

Involvement of bleomycin hydrolase and poly(ADP-ribose) polymerase-1 in Ubc9-mediated resistance to chemotherapy agents

YANG CHEN, HUIXIAN ZHANG and QIYANG HE

Institute of Medicinal Biotechnology, Peking Union Medical College and
Chinese Academy of Medical Sciences, Beijing 100050, P.R. China

Received September 19, 2016; Accepted November 6, 2016

DOI: 10.3892/ijo.2016.3777

Abstract. Ubiquitin-conjugating protein 9 (Ubc9), the sole enzyme for sumoylation, plays critical roles in many physiological functions, such as DNA damage repair and genome integrity. Its overexpression led to poor prognosis and drug resistance in tumor chemotherapy. However, the underlying mechanism by which Ubc9 promotes tumor progress and influences the susceptibility to antitumor agents remains elusive. In this study, we used nine antitumor agents with distinct actions to explore Ubc9-mediated resistance in human breast carcinoma MCF-7 cells. Increase of susceptibility, respectively, to bleomycin, hydroxycamptothecin, *cis*-dichlorodiamineplatinum, 5-fluorouracil, irinotecan and gemcitabine, but not for doxorubicin, vincristine and norcantharidin, was observed after the knockdown of Ubc9 protein level with RNA interference. Reduction of bleomycin hydrolase and poly(ADP-ribose) polymerase-1 levels after knockdown of Ubc9 suggests their contribution to Ubc9-mediated drug resistance. This is the first report on the sensitivity to hydroxycamptothecin, *cis*-dichlorodiamineplatinum and gemcitabine that increased after knockdown of bleomycin hydrolase at protein level. In conclusion, Ubc9 plays different roles of action in antitumor agents in chemotherapy. The process requires bleomycin hydrolase and poly(ADP-ribose) polymerase-1. The results are beneficial to deeply understanding of Ubc9 functions and for precise prediction of chemotherapy outcomes in tumors.

Introduction

Sumoylation, one mode of post-translational modification for proteins, has been shown to play an important role in the DNA

damage repair, genome maintenance, protein transportation, activity and stability of proteins (1,2). The process includes three steps that attach SUMO to other proteins by activating, conjugating and ligating of enzymes. Among them, ubiquitin-conjugating protein 9 (Ubc9) is the sole SUMO-conjugating enzyme. Accumulating evidence has shown that it participates in many physiological functions, such as oxidative stress, genome integrity, protein quality control in cardiomyocytes and regulation of meiotic synapsis (3-7).

Ubc9 is also closely related to tumorigenesis and neoplastic metastasis, suggesting a molecular biomarker of tumors (8). It is found to highly express in various cancers, such as colon, lung, head and neck carcinoma, melanoma, and breast cancers (9-13). Ubc9 overexpression is greatly associated with neoplastic grades of breast cancer (14,15). Transfection of the Ubc9 dominant-negative mutant into the human breast MCF-7 tumor cells in nude mice led to inhibition of tumor growth *in vivo* (9). The mechanism underlying this action can increase the expression of Daxx, a protein that mediates Fas-associated apoptosis in the cytoplasm (16). Furthermore, compared with wild-type cells, the expression of bcl-2 proto-oncogene significantly decreased in the cells transfected with dominant-negative mutant of Ubc9 (17).

Ubc9 overexpression involves drug resistance to chemotherapy agents in tumors. It predicts chemoresistance in breast cancer (18). The sensitivity to cisplatin, paclitaxel and temozolomide has been reported increased by knockdown of Ubc9 with specific siRNA in melanoma (10). It can also increase the sensitivity of cells to topoisomerase I inhibitor topotecan and topoisomerase II inhibitor MV-26 (16). Because topoisomerase I ubiquitination and sumoylation share the same amino acid position, the sumoylation can prevent the degradation of topoisomerase I through ubiquitination pathway, which is caused by topoisomerase I inhibitor, and then decrease the cytotoxicity of topoisomerase I inhibitors (19). In addition, SUMO-Ubc9 complexes can modify multiple proteins related with DNA damage, promote DNA damage repair, and lead to the reduction of sensitivity to some DNA damage targeting drugs in tumor cells (20).

Although there are several reports on the Ubc9-mediated resistance to antitumor drugs, it remains unclear how it affects different drugs with various actions on tumor cells. It requires

Correspondence to: Dr Qiyang He, Institute of Medicinal Biotechnology, Peking Union Medical College and Chinese Academy of Medical Sciences, 1 Tiantan Xili, Beijing 100050, P.R. China
E-mail: qiyang_he@vip.163.com

Key words: ubiquitin-conjugating protein 9, bleomycin hydrolase, poly(ADP-ribose) polymerase-1, drug resistance, antitumor agent

to be further clarified whether Ubc9 is one of the biomarkers for the prediction of individual tumor response to drug treatment in clinic. Ubc9 can bind several proteins *in vivo*, such as bleomycin hydrolase (BLH) and poly(ADP-ribose) polymerase-1 (PARP-1) (21,22), which are associated with drug resistance. BLH can deactivate antitumor antibiotic bleomycin (23) and was confirmed as one of the biomarkers for determination of bleomycin action in our previous study (24). PARP-1 is a critical enzyme in DNA damage repair (25). Its targeted inhibitors, such as olaparib, have been proved as very effective therapy for advanced ovarian cancers with BRCA1/BRCA2 mutation (26,27).

Here, we provide the evidence on the sensitivity to nine antitumor drugs after knockdown of Ubc9 in human breast carcinoma MCF-7 cells. Our findings reveal that Ubc9-mediated resistance to them involves BLH and PARP-1.

