Suppression of MG132-mediated cell death by peroxiredoxin 1 through influence on ASK1 activation in human thyroid cancer cells

Zhen-Xian Du, Ying Yan¹, Hai-Yan Zhang², Bao-Qin Liu³, Yan-Yan Gao³, Xiao-Fang Niu³, Yifu Guan³, Xin Meng³ and Hua-Qin Wang³

Department of Endocrinology and Metabolism, The First Affiliated Hospital, China Medical University, Shenyang 110001, People's Republic of China

¹Department of Radiotherapy, Shenyang Northern Hospital, Shenyang 110016, People's Republic of China

²Department of Geriatrics, The First Affiliated Hospital and ³Department of Biochemistry and Molecular Biology, China Medical University, Shenyang 110001, People's Republic of China

(Correspondence should be addressed to H-Q Wang; Email: wanghq_doctor@hotmail.com)

Abstract

Proteasome inhibitors represent a novel class of antitumor agents with pre-clinical and clinical evidence of activity against hematologic malignancies and solid tumors. However, emerging evidence indicates that antiapoptotic factors may also accumulate as a consequence of exposure to these drugs, thus it seems plausible that the activation of survival signaling cascades might compromise their antitumoral effects. Peroxiredoxins (PRDXs) are a family of thiol-containing peroxidases identified primarily by their ability to remove cellular hydroperoxides. The function of PRDX1 in particular has been implicated in regulating cell proliferation, differentiation, and apoptosis. Another important finding is that aberrant upregulation of PRDX1 has been discovered in various cancers. Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase (MAPK) kinase kinase that is regulated under conditions of cellular stress. ASK1 phosphorylates c-Jun N-terminal kinase and p38 MAPK, and elicits an apoptotic response. ASK1 activity is regulated at multiple levels, one of which is through interaction with PRDX1. In this study, for the first time we report that upregulation of PRDX1 expression was found in thyroid cancer cells treated with proteasome inhibitors, and PRDX1 knockdown resulted in accelerated proteasome inhibitor-induced cell death. In addition, we demonstrated that ASK1 activity was implicated in the PRDX1-dependent response of thyroid cancer cells to proteasome inhibitor-mediated cell death.

Endocrine-Related Cancer (2010) 17 553–560

Introduction

Oxidative stress is widely recognized as a major pathophysiologic mechanism in various injuries that lead to either acute or chronic organ failure. This stress is characterized by the increased production of reactive oxygen species (ROS) such as superoxide radicals, H₂O₂, and hydroxyl radicals, which can result in the oxidation of macromolecules including nucleic acids, lipids, and proteins. Endogenous ROS are usually byproducts of cellular metabolism, and can be induced to high levels. Mammalian cells have developed many

antioxidative systems to maintain an appropriate level of ROS and regulate their action. Peroxiredoxins (PRDXs), a family of peroxidases that reduce intracellular peroxides (one type of ROS) with the thioredoxin system as the electron donor, are highly expressed in various cellular compartments. PRDXs are currently known to possess six mammalian isoforms. Although their individual roles in cellular redox regulation and antioxidant protection are quite distinct, they all catalyze peroxide reduction to balance cellular H₂O₂ levels essential for signaling and metabolism (Shi *et al.* 2008). Besides their cytoprotective

antioxidant function, PRDXs appear to play a role in cell proliferation, differentiation, immune response, protection of oxidant-sensitive proteins, regulation of cellular H₂O₂, control of apoptosis, and processes involving redox signaling (Shi *et al.* 2008).

