mice (Fig. 3B). In addition, histological and TUNEL staining assays showed more apparent apoptosis induction in tumor tissues from the treated SW872-S-bearing mice (Fig. 3C and D).

**Inhibition of JNK or blockade of protein synthesis almost completely reverses apoptosis induction of bortezomib in SW872-S cells**

A large body of literatures has shown that JNK activation plays critical roles in mediating bortezomib-induced apoptosis (Chauhan et al., 2004; Nawrocki et al., 2005; Lauricella et al., 2006; Carew et al., 2008). As expected, the treatment of SW872 cells with bortezomib led to phosphorylation of JNK1/2 in a dose-dependent manner (Fig. 4A). This response was greatly augmented in the treated SW872-S cells. In these cells, the pan-JNK inhibitor SP60015 suppressed the bortezomib-induced cleavage of caspase 9 completely, and the cleavage of caspase 3 and PARP partially (Fig. 4B).

Proteasome inhibitor bortezomib can induce the accumulation of misfolded protein in endoplasmic reticulum (ER), leading to ER stress-associated apoptosis. We hypothesized that the amelioration of ER protein load might reduce the bortezomib-induced apoptosis in SW872-S cells. As shown in Fig. 4C and D, the translation inhibitor cycloheximide almost completely blocked the effect of bortezomib on the cells including cell proliferation, the accumulation of ubiquitinated proteins, JNK activation and apoptotic signaling in SW872-S cells.

**Bortezomib shows stronger suppression on the expression and function of MDR1 in SW872-S cells than in SW872 cells**

The results, thus far, suggested that preferential sensitivity of SW872-S cells to bortezomib could be attributable to a higher degree of proteasome inhibition. However, there was no difference in the chymotrypsin-like activity of the 26S proteasome between these two cell lines (Supplementary Fig. S1), indicating that the increased sensitivity of SW872-S cells to bortezomib was not due to the difference of intrinsic proteasome activity. To investigate the mechanism underlymg, the effects of bortezomib on the expression and function of MDR1 were tested in SW872 and SW872-S cells. Bortezomib decreased the levels of MDR1 protein in a dose-dependent manner, which was more effective in SW872-S cells (Fig. 5A). In comparison, sunitinib showed a weak inhibition on the MDR1 protein expression in SW872 cells but lost the activity completely in SW872-S cells. Real-time PCR analysis also demonstrated the significantly increased the inhibition of bortezomib on MDR1 gene expression in SW872-S cells compared with SW872 cells (Fig. 5B). When a rhodamine-123 (Rh-123) efflux assay was used to determine the MDR1 function, bortezomib treatment resulted in a reduction in Rh-123 efflux, indicating a reduction in MDR1 function (Fig. 5C). This effect was more marked in SW872-S cells. Consistently, the chymotrypsin-like activity of the 26S proteasome was more strongly inhibited in SW872-S cells after bortezomib treatment (Fig. 5D).

**Inhibition of SERCA2 protects SW872-S cells from bortezomib**

We previously found that SW872-S cells exhibited greatly higher mRNA and protein levels of SERCA 2b expression than SW872 cells did, and the elevated expression of SERCA2 was closely related to the malignant degree of different types of human liposarcoma (Wang et al., 2011a, 2011b, 2014). To test the possible role of SERCA 2 in the increased sensitivity to bortezomib in the malignantly progressed liposarcoma subpopulation, we compared the effect of thapsigargin (TG), a pharmacological inhibitor of the SERCA pump, on bortezomib-treated SW872 and SW872-S cells. As shown in Fig. 6A, 10 nM of TG greatly ameliorated the effect of bortezomib, especially in SW872-S cells. Knocking down the expression of SERCA 2 ameliorated downregulation of MDR1 expression and the cleavage of PARP (Fig. 6B), and blocked the anti-proliferation effect of bortezomib with an increased...
inhibition rate in SW872-S cells compared with that in SW872 cells (Fig. 6C). Furthermore, the in vivo effect of bortezomib was examined on SERCA2 and MDR1 expression in the tumor samples from the bortezomib-treated mice. High levels of SERCA2 and more apparent reduction of MDR1 expression were observed in the tumor tissues from the treated SW872-S-bearing mice than those from the treated SW872-bearing mice (Fig. 6D).