Materials and methods

Drugs and chemicals. Boningmycin (BON) was kindly provided by Professor Ruxian Chen at our institute, and its purity was >95%. It was prepared into a 2-mM solution dissolved in PBS buffer and stored at -20°C before use. Hydroxycamptothecin (HCPT), *cis*-dichlorodiamineplatinum (DDP), 5-fluorouracil (5-FU), doxorubicin (DOX), vepeside (VP-16), vincristine (VCR), norcantharidin (NCTD), 3-(4,5-dimethyl-2-thiazoyl)-2,5-2H-tetrazolium bromide (MTT) and S-adenosyl methionine (SAMe) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gemcitabine (GEM) hydrochloride was obtained from Lilly France. Unless otherwise stated, all other chemicals were obtained from Beijing Chemical Factory.

Cell lines and cell culture. Human breast cancer MCF-7 cells were cultured in RPMI-1640 medium (Hyclone, UT, USA). The media were supplemented with 10% (v/v) fetal bovine serum (Thermo Fisher Scientific, CA, USA). The cells were incubated at 37°C in humidified 5% CO₂.

Measurement of cellular viability assessed using MTT method (28). In brief, the cells were seeded into a 96-well plate at a cell density of 3,000 per well for 24 h, followed by drug treatment for 48 h. Consequently, MTT was added to the medium and incubated for 2 h, and the crystals were dissolved with dimethyl sulfoxide. The plates were read using an enzyme-linked immunosorbent assay plate reader at 570 nm. The control group was set as 100%, and IC₅₀ values were calculated and plotted with GraphPad Prism 5.

RNA interference siRNAs duplex against Ubc9 (sense sequence: GCCUGUCCAUCUAGAGGAGGACAA), BLH (sense sequence: UACCCAAACAGAUGCACACCACUCG) was synthesized by Life Technologies (Carlsbad, CA, USA). The siRNAs duplex against PARP-1 was purchased from Cell Signaling Technology (Beverly, MA, USA). The RNA interference with the concentration of 100 pmol RNAi duplex was performed with Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions.

Western blot analysis. Western blot assay was described previously (28). The sources of primary antibodies against Ubc9,

caspase-3 and PARP-1 were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). The antibodies against p53 and actin were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The mouse anti-BLH antibody was from Abcam (Cambridge, UK).

Cell cycle analysis by flow cytometry. The cells were trypsinized and fixed with cold 70% ethanol overnight. The fixed cells were washed twice with PBS and incubated with 100 µg/ml of ribonuclease A at 37°C for 30 min and then stained in PBS containing 50 µg/ml propidium iodide (Sigma) for 1 h. The fluorescence intensity was detected using BD FACSCalibur cytometer (BD Biosciences, CA, USA) and the cell cycle distribution was assayed with the ModFit LT software (BD Biosciences).

Detection of apoptotic cells by flow cytometry. The apoptotic cells were stained with Annexin V-FITC/PI Apoptosis kit (BD Biosciences), following the protocol provided by the manufacturer. The fluorescence intensities were measured using a BD FACSCalibur flow cytometer.

The chromosome condensation was stained with Hoechst 33342 bisbenzimidazole. To distinguish the cells at mitosis phase from G₂ phase, DNA specific fluorescent dye Hoechst 33342 was used to stain the cells with the concentration of 2 µg/ml at room temperature for 15 min. Images were captured with a fluorescence microscope (Olympus, Japan).

Statistical analysis. Results were expressed as the mean ± SD from at least three independent experiments. Statistical analysis was performed with the Student's t-test using SPSS 16.0. The criterion for statistical significance was set at P<0.05. Combination index was performed using CompuSyn.

Results

Reduction of cell proliferation by knockdown of Ubc9. To investigate the functionality of Ubc9 in the role of cell proliferation, we performed RNA interference experiments. Three different Ubc9-siRNAs were used to knock down Ubc9 mRNA and analyzed the levels of Ubc9 by western blotting after MCF-7 cells were transfected with siRNAs for 72 h. Ubc9 protein levels were obviously reduced <20%, whereas no such reduction was seen with treatment of negative siRNA (Fig. 1A). The Ubc9 siRNA-1 and siRNA-2 were chosen for the following experiments. The MCF-7 cells were transfected for 24 h, and cell numbers were counted every day with Beckman Coulter for a week. Comparing with the control group, the rate of cell proliferation was significantly decreased in Ubc9 siRNA-treated group (Fig. 1B). In order to further confirm that the knockdown of Ubc9 can affect cell proliferation, cell cycle distributions in the MCF-7 cells were analyzed by flow cytometry following knockdown of Ubc9. As shown in Fig. 1C, knockdown of Ubc9 had increased accumulation of cells at G₂/M-phase from 25.2% in non-transfected cells to 38.7 and 33.7% in transfected cells, respectively. To distinguish the cells at G₂ with M phase, we observed the staining by the specific DNA dye Hoechst 33342 (Fig. 1D). The chromosome condensations showed in Ubc9-interfered group, suggesting the arrest of cells at M phase. It is consistent with previous