PRDX1, whose function has been implicated in regulating cell proliferation, differentiation, and apoptosis, is the most abundant and ubiquitously distributed member of the mammalian PRDX family, having been identified in a large variety of organisms. Analysis of cells and mice lacking PRDX1 suggest that it not only regulates H₂O₂ levels, but also possesses the properties of a tumor suppressor (Neumann *et al.* 2003), which was further supported by the finding that PRDX1 interacts with c-Myc, thereby selectively inhibiting c-Myc transcriptional activity (Egler *et al.* 2005). However, many studies indicated that aberrant increased expression of PRDX1 is found in various kinds of cancers including thyroid, lung, and breast (Yanagawa *et al.* 1999, 2000, Chang *et al.* 2001, Kim *et al.* 2003, 2007, 2008, Alfonso *et al.* 2004, Lehtonen *et al.* 2004, Xin *et al.* 2005, Park *et al.* 2006), which obscures its actual role in tumorigenesis. As the hypoxic and unstable oxygenation microenvironment of a tumor is one of the key factors influencing tumor growth and progression, this increase in PRDX1 expression might be an adaptive response. In addition, the expression of PRDX is inducible by various stimuli including H₂O₂, okadaic acid (OA), 12-O-tetradecanoylphorbol 13-acetate (TPA), and butylated hydroxyanisole, which protects cells from these stimuli (Ishii *et al.* 2000, Immenschuh *et al.* 2002, Wijayanti *et al.* 2008). Furthermore, an increased level of PRDX1 was reported to be involved in the protection of cancer cells against various therapeutic challenges by neutralizing ROS and modulating the related signaling pathways (Butzke *et al.* 2004, Kim *et al.* 2006, Song *et al.* 2009). These findings indicate that PRDX1 functions as an important regulator of resistance against cancer therapy, in which induction might be an adaptive response of cells to various stimuli.

Apoptosis signal-regulating kinase 1 (ASK1) is a ubiquitously expressed serine–threonine protein kinase that functions as a mitogen-activated protein kinase (MAPK) kinase kinase to activate the c-Jun N-terminal kinase (JNK) and p38 MAPK signaling cascades (Nishitoh *et al.* 1998, Saitoh *et al.* 1998, Tobiume *et al.* 2001). ASK1 is activated in response to various stresses and plays a critical role in the regulation of signaling in response to oxidative stress, which is a major contributor to proteotoxic damage and cell death (Nishitoh *et al.* 1998, Saitoh *et al.* 1998, Tobiume *et al.* 2001, Goldman *et al.* 2004). Recently, PRDX1 has

been reported to function as a negative regulator of ASK1 activity (Kim *et al.* 2008), further suggesting a role for PRDX1 in protecting cancer cells from harmful stimuli.

Proteasome inhibitors represent a novel class of antitumor agents with pre-clinical and clinical evidence of activity against hematologic malignancies and solid tumors. However, it has to be noted that proteasome inhibitors paradoxically also trigger accumulation or even *de novo* synthesis of antiapoptotic factors and can compromise the cytotoxic effect of the drug (Yang *et al.* 2006, Zaarur *et al.* 2006, Podar *et al.* 2008, Wang *et al.* 2007, 2008). Suppression of these unwanted proteins in combination with an effective conventional cancer chemotherapeutic agent is therefore a rational approach to improve cancer therapy. In this study, we demonstrated for the first time that PRDX1 is another molecule which is induced by proteasome inhibitors and compromised the anticancer effects of proteasome inhibition. In addition, we showed that the specific, potent proteasome inhibitor MG132 activated ASK1 as well as its downstream effectors p38 and JNK, and induction of PRDX1 suppressed MG132-mediated thyroid cancer cell death by its influence on ASK1 activity.

Materials and methods

Culture of multiple cancer cell lines

FRO82-1 (simply FRO) cell lines were initially obtained from Dr James A Fagin (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) and were provided to us by Dr Shunichi Yamashita (Nagasaki University Graduate School of Biomedical Sciences, Japan). KTC1 and KTC3 cell lines were generously provided by Dr Junichi Kurebayashi (Kawasaki Medical School, Japan). 8305C and 8505C cells were obtained from the European Collection of Animal Cell Cultures. All cell lines were maintained in DMEM (Sigma–Aldrich) supplemented with 10% fetal bovine serum (Sigma–Aldrich).

Chemicals

MG132, epoxomicin, Z-Ile-Glu(OtBu)-Ala-Leu-H (PSI), and lactacystin were purchased from Calbiochem (La Jolla, CA, USA). Actinomycin D and tumor necrosis factor α (TNF α) were obtained from Sigma and PeproTech (London, UK) respectively. As a vehicle control, 0.02% dimethyl sulfoxide (DMSO) was used.