Discussion

In general, tumor malignancies increase cellular resistance to various chemotherapeutic agents (Honoki, 2010; Crawford, 2013; Homet and Ribas, 2014). In this study, we compared the sensitivity to several clinically used anticancer agents between malignantly progressed liposarcoma SW872-S cells and its parental SW872 cells. Bortezomib was the most potent among these agents in killing SW872-S cells. The IC50 of bortezomib in SW872-S cells was about 7-fold lower than that in SW872 cells, while SW872-S cells exhibited higher resistance to most of the cytotoxic agents including 5-FU, methotrexate, cisplatin and tyrosine kinase inhibitor sunitinib. SW872-S cells are also preferentially sensitive to another proteasome inhibitor MG132 (data not shown). These findings may allow to better exploit the therapeutic potency of proteasome inhibitors in aggressive or metastatic liposarcoma.

To investigate the mechanism of such preferentially chemosensitivity, apoptotic signaling was compared between bortezomib-treated SW872 and SW872-S cells. The proteasome is involved in the ER-associated machinery for protein degradation that removes misfolded protein from the ER for cytosolic degradation. Proteasome inhibition thus results in the accumulation of misfolded proteins in the ER, which can induce excessive activation of unfolded protein response (UPR) leading to apoptosis via mitochondria-dependent or -independent pathways involving CHOP, JNK and caspase 4 (Zhang and Kaufman, 2006; Kraus et al., 2008). Indeed, bortezomib treatment induced the accumulation of ubiquitinated proteins in both SW872 and SW872-S cells. Robustly enhanced apoptotic signaling was found in SW872-S cells and SW872-S xenograft after bortezomib treatment. Taking into account the role of JNK activation in mediating bortezomib-induced apoptosis, we detected higher levels of phosphorylated JNK in treated SW872-S cells than SW872 cells. The inhibition of JNK with a pharmacological inhibitor or blockade of protein synthesis with a translation inhibitor ameliorated this enhanced response.
apoptosis, suggesting that the preferential sensitivity of SW872-S cells to bortezomib could be attributable to a higher degree of proteasome inhibition.

Recent studies have shown that bortezomib is a substrate for MDR-1 (also named as P-glycoprotein; ABCB1), but not for the other drug efflux transporters (Nakamura et al., 2007; Rumpold et al., 2007; O'Connor et al., 2007).

**Fig. 4.** Bortezomib-induced apoptotic signaling was partially reversed by the inhibition of JNK or blockade of protein synthesis in SW872-S cells. (A) SW872 and SW872-S cells were treated with various concentrations of bortezomib for 24 h. The protein levels of phosphorylated JNK and total JNK were determined by western blot. (B) SW872-S cells were pre-treated with or without 10 μM of SP600125 for 2 h, followed by incubation with indicated concentrations of bortezomib for 24 h. The protein levels of phosphorylated JNK, cleaved caspase 9, caspase 3 and PARP were detected. (C, D) SW872-S cells were treated with 5 μg/mL of cycloheximide (CHX) for 2 h, followed by incubation with indicated concentrations of bortezomib for 24 h. The survival cell number was counted. The data are mean ± SEM of three independent experiments. (D) The protein levels of ubiquitin-linked proteins, phosphorylated JNK, cleaved caspase 9, caspase 3 and PARP were detected. Data are representative of at least 3 experiments.

**Fig. 5.** Bortezomib suppressed MDR1 expression and function in SW872-S cells more effectively. SW872 and SW872-S cells were treated with indicated concentrations of bortezomib or sunitinib for 72 h. (A) The protein levels of MDR1 were determined by western blot. Bands were analyzed by densitometry. The data are mean ± SEM of three independent experiments. *P < 0.05, **P < 0.01 versus the control group without treatment. (B) The mRNA levels of MDR1 were determined by real-time PCR in bortezomib-treated cells. GAPDH was used as loading control. *P < 0.05 (C) Rhodamine-123 efflux was monitored in bortezomib-treated cells. The results were expressed relative to control at each time point (mean of the median intensity of rhodamine-123 fluorescence ± SEM). (D) The CT-like proteasome activity of bortezomib-treated cells was measured using a proteasome activity kit from BD according to the manufacturer’s instructions. *P < 0.05 versus the control group without treatment.
et al., 2013). Bortezomib activity is affected by the expression levels of MDR-1 expression. In the present study, bortezomib decreased MDR1 expression at both mRNA and protein levels more effectively in SW872-S cells. We also demonstrated that treatment with bortezomib caused an evident reduction in MDR1 function by showing an accumulation of Rh-123 in SW872-S cells. Accordingly, the chymotrypsin-like activity of the 26S proteasome was more strongly inhibited in SW872-S cells after bortezomib treatment. It is likely that more effective suppression on MDR1 expression and function might promote the intracellular bortezomib accumulation, resulting in the enhanced degree of proteasome inhibition in treated SW872-S cells. The finding that bortezomib directly inhibits the expression and function of MDR1 has also been reported in lung cancer and multiple myeloma cell lines (O’Connor et al., 2013). Similarly, proteasome inhibitors decrease the expression of MDR1 at both mRNA and protein levels in breast cancer and gastric cancer cells (Fujita et al., 2005; Zhang et al., 2008). Some studies suggest that MDR1 activation is dependent on NF-κB activation that requires an NF-κB-binding site located distal to the MDR1 promoter, and that inhibiting the NF-κB pathway with bortezomib will downregulate MDR1 expression (Kuo et al., 2002; Bentires-Alj et al., 2003; O’Connor et al., 2013). However, we found that the phosphorylation of IKKα/β, IκBα and NF-κB/p65 was evidently increased in bortezomib-treated SW872-S cells (supplementary Figure S2), which is in agreement with recent results (Hideshima et al., 2009). Therefore, the mechanism other than NF-κB inhibition could be responsible for the downregulation of MDR1 by bortezomib.