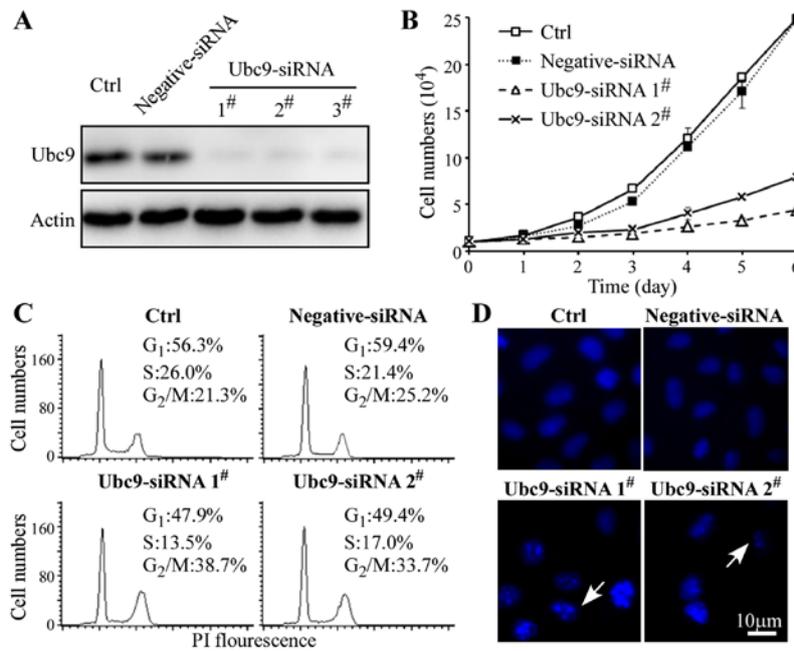


Figure 1. Inhibitory action of cell proliferation by knockdown of Ubc9 in MCF-7 cells. (A) Ubc9 expression by three different siRNAs. (B) The cell proliferation curve of MCF-7 cell line after knockdown of Ubc9 siRNA-1 and siRNA-2 during 7 days. (C) The effects on distribution of cell cycle by knockdown of Ubc9 siRNA-1 and siRNA-2. The cells were subjected to cell cycle analysis after knockdown of Ubc9 with RNA interference for 24 h. (D) Bisbenzimidazole H 33342 trihydrochloride staining to detect chromosome condensation. A representative result of three independent experiments is shown.

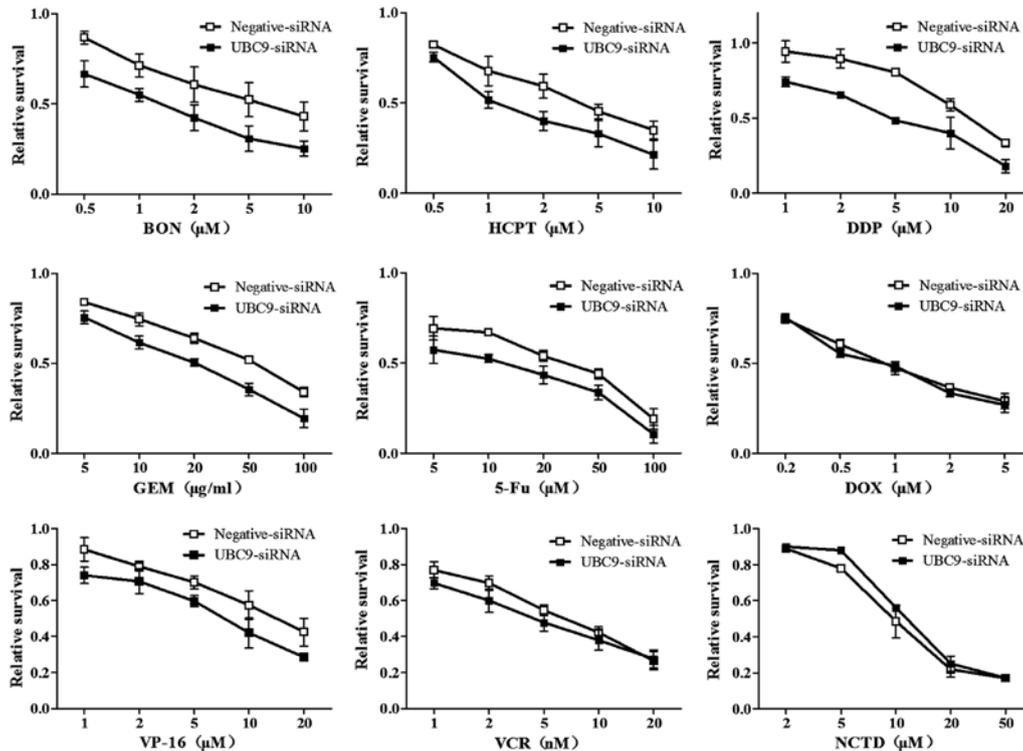


Figure 2. Increase of susceptibility to antitumor agents by knockdown of Ubc9 in MCF-7 cells. The cell viability was determined by MTT assay after treatment with drugs for 48 h. One representative result from three independent experiments is shown.

research results in yeast cells (29). The results suggested that Ubc9 is necessary for cell proliferation.

Increase of susceptibility to antitumor agents by knockdown of Ubc9. In order to evaluate whether Ubc9 is related to the

susceptibility to antitumor agents, nine types of antitumor agents with different actions were chosen to treat MCF-7 cells. As illustrated in Fig. 2, the susceptibility to BON, HCPT, DDP, GEM, 5-Fu and VP-16 increased after knockdown of Ubc9. In contrast, the sensitivity to VCR, NCTD, or DOX did not

Table I. Potentiation of actions of antitumor drugs after knock-down of Ubc9 in MCF-7 cells.

Drugs	Negative-siRNA	Ubc9-siRNA
BON (μM)	5.6 \pm 1.1	1.3 \pm 0.4 ^b
HCPT (μM)	3.7 \pm 0.8	1.5 \pm 0.4 ^b
DDP (μM)	12.6 \pm 1.6	4.5 \pm 1.1 ^b
GEM ($\mu\text{g/ml}$)	46.3 \pm 7.1	20.0 \pm 3.2 ^b
5-Fu (μM)	23.9 \pm 3.6	10.4 \pm 4.3 ^a
DOX (μM)	0.2 \pm 0.1	0.2 \pm 0.1
VP-16 (μM)	14.3 \pm 2.0	6.7 \pm 1.8 ^a
VCR (nM)	6.1 \pm 1.4	4.1 \pm 0.8 ^a
NCTD (μM)	9.7 \pm 2.4	11.6 \pm 2.3

The cells were treated with various concentrations of drugs for 48 h. Cell survival was determined by MTT assay. The data represent the mean \pm standard deviation from three independent experiments. ^aP<0.05, ^bP<0.01 versus negative-siRNA groups.

change obviously. The IC₅₀ values of negative siRNA and Ubc9 siRNA are summarized in Table I. Among them, the sensitivity to BON is mostly augmented, reaching 4-fold. The results revealed that Ubc9 is involved in the susceptibility to various types of antitumor agents.