Detection of cell death

For cell death assays, cells were washed twice in PBS, and were then stained with Annexin V-FITC (Biovision, Mountainview, CA, USA) and propidium iodide (PI, Sigma–Aldrich) according to the manufacturer's instructions. After staining with annexin V-FITC and PI, samples were analyzed by fluorescence-activated cell scanner (FACScan) flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

RNA isolation and real-time reverse transcription-PCR

RNA isolation and real-time reverse transcription (RT)-PCR were performed as previously reported (Wang *et al.* 2007). 5'-CACAGCTGTTATGCCAGATG-3' and 5'-ACTGAAAGCAATGATCTCCG-3', 5'-AGATCATCGCGTTCAGCAAC-3' and 5'-ATCCTCAGACAAGCGTCTGG-3', 5'-GTCGCAGTCTCAGTGGATTC-3' and 5'-AACAGCACACCGTAGTCTCG-3', 5'-AACAGCTGTGATCGATGGAG-3' and 5'-TCAAGTCTGTCGCCAAAAGC-3', 5'-CAAGAAGGGTGTGCTGTTTG-3' and 5'-TAACACTCAGACAGGCCACC-3', and 5'-ATGCCTGTGACAGCTCGTGTG-3' and 5'-TCTTCTTCAGGGATGGTTGG-3' primer pairs were used to amplify PRDX1-6 respectively. For β -actin, the forward primer was 5'-GAGACCTTCAACACCCCAGCC-3', and the reverse primer was 5'-GGATCTTCATGAGGTAGTCAG-3'. Results were normalized against those of β -actin and presented as arbitrary unit.

Western blot analysis

Cells were lysed in lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, and protease inhibitor cocktail (Sigma–Aldrich). Cell extract protein amounts were quantified using the BSA protein assay kit. Equivalent amounts of protein (25 μ g) were separated using 12% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore Corporation, Billerica, MA, USA).

Construction of PRDX1 plasmid and cell transfection

A cDNA encoding human PRDX1 was generated by PCR from human brain cDNA library (Invitrogen) and subcloned into the EcoRI/XhoI sites of the eukaryotic expression plasmid pcDNA3. The construct was verified by DNA sequencing. Cells were transfected with Lipofectamine 2000 reagent (Invitrogen) as instructed by the supplier.

Small interfering RNA

The small interfering RNA (siRNA) sequences used here were as follows: siRNA against PRDX1 (siPRDX1), CAGAUGGUCAGUUUAAAGAU, siRNA against ASK1 (siASK1), AUGUGCUCACACGCCGGCUGUU. The scramble nonsense siRNA (scramble, CCGUAUCGUAAGCAGUACU) that has no homology to any known genes was used as control. Transfection of siRNA oligonucleotide was performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's recommendations.

Statistical analysis

Statistical significance was analyzed by ANOVA and *post hoc* Dunnett's test. Statistical significance was defined as $P < 0.05$. All experiments were repeated three times, and data were expressed as the mean $\pm$ s.d. from a representative experiment.

Results

Induction of PRDX1 expression by proteasome inhibitors in thyroid cancers

We first performed real-time RT-PCR to study the regulation of PRDX mRNA expression by MG132 in a panel of thyroid cancer cell lines. Incubation with MG132 significantly increased PRDX1 mRNA levels in the panel of thyroid cancer cells (Fig. 1A). Although to a much lesser extent, MG132 also enhanced PRDX6 mRNA levels (Fig. 1A). MG132 had no obvious effect on the mRNA expression levels of PRDX2–5 (Fig. 1A). Western blot analysis also demonstrated a marked increase in PRDX1 protein expression by MG132 treatment (Fig. 1B). Paradoxically, MG132 exposure had no stable effects on PRDX6 in the panel of thyroid cancer cells (Fig. 1B). The PRDX6 protein levels were slightly increased in 8305C and 8505C cells, no clear alteration was observed in KTC1 and KTC3 cells, and MG132 caused a decrease in FRO cells (Fig. 1B). To examine whether the increase in PRDX1 mRNA on MG132 treatment was due to an increase in PRDX1 mRNA stability or a transcriptional induction, we pretreated FRO cells with 10 μ g/ml actinomycin D for 1 h, and then treated with vehicle or MG132 for an additional 8 h. Pretreatment with the transcription inhibitor actinomycin D completely blocked the upregulation of PRDX1 by MG132 (Fig. 1C), indicating that MG132 induced increased PRDX1 expression at the transcriptional level. To further assess whether upregulation of PRDX1 was a general response to proteasome inhibition, we investigated PRDX1 expression in thyroid cancer cells