Malignant cells are characterized by increased basal ER stress and UPR activation, possibly due to their higher rate of production of defective protein, compared with nonmalignant cells (Scriven et al., 2007; Kraus et al., 2008). This may allow one to selectively eliminate malignant cells by appropriately increasing the degree of ER stress with bortezomib (Fels et al., 2008). However, more accumulation of ubiquitinated proteins was not observed in SW872-S cells than in SW872 cells (Fig. 2A). SW872-S cells seemed not more sensitive to the ER stressor TG compared with SW872 cells (Fig. 6A).

Our previous results demonstrated that greatly higher mRNA and protein levels of SERCA 2b expression were detected in SW872-S cells than in parental SW872 cells (Wang et al., 2014). SERCA acts as a Ca2+-ATPase that transfers Ca2+ from the cytosol to the lumen of the sarcoplasmic/endoplasmic reticulum, and SERCA-dependent calcium transport is the only calcium uptake mechanism in this organelle. ER calcium homeostasis is involved in a multitude of signaling (Monteith et al., 2007). It was previously shown that increased intracellular calcium concentration may regulate the transcription of the mdr1 gene and enhance the expression of MDR1 protein (Baudouin-Legros et al., 2003; Riganti et al., 2009). In our study, knocking down SERCA 2 increased the MDR1 expression in SW872-S cells (Fig. 6). The pharmacological or genetic inhibition of SERCA 2 resulted in partial reversal of cell growth inhibition induced by bortezomib. It is likely that the intracellular calcium concentration regulated by SERCA2 may play an important role in bortezomib-induced MDR1 suppression and finally lead to enhanced bortezomib sensitivity in SW872-S cells.

Fig. 6. The inhibition of SERCA2 attenuated the sensibility of SW872-S cells to bortezomib. (A) SW872 and SW872-S cells were treated with indicated concentrations of bortezomib and thapsigargin (TG) individually or in combination. Cell viability was determined by MTT assay after 72 h treatment. (B) SW872 and SW872-S cells were transfected with luciferase (NC) or SERCA2 siRNA for 24 h, followed by bortezomib treatment for 24 h. The protein levels of SERCA2, MDR1 and PARP were determined by western blot. Data are representative of at least 3 experiments. (C) SW872 and SW872-S cells were transfected with luciferase or SERCA2 siRNA for 24 h, followed by 100 nM bortezomib treatment for 72 h. Cell viability was determined. The data are mean ± SEM of three independent experiments. **P < 0.005, ***P < 0.001. (D) The protein levels of SERCA2 and MDR1 in the implanted tumors were determined by western blot. The tumor-bearing mice were treated with bortezomib as described in Fig. 3. Data are representative of at least 3 experiments.

In summary, our findings demonstrate that highly malignant human liposarcoma SW872-S cells, which were more resistant to cytotoxic agents, showed preferential sensitivity to bortezomib compared with its parental SW872 cells. The reliance of such sensitivity on high SERCA2 expression may implicate a unique therapeutic opportunity. Proteasome inhibitor bortezomib may serve as a potent agent for treating advanced liposarcoma via suppression of MDR1 expression and function.

Conflict of interest statement

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.taap.2014.12.015.

References