Increase of apoptosis by knockdown of Ubc9. In order to clarify the mechanism by which Ubc9 protein level affects the sensitivity of antitumor agents, the actions of HCPT and DDP were further observed to detect apoptotic event with Annexin V/PI staining and western blotting. The rates of early apoptotic cells were 86.5 and 29.7%, respectively, after the Ubc9-siRNA transfected cells were exposed to 5 μM HCPT or 10 μM DDP (Fig. 3A), higher in comparison with the groups of negative siRNA transfected cells. The apoptotic signaling pathways were also detected by western blotting at time-points after exposure to HCPT for 6, 12, 24, 36 and 48 h (Fig. 3B). The cleaved PARP-1 fragment was obviously observed at 24 h in the Ubc9-siRNA transfected cells. In addition, the

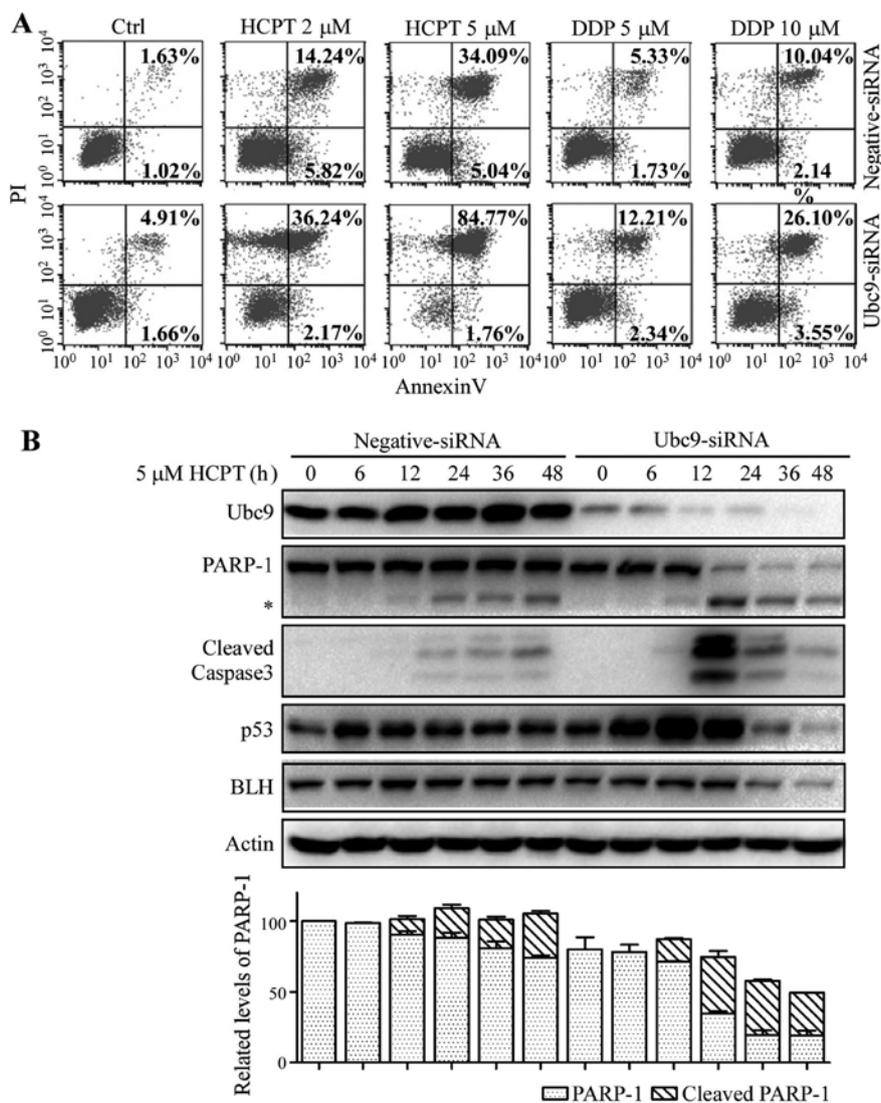


Figure 3. Potentiation of apoptotic rate by reduction of Ubc9 protein level. (A) Knockdown of Ubc9 expression by siRNA for 24 h, and exposure to 2 and 5 μM HCPT or 5 and 10 μM DDP. Apoptotic cells were detected by Annexin V/PI staining after the MCF-7 cells were treated with drugs for 48 h. (B) Activation of the apoptotic pathway after exposure to 2 μM HCPT was aggravated by reduction of Ubc9 expression. Protein lysates were analyzed by western blotting at various time-points. The levels of actin are shown as loading controls. One representative result from three independent experiments is shown.