Figure 1 Induction of PRDX1 by proteasome inhibitors in thyroid cancer cells. (A) A panel of thyroid cancer cell lines were treated with 2 μ M MG132 for 8 h, total RNA was then isolated, and real-time RT-PCR was performed. (B) Cells were treated with 2 μ M MG132 for 24 h, and western blot analysis was performed using the indicated antibodies. (C) FRO cells were pretreated with vehicle control or 10 μ g/ml actinomycin D for 1 h, followed by treatment with MG132 for additional 8 h, PRDX1 mRNA expression was then investigated using real-time RT-PCR. (D) FRO cells were treated with vehicle control, 100 nM bortezomib, 10 nM PSI, 10 μ M lactacystin, 20 nM epoxomycin, or 2 μ M MG132 for 8 h, PRDX1 mRNA expression levels then were analyzed using real-time RT-PCR. (E) FRO cells were treated as in (D), and western blot analysis was performed. (F) 8305C and 8505C cells were treated as in (D), and western blot analysis was performed. * $P < 0.05$; ** $P < 0.01$; NS, not significant compared with vehicle-treated cells.

treated with different proteasome inhibitors. Similar to MG132, four other proteasome inhibitors bortezomib, PSI, lactacystin, and epoxomycin also elevated PRDX1 mRNA (Fig. 1D) and protein (Fig. 1E) expression levels in FRO cells. In addition, these different proteasome inhibitors also increased PRDX1 expression in 8305C and 8505C cells (Fig. 1F), indicating that induction of PRDX1 is a general effect of proteasome inhibition.

Promotion of MG132-mediated thyroid cancer cell death by suppression of PRDX1 induction

We next examined the functional roles of PRDX1 in MG132-mediated apoptosis in thyroid cancer cells. FRO cells were transfected with PRDX1 siRNA (siPRDX1), the level of knockdown was then determined by real-time RT-PCR (Fig. 2A) and western blot analysis (Fig. 2B). siPRDX1 efficiently suppressed MG132-mediated upregulation of PRDX1,

~30–40% of the PRDX1 protein detected in the control cells (scramble) was detected in siPRDX1-transfected cells (Fig. 2B). To determine the effect of PRDX1 knockdown on proteasome inhibitor-mediated cell death, siPRDX1-transfected cells were cultured for 24 h in the presence of MG132, labeled with annexin V-FITC/PI, and subjected to flow cytometry. Cells transfected with siPRDX1 displayed an abrupt increase in cell death with MG132 treatment (Fig. 2C). siPRDX1 also increased the sensitivity of FRO cells to other proteasome inhibitors including lactacystin and epoxomycin (Fig. 2D). In addition, siPRDX1 also increased the sensitivity of two other thyroid cancer cell lines, 8305C and 8505C cells, to MG132-induced cell toxicity (Fig. 2E). To further confirm whether the exogenously expressed PRDX1 could compromise the sensitizing effect of siPRDX1 on MG132, we investigated the effect of PRDX1 overexpression (Fig. 2F). Overexpression of PRDX1 *per se* had no

Figure 2 Promotion of MG132-mediated apoptosis by knockdown of PRDX1 by siRNA. (A) FRO cells were transfected with scrambled siRNA or siRNA against PRDX1 (siPRDX1) for 48 h, and were then treated with vehicle control or MG132 for additional 24 h, PRDX1 mRNA was then measured using real-time RT-PCR. (B) FRO cells were treated as in (A), and western blot analysis was performed. (C) FRO cells were treated as in (A), and apoptotic cells were analyzed using flow cytometry. (D) FRO cells were transfected with scramble or siPRDX1 for 48 h, and were then treated with vehicle control, lactacystin or epoxomycin for an additional 24 h, and apoptotic cells were analyzed. (E) 8305C and 8505C cells were transfected with scramble or siPRDX1 for 48 h, and were then treated with vehicle control or MG132 for an additional 24 h, and apoptotic cells were analyzed using flow cytometry. (F) FRO cells transfected with mock (1), siPRDX1 (2), PRDX1 expression vector (3) or both siPRDX1 and PRDX1 expression vector (4), were then treated with vehicle control or MG132 for 24 h, and western blot was performed. (G) FRO cells were treated as in (E), and apoptotic cells were analyzed. * $P < 0.01$ and NS, not significant.

significant effect on MG132-mediated apoptosis, but it recovered the responsiveness of siPRDX1 to MG132-induced cell death (Fig. 2G).