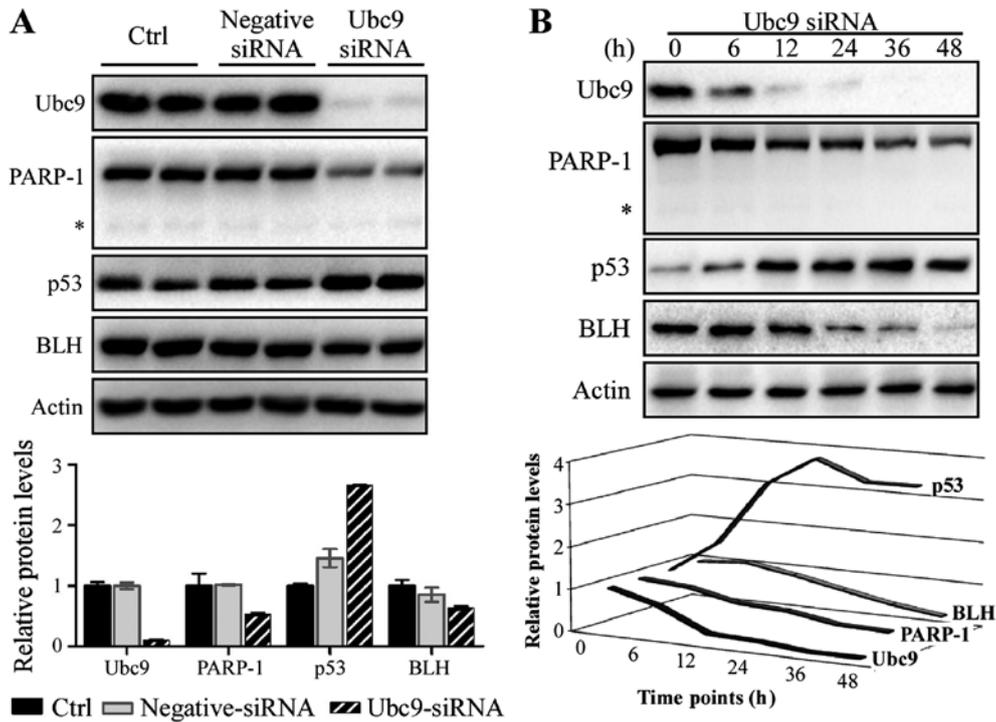


Figure 4. Reduction of Ubc9 expression causes alterations in protein expression of PARP-1, p53 and BLH. (A) Western blot results showed knockdown of Ubc9 led to reduction of protein levels of PARP-1, p53 and BLH by the interfering Ubc9 siRNA for 48 h. (B) The protein levels of the above-mentioned proteins were detected by western blotting after the cells were harvested at the indicated time points with transfection of Ubc9-siRNA. One representative result from three independent experiments is shown.

protein levels of cleaved caspase-3 and p53 in Ubc9-siRNA transfected cells significantly increased comparing with the negative siRNA transfected cells. Reduction of BLH level in apoptosis was also clearly detected after knockdown of Ubc9.

Reduction of BLH and PARP-1 levels in the Ubc9-siRNA transfected cells. To explore the mechanism of Ubc9-mediated resistance to antitumor agents, the binding proteins with Ubc9, such as BLH and PARP-1 were also detected. The protein levels of PARP-1 and BLH in MCF-7 cells were greatly reduced after the knocking down of Ubc9 protein level (Fig. 4A). Similar phenomena were also observed time-dependently (Fig. 4B). It is not due to apoptotic event as the fragment of cleaved PARP-1 was not detected. Interestingly, increase of p53 protein level was significantly observed after reduction of Ubc9. These results suggest that BLH and PARP-1 are related to Ubc9 action in the resistance to antitumor agents.

Increase of the sensitivity to antitumor agents by knockdown of BLH. In our previous study, the susceptibility to bleomycin was obviously augmented after knockdown of BLH protein level (24). BLH levels were maintained at a lower level after the cells were transfected with BLH siRNAs for 72 h (Fig. 5A). The antitumor agents, BON, HCPT, DDP and GEM were used to treat the MCF-7 cells (Fig. 5B). The IC₅₀ values of negative siRNA and BLH siRNA groups were 2.5±0.3 and 0.5±0.1 μM, respectively, showing the increased sensitivity to BON as 5-fold after knockdown of BLH. Importantly, the reduction of BLH protein level was affected the sensitivity to HCPT, DDP and GEM (Fig. 5B). The results suggested that BLH meditates

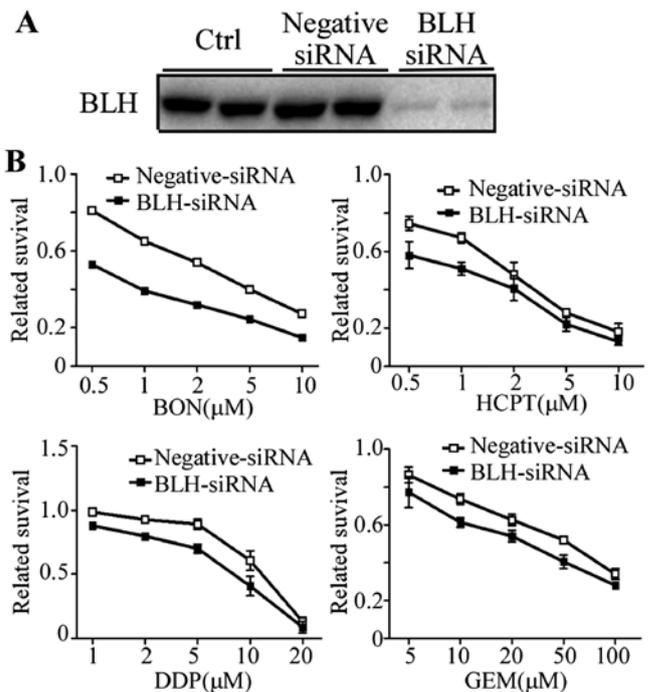


Figure 5. Reduction of BLH protein level increases the sensitivity to BON, HCPT, DDP and GEM in the MCF-7 cells. (A) BLH protein levels after knockdown of BLH siRNA. (B) The cell viability was determined by MTT assay after knockdown of BLH and then treatment with drugs for 48 h. The results are expressed as the mean ± SD from three separate experiments.

the Ubc9-associated resistance to antitumor agents in addition to direct metabolism of bleomycin.