Implication of ASK1 activation in MG132-mediated thyroid cancer cell death

ASK1 is activated in response to various stresses including H_2O_2 , serum withdrawal, endoplasmic reticulum stress, and $TNF\alpha$ (Ichijo *et al.* 1997, Tobiume *et al.* 1997, Chang *et al.* 1998, Morita *et al.* 2001). Therefore, we investigated whether ASK1 was also involved in MG132-mediated thyroid cancer cell death. We first examined the effect of MG132 on the catalytic activity of ASK1 and its downstream effectors p38 and JNK by using their respective anti-phosphorylated antibodies. Similar to $TNF\alpha$, the well-known activator of ASK1, treatment of FRO cells with MG132 activated endogenous ASK1, as well as its downstream effectors p38 and JNK (Fig. 3A), indicating that MG132 activates the ASK1–p38 and the ASK1–JNK pathways. We next examined whether ASK1 is required for the activation of p38 and JNK, as well as whether ASK1 is required for the induction of cell death mediated by proteasome inhibition, by using siRNA raised against ASK1 (siASK1) to knock down endogenous ASK1. siASK1 successfully

reduced the expression of total and phosphorylated ASK1 (Fig. 3B). siASK1 also dramatically decreased the activation of p38 and JNK mediated by MG132, as well as by lactacystin (Fig. 3B). In addition, the apoptotic FRO cell death induced by MG132 or lactacystin was clearly suppressed by siASK1 (Fig. 3C), indicating that ASK1 is an essential component of p38 and JNK activation, as well as FRO cell death induced by proteasome inhibition. To investigate the general involvement of ASK1 activation in proteasome inhibition-mediated thyroid cancer cell death, we checked the effect of siASK1 in thyroid cancer KTC1 and 8505C cells, in which cell death could be induced by proteasome inhibitors (Wang *et al.* 2007). siASK1 treatment significantly protected both KTC1 and 8505C cells from MG132-induced cell death when compared with scrambled siRNAs (Fig. 3D).

Suppression of MG132-mediated ASK1 activation by PRDX1

It has been reported that PRDX1 negatively regulates ASK1 activity (Kim *et al.* 2008), we hypothesized that induction of PRDX1 may affect MG132-induced apoptosis via its influence on the activation of ASK1. To explore this question, we tested whether PRDX1

knockdown can affect the activation of ASK1-mediated signaling by MG132. FRO cells were transiently transfected with scramble or siPRDX1 and stimulated with MG132 for various time periods, and then the level of phosphorylation of ASK1, p38, and JNK was evaluated. Upon the stimulation of MG132, phosphorylated ASK1 was first detected after 2 h, the levels gradually increased in a time-dependent manner and peaked at 8–12 h (Fig. 4A). Similarly, phosphorylation of p38 and JNK increased in a time-dependent manner and peaked at 8–12 h (Fig. 4A). Interestingly, the levels of phosphorylated ASK1 quickly and strongly appeared at 1 h, peaked at 4–8 h, and were then continuously maintained in PRDX1 knockdown cells (Fig. 4A). Similar patterns of p38 and JNK phosphorylation could be detected in PRDX1 knockdown cells (Fig. 4A). To further confirm the role of ASK1 in the PRDX1-dependent response of thyroid cancer cells to MG132-mediated apoptosis, we knocked down ASK1 in FRO cells transfected with siPRDX1. Suppression of ASK1 clearly decreased siPRDX1-mediated promotion of apoptotic cell death induced by MG132 (Fig. 4B),

Figure 4 Implication of ASK1 in PRDX1-dependent effects on MG132-mediated cell death. (A) FRO cells were transfected with scramble or siPRDX1 for 24 h, treated with MG132 for the indicated time, and western blot analysis was then performed. (B) FRO cells were treated with siPRDX1 or siASK1 alone or combination, and apoptotic cells were analyzed using flow cytometry. * $P < 0.05$; ** $P < 0.01$.

Figure 3 Involvement of ASK1 in MG132-mediated cell death in FRO cells. (A) FRO cells were treated with MG132 or TNF α for 24 h, and western blot analysis was then performed using the indicated antibodies. (B) FRO cells were treated with scramble or siASK1 for 24 h, followed by treatment with MG132 or lactacystin for an additional 24 h, and western blot analysis was then performed. (C) Cells were treated as in (B), and apoptotic cells were analyzed using flow cytometry. (D) KTC1 or 8505C cells were transfected with scramble or siASK1 for 24 h, followed by treatment with MG132 for an additional 24 h, and apoptotic cells were analyzed. * $P < 0.01$.

these results strongly suggest that the induction of PRDX1 as a negative regulator plays a key role for ASK1-mediated cell death in response to MG132 in undifferentiated thyroid cancer cells.