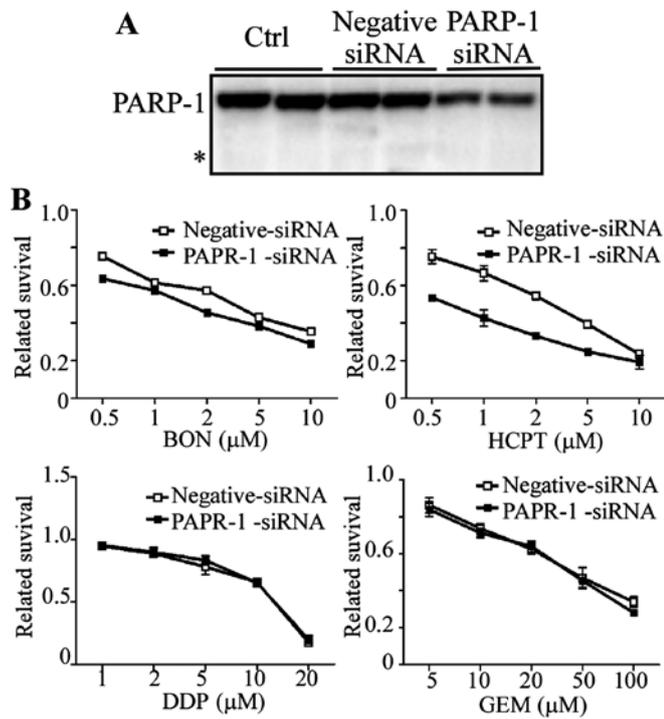


Figure 6. Reduction of PARP-1 protein level influences the sensitivity to antitumor agents in the MCF-7 cells. (A) PARP-1 protein levels after knockdown of BLH siRNA. (B) The cell viability was determined by MTT assay after knockdown of PARP-1 and then treatment with drugs for 48 h. The results are expressed as the mean ± SD from three separate experiments.

Increase of the sensitivity to antitumor agents by knockdown of PARP-1. The PARP-1 protein level was maintained at a lower level after the cells were transfected with PARP-1 siRNAs for 72 h (Fig. 6A). At the same time, the sensitivity to BON, HCPT, DDP and GEM were determined (Fig. 6B). The IC₅₀ values of BON in the negative siRNA and PARP-1 siRNA groups were 3.1±0.3 and 1.6±0.2 μM, respectively, indicating increase of the sensitivity to BON after knockdown of PARP-1. Interference on PARP-1 led to 4.1-fold increase of the sensitivity to HCPT. To our surprise, the sensitivity to DDP and GEM did not change after reduction of PARP-1 protein level. Thus, PARP-1 partly contributed to Ubc9-mediated resistance to antitumor agents.

Potential of actions of antitumor agents in combination with Ubc9 inhibitor SAME. In order to further demonstrate the action of the Ubc9-mediated drug resistance, Ubc9 inhibitor SAME was used to treat MCF-7 cells. It is a common substrate involved in methyl group transfers and reduction of Ubc9 expression (30). Treatment with SAME for 48 h led to reduction of protein levels of Ubc9 and p53 (Fig. 7A). However, the PARP-1 and BLH protein levels were less reduced after treatment with non-cytotoxic concentrations of 0.1 and 0.2 mM SAME. The combination results of SAME and antitumor drugs are shown in Fig. 7B. The survival rate had no significant difference between BON alone and the combined group. The cell survival rates were greatly decreased in the combination of HCPT or DDP with SAME.

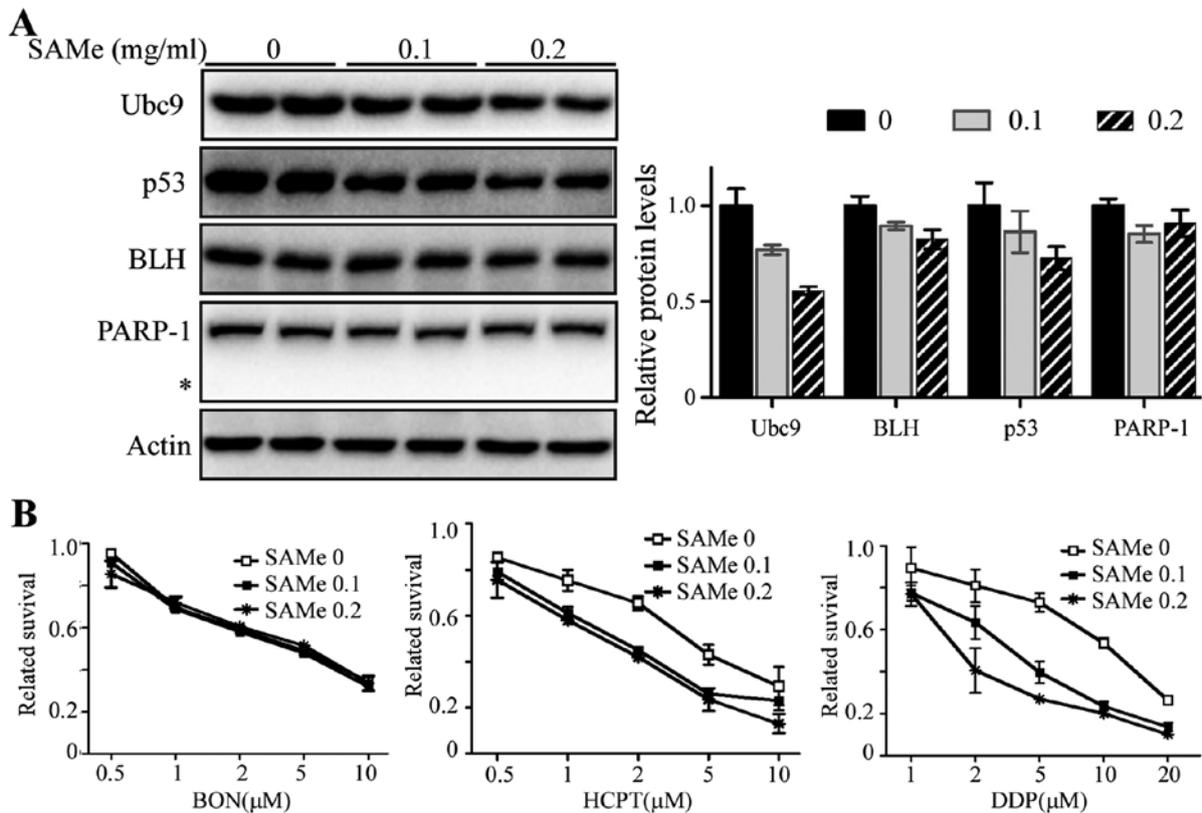


Figure 7. Potentiation of the action of HCPT and DDP in combination with SAME in the MCF-7 cells. (A) The protein levels of Ubc9, p53, BLH and PARP-1 were detected by western blotting after the MCF-7 cells were treated with 0.1 and 0.2 mg/ml SAME for 48 h. (B) Effects of BON, HCPT or DDP in combination with SAME on the proliferation of MCF-7 cells determined by MTT assay. The results are representative of three separate experiments. *CI<1 and **CI<0.8 drugs alone versus combination with 0.1 mg/ml SAME. #CI<1 and ##CI<0.8 drugs alone versus combination with 0.2 mg/ml SAME.

Discussion

In this study, we present evidence to demonstrate the role of Ubc9 in drug resistance to different actions of chemotherapy agents. The underlying mechanism involves Ubc9 binding proteins BLH and PARP-1. These results further support the hypothesis that Ubc9 overexpression is a biomarker for the prediction of tumor progression and drug resistance.

In order to systemically illuminate the Ubc9-mediated drug resistance, we assayed the susceptibility to nine anti-tumor agents with different actions. BON, a new member of bleomycin family, showed more potent suppression of human hepatoma growth in a mouse model (31). HCPT and VP-16 are topoisomerase I and topoisomerase II inhibitors, respectively. DOX and DDP act directly blocking DNA replication and transcription. 5-Fu and GEM inhibited thymidylate synthase and ribonucleotide reductase, respectively, which were the key enzyme in DNA synthesis. VCR inhibits mitosis by suppressing microtubule association. NCTD increase death rate of cancer cells and activity of lysosomes. In this study, knockdown of Ubc9 protein level led to different increase of susceptibility to the above-mentioned drugs. The increases of susceptibility to HCPT and DDP have been observed after knockdown of Ubc9 or treatment with Ubc9 specific inhibitor in MCF-7 cells. It is consistent with the result in melanoma cells that cisplatin and paclitaxel augmented the rates of Ubc9-related apoptosis by as much as 50%, but temozolomide by only 10-15% (10).

Reduction of Ubc9 augments the sensitivity to BON, 5-FU, VP-16 and GEM, but showed no action with DOX. No association of Ubc9-mediated resistance with DOX is consistent with the DOX resistance in sumoylation systems of yeast cells (32). It may be the reason why DOX is widely used for first line tumor therapy in clinic as Ubc9 overexpression in tumor cells has no effect on DOX action. Although DOX, BON, DDP and VP-16 can cause DNA damage response, the roles of Ubc9 in the process are totally different. Another report revealed that depletion of Ubc9 protein level did not affect the homologous recombination or alternative non-homologous end joining, but required conservative non-homologous end joining in DNA double-strand break response (33).

To our knowledge, this is the first report on knockdown of BLH increasing the sensitivity to antitumor drugs other than bleomycin in MCF-7 cells (Fig. 5). BLH can inactivate bleomycin action and is one of the biomarkers for the determination of bleomycin action (24). Accumulating data have shown that BLH can play multiple roles under different physiological and pathological conditions, such as preparation of peptides for antigen presentation (34), the pathogenesis of Alzheimer's disease (35) and skin moisture (36). Furthermore, BLH protects mice against L-homocysteine thiolactone toxicity by metabolizing it to homocysteine, suggesting a mechanism by which it has a role in cellular detoxification (37,38). In our previous report, we found that BLH is cleaved by caspase-3 in the process of apoptosis (28). In this study, we demonstrated that knockdown of Ubc9 expression can cause BLH content to decrease (Fig. 4), and knockdown of BLH protein level directly affected the sensitivity to several antitumor agents in MCF-7 cells (Fig. 6). These findings indicate that BLH may act as a protective role in cells that degrades intracellular toxic substances and maintains cell survival. The function of Ubc9/

BLH protein complex in the cells is being investigated in our laboratory.

PARP-1 plays very important roles in DNA damage repairs, especially in the repair of single-strand DNA breaks. The results that reduction of PARP-1 protein level after knocking down of Ubc9 levels and increase of the sensitivity to antitumor drugs by knockdown of PARP-1 suggest its role in the Ubc9-mediated drug resistance. Modification of PARP-1 with the small ubiquitin-related modifier affects its function as a transcriptional co-activator of hypoxia-responsive genes (39). The affinity of PARP-1 is enhanced by Ubc9 upon binding to DNA (40). The PARP-1-targeted agents have been approved for use in clinic as tumor therapy. This encourages development of new PARP-1 inhibitors (41). Our results presented here suggest that wide applications of them should be considered based on the effects of cellular interacting proteins, such as Ubc9.

It is a very important to understand the way in which Ubc9 binds BLH and PARP-1 in the cells. There is no evidence to show their binding is in a competitive manner. As a matter of fact, only one percent of sumoylated Ubc9 can function well in yeast meiosis (7), suggesting the high efficiency to exact its role. It has been observed that distinct Ubc9 protein complexes formed in response to DNA double-strand breaks (33). Therefore, it will be important to determine whether the levels or activities of these protein complexes are regulated in response to drug treatment, thereby altering cellular processes through global or local changes in SUMO modification and binding proteins.