Discussion

The proteasome represents the major degradation pathway for proteins involved in the regulation of cell survival, proliferation, apoptosis, and other critical cellular functions (Adams 2004). The proteasome also has a critical role in the selective removal of mutant, damaged, and misfolded proteins. Based on these unique properties of the ubiquitin-proteasome system, the proteasome pathway has recently emerged as an attractive target for the development of anticancer agents. Paradoxically, proteasome inhibitors also activate survival signaling cascades, thus compromising their anticancer effects (Mimnaugh et al. 2004, Nencioni et al. 2005). Therefore, the identification of the antiapoptotic activities of proteasome inhibitors could lead to the design of novel, rational combination regimens with enhanced antitumoral efficacy.

The PRDX family includes six known members (PRDX1–6). In the current study, we found that MG132 specifically upregulated expression of PRDX1 and PRDX6 at the transcriptional level. At the protein level, consistent with mRNA expression, MG132

increased PRDX1 protein levels. MG132 had no stable effects on PRDX6 protein levels in the panel of cell lines investigated. MG132 caused a slight increase in PRDX6 a protein in KTC3, 8305C, and 8505C cells. However, MG132 resulted in a decrease in PRDX6 protein levels in FRO and KTC1 cells. Currently, the mechanisms underlying the differential mRNA and protein expressions of PRDX6, as well as the functional effects in the panel of thyroid cancer cell lines, are under investigation in our laboratory. Besides proteasome inhibitors, H₂O₂, OA, TPA, and butylated hydroxyanisole could also induce the expression of PRDX1 (Ishii *et al.* 2000, Immenschuh *et al.* 2002, Wijayanti *et al.* 2008), indicating the expression of PRDX1 is inducible by various stimuli. PRDX1 expression is also elevated in most cancers, although the physiological mechanism is still unclear (Yanagawa *et al.* 1999, 2000, Chang *et al.* 2001, Kim *et al.* 2003, 2007, 2008, Alfonso *et al.* 2004, Lehtonen *et al.* 2004, Xin *et al.* 2005, Park *et al.* 2006). Previous studies have reported that an increased level of PRDX1 might be involved in the protection of cancer cells against various therapeutic challenges (Butzke *et al.* 2004, Kim *et al.* 2006, Song *et al.* 2009). Consistent with these reports, this study demonstrated that the induction of PRDX1 by proteasome inhibition compromised any antitumoral cytotoxicity. Based on these results, it is tempting to propose that increased PRDX1 in cancer cells may play a critical role in providing protection against harmful stimuli.

In mammalian cells, ASK1 participates in the JNK and p38 MAPK signaling cascades by phosphorylating MKK4/MKK7 and MKK3/MKK6 respectively (Nishitoh *et al.* 1998). ASK1 is activated in response to various extracellular and intracellular stimuli, such as lipopolysaccharide, ROS, endoplasmic reticulum (ER) stress, and signaling through Fas and TNF α (Matsukawa *et al.* 2004). ASK1 is well known as a proapoptotic, stress-activated signaling molecule, and it is under tight regulation at multiple levels, one of which is negatively regulated by PRDX1 (Kim *et al.* 2008). In this study, we demonstrated that ASK1 played an essential role in MG132-mediated thyroid cancer cell death, as ASK1 knockdown by siRNA markedly suppressed the cytotoxic effects of MG132. Consistent with previous reports, we detected that induction of PRDX1 reduced MG132-mediated cell death via suppression of ASK1 activation.

In conclusion, we have described that PRDX1 is another ‘unwanted molecule’ in cancer therapy with proteasome inhibitors, which compromises the antitumoral cytotoxic responses of thyroid cancer cells treated with MG132 via the negative modulation of ASK1 activity and inhibited ASK1-mediated apoptosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by National Natural Science Foundation of China (30740086, 30870522) and Shenyang Outstanding Talent Foundation to H-Q Wang.

References

- Adams J 2004 The proteasome: a suitable antineoplastic target. *Nature Reviews. Cancer* **4** 349–360.
- Alfonso P, Catala M, Rico-Morales ML, Durante-Rodriguez G, Moro-Rodriguez E, Fernandez-Garcia H, Escribano JM, Alvarez-Fernandez E & Garcia-Poblete E 2004 Proteomic analysis of lung biopsies: differential protein expression profile between peritumoral and tumoral tissue. *Proteomics* **4** 442–447.
- Butzke D, Machuy N, Thiede B, Hurwitz R, Goedert S & Rudel T 2004 Hydrogen peroxide produced by Aplysia ink toxin kills tumor cells independent of apoptosis via peroxiredoxin I sensitive pathways. *Cell Death and Differentiation* **11** 608–617.
- Chang HY, Nishitoh H, Yang X, Ichijo H & Baltimore D 1998 Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* **281** 1860–1863.
- Chang JW, Jeon HB, Lee JH, Yoo JS, Chun JS, Kim JH & Yoo YJ 2001 Augmented expression of peroxiredoxin I in lung cancer. *Biochemical and Biophysical Research Communications* **289** 507–512.
- Egler RA, Fernandes E, Rothermund K, Sereika S, de Souza-Pinto N, Jaruga P, Dizdaroglu M & Prochownik EV 2005 Regulation of reactive oxygen species, DNA damage, and c-Myc function by peroxiredoxin 1. *Oncogene* **24** 8038–8050.
- Goldman EH, Chen L & Fu H 2004 Activation of apoptosis signal-regulating kinase 1 by reactive oxygen species through dephosphorylation at serine 967 and 14-3-3 dissociation. *Journal of Biological Chemistry* **279** 10442–10449.
- Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K & Gotoh Y 1997 Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* **275** 90–94.
- Immenschuh S, Iwahara S & Schwennen B 2002 Induction of heme-binding protein 23/peroxiredoxin I gene expression by okadaic acid in cultured rat hepatocytes. *DNA and Cell Biology* **21** 347–354.
- Ishii T, Itoh K, Akasaka J, Yanagawa T, Takahashi S, Yoshida H, Bannai S & Yamamoto M 2000 Induction of murine intestinal and hepatic peroxiredoxin MSP23 by dietary butylated hydroxyanisole. *Carcinogenesis* **21** 1013–1016.
- Kim HJ, Chae HZ, Kim YJ, Kim YH, Hwang TS, Park EM & Park YM 2003 Preferential elevation of Prx I and Trx