The high expression of Ubc9 in tumor tissues may be a driving force for tumorigenesis and metastasis as it regulates the function of many growth-associated oncoproteins. In K-ras mutant colorectal cancer cells, oncogenesis by Ras/Raf pathway required Ubc9-mediated sumoylation (42). The phosphorylation of Ubc9 and SUMO-1 by AKT modulates the substrate sumoylation specificity in tumor cells (43). The expression of Ubc9 is regulated by estrogen receptor α and nuclear factor Y in MCF-7 cells (44). Because of the complexity and difference of Ubc9 regulation in various tumors, it will be conducive to the prediction of individual tumor response to drug treatment in clinic when using Ubc9 as one of the biomarkers. It is rational to design the compound to target Ubc9 as it is overexpressed in arrays of tumors and a unique conjugating enzyme for somoylation. In this study, SAME inhibited the expression of Ubc9 and augmented the sensitivity to HCPT and DDP (Fig. 7). Another report showed that antibiotic spectinomycin B1 can directly inhibit Ubc9 *in vitro* and *in vivo* (45). Developing Ubc9-targeted inhibitors for treatment of various types of cancers shows promise.

In conclusion, Ubc9 overexpression leads to resistance of antitumor agents and failure in tumor chemotherapy. The evidence that BLH and PARP-1 as binding proteins participate in the process provides new way to overcome this. Ubc9 is a biomarker for tumorigenesis and the progression in some types of cancer. It is valuable to precisely detect Ubc9 levels in tumor cells and screen the compound targeting Ubc9.

Acknowledgements

This study was supported by grants from National Scientific Foundation of China (81273553, 31471150).

References

- Gareau JR and Lima CD: The SUMO pathway: Emerging mechanisms that shape specificity, conjugation and recognition. *Nat Rev Mol Cell Biol* 11: 861-871, 2010.
- Schwertman P, Bekker-Jensen S and Mailand N: Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat Rev Mol Cell Biol* 17: 379-394, 2016.
- Stankovic-Valentin N, Drzewicka K, König C, Schiebel E and Melchior F: Redox regulation of SUMO enzymes is required for ATM activity and survival in oxidative stress. *EMBO J* 35: 1312-1329, 2016.
- Guervilly JH, Takedachi A, Naim V, Scaglione S, Chawhan C, Lovera Y, Despras E, Kuraoka I, Kannouche P, Rosselli F, *et al*: The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. *Mol Cell* 57: 123-137, 2015.
- Ouyang J, Garner E, Hallet A, Nguyen HD, Rickman KA, Gill G, Smogorzewska A and Zou L: Noncovalent interactions with SUMO and ubiquitin orchestrate distinct functions of the SLX4 complex in genome maintenance. *Mol Cell* 57: 108-122, 2015.
- Gupta MK, McLendon PM, Gulick J, James J, Khalili K and Robbins J: Ubc9-mediated sumoylation favorably impacts cardiac function in compromised hearts. *Circ Res* 118: 1894-1905, 2016.
- Klug H, Xaver M, Chaugule VK, Koidl S, Mittler G, Klein F and Pichler A: Ubc9 sumoylation controls SUMO chain formation and meiotic synapsis in *Saccharomyces cerevisiae*. *Mol Cell* 50: 625-636, 2013.
- Mattosco D and Chiocca S: SUMO pathway components as possible cancer biomarkers. *Future Oncol* 11: 1599-1610, 2015.
- Mo YY, Yu Y, Theodosiou E, Ee PL and Beck WT: A role for Ubc9 in tumorigenesis. *Oncogene* 24: 2677-2683, 2005.
- Moschos SJ, Smith AP, Mandic M, Athanassiou C, Watson-Hurst K, Jukic DM, Edington HD, Kirkwood JM and Becker D: SAGE and antibody array analysis of melanoma-infiltrated lymph nodes: Identification of Ubc9 as an important molecule in advanced-stage melanomas. *Oncogene* 26: 4216-4225, 2007.
- Ronen O, Malone JP, Kay P, Bivens C, Hall K, Paruchuri LP, Mo YY, Robbins KT and Ran S: Expression of a novel marker, Ubc9, in squamous cell carcinoma of the head and neck. *Head Neck* 31: 845-855, 2009.
- Moschos SJ, Jukic DM, Athanassiou C, Bhargava R, Dacic S, Wang X, Kuan SF, Fayewicz SL, Galambos C, Acquafondata M, *et al*: Expression analysis of Ubc9, the single small ubiquitin-like modifier (SUMO) E2 conjugating enzyme, in normal and malignant tissues. *Hum Pathol* 41: 1286-1298, 2010.
- Li H, Niu H, Peng Y, Wang J and He P: Ubc9 promotes invasion and metastasis of lung cancer cells. *Oncol Rep* 29: 1588-1594, 2013.
- Synowiec E, Krupa R, Morawiec Z, Wasylecka M, Dziki L, Morawiec J, Blasiak J and Wozniak K: Efficacy of DNA double-strand breaks repair in breast cancer is decreased in carriers of the variant allele of the UBC9 gene c.73G>A polymorphism. *Mutat Res* 694: 31-38, 2010.
- Zhu S, Sachdeva M, Wu F, Lu Z and Mo YY: Ubc9 promotes breast cell invasion and metastasis in a sumoylation-independent manner. *Oncogene* 29: 1763-1772, 2010.
- Mo YY, Yu Y, Ee PL and Beck WT: Overexpression of a dominant-negative mutant Ubc9 is associated with increased sensitivity to anticancer drugs. *Cancer Res* 64: 2793-2798, 2004.
- Lu Z, Wu H and Mo YY: Regulation of bcl-2 expression by Ubc9. *Exp Cell Res* 312: 1865-1875, 2006.
- Chen SF, Gong C, Luo