- expression in lung cancer cells following hypoxia and in human lung cancer tissues. *Cell Biology and Toxicology* **19** 285–298.
- Kim YJ, Lee WS, Ip C, Chae HZ, Park EM & Park YM 2006 Prx1 suppresses radiation-induced c-Jun NH2-terminal kinase signaling in lung cancer cells through interaction with the glutathione S-transferase Pi/c-Jun NH2-terminal kinase complex. *Cancer Research* **66** 7136–7142.
- Kim JH, Bogner PN, Ramnath N, Park Y, Yu J & Park YM 2007 Elevated peroxiredoxin 1, but not NF-E2-related factor 2, is an independent prognostic factor for disease recurrence and reduced survival in stage I non-small cell lung cancer. *Clinical Cancer Research* **13** 3875–3882.
- Kim J, Lowe T & Hoppe T 2008 Protein quality control gets muscle into shape. *Trends in Cell Biology* **18** 264–272.
- Lehtonen ST, Svensk AM, Soini Y, Paakko P, Hirvikoski P, Kang SW, Saily M & Kinnula VL 2004 Peroxiredoxins, a novel protein family in lung cancer. *International Journal of Cancer* **111** 514–521.
- Matsukawa J, Matsuzawa A, Takeda K & Ichijo H 2004 The ASK1–MAP kinase cascades in mammalian stress response. *Journal of Biochemistry* **136** 261–265.
- Minnaugh EG, Xu W, Vos M, Yuan X, Isaacs JS, Bisht KS, Gius D & Neckers L 2004 Simultaneous inhibition of hsp 90 and the proteasome promotes protein ubiquitination, causes endoplasmic reticulum-derived cytosolic vacuolization, and enhances antitumor activity. *Molecular Cancer Therapeutics* **3** 551–566.
- Morita K, Saitoh M, Tobiume K, Matsuura H, Enomoto S, Nishitoh H & Ichijo H 2001 Negative feedback regulation of ASK1 by protein phosphatase 5 (PP5) in response to oxidative stress. *EMBO Journal* **20** 6028–6036.
- Nencioni A, Hua F, Dillon CP, Yokoo R, Scheiermann C, Cardone MH, Barbieri E, Rocco I, Garuti A, Wesselborg S *et al.* 2005 Evidence for a protective role of Mcl-1 in proteasome inhibitor-induced apoptosis. *Blood* **105** 3255–3262.
- Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH & Van Etten RA 2003 Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* **424** 561–565.
- Nishitoh H, Saitoh M, Mochida Y, Takeda K, Nakano H, Rothe M, Miyazono K & Ichijo H 1998 ASK1 is essential for JNK/SAPK activation by TRAF2. *Molecular Cell* **2** 389–395.
- Park JH, Kim YS, Lee HL, Shim JY, Lee KS, Oh YJ, Shin SS, Choi YH, Park KJ, Park RW *et al.* 2006 Expression of peroxiredoxin and thioredoxin in human lung cancer and paired normal lung. *Respirology* **11** 269–275.
- Podar K, Gouill SL, Zhang J, Opferman JT, Zorn E, Tai YT, Hideshima T, Amiot M, Chauhan D, Harousseau JL *et al.* 2008 A pivotal role for Mcl-1 in Bortezomib-induced apoptosis. *Oncogene* **27** 721–731.
- Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K & Ichijo H 1998 Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO Journal* **17** 2596–2606.
- Shi J, Tricot GJ, Garg TK, Malaviarachchi PA, Szmania SM, Kellum RE, Storrie B, Mulder A, Shaughnessy JD Jr, Barlogie B *et al.* 2008 Bortezomib down-regulates the cell-surface expression of HLA class I and enhances natural killer cell-mediated lysis of myeloma. *Blood* **111** 1309–1317.
- Song IS, Kim SU, Oh NS, Kim J, Yu DY, Huang SM, Kim JM, Lee DS & Kim NS 2009 Peroxiredoxin I contributes to TRAIL resistance through suppression of redox-sensitive caspase activation in human hepatoma cells. *Carcinogenesis* **30** 1106–1114.
- Tobiume K, Inage T, Takeda K, Enomoto S, Miyazono K & Ichijo H 1997 Molecular cloning and characterization of the mouse apoptosis signal-regulating kinase 1. *Biochemical and Biophysical Research Communications* **239** 905–910.
- Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K, Minowa O, Miyazono K, Noda T & Ichijo H 2001 ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. *EMBO Reports* **2** 222–228.
- Wang HQ, Du ZX, Zhang HY & Gao DX 2007 Different induction of GRP78 and CHOP as a predictor of sensitivity to proteasome inhibitors in thyroid cancer cells. *Endocrinology* **148** 3258–3270.
- Wang HQ, Liu HM, Zhang HY, Guan Y & Du ZX 2008 Transcriptional upregulation of BAG3 upon proteasome inhibition. *Biochemical and Biophysical Research Communications* **365** 381–385.
- Wijayanti N, Naidu S, Kietzmann T & Immenschuh S 2008 Inhibition of phorbol ester-dependent peroxiredoxin I gene activation by lipopolysaccharide via phosphorylation of RelA/p65 at serine 276 in monocytes. *Free Radical Biology & Medicine* **44** 699–710.
- Xin H, Xu X, Li L, Ning H, Rong Y, Shang Y, Wang Y, Fu XY & Chang Z 2005 CHIP controls the sensitivity of transforming growth factor-beta signaling by modulating the basal level of Smad3 through ubiquitin-mediated degradation. *Journal of Biological Chemistry* **280** 20842–20850.
- Yanagawa T, Ishikawa T, Ishii T, Tabuchi K, Iwasa S, Bannai S, Omura K, Suzuki H & Yoshida H 1999 Peroxiredoxin I expression in human thyroid tumors. *Cancer Letters* **145** 127–132.
- Yanagawa T, Iwasa S, Ishii T, Tabuchi K, Yusa H, Onizawa K, Omura K, Harada H, Suzuki H & Yoshida H 2000 Peroxiredoxin I expression in oral cancer: a potential new tumor marker. *Cancer Letters* **156** 27–35.
- Yang W, Monroe J, Zhang Y, George D, Bremer E & Li H 2006 Proteasome inhibition induces both pro- and anti-cell death pathways in prostate cancer cells. *Cancer Letters* **243** 217–227.
- Zaarur N, Gabai VL, Porco JA Jr, Calderwood S & Sherman MY 2006 Targeting heat shock response to sensitize cancer cells to proteasome and Hsp90 inhibitors. *Cancer Research* **66** 1783–1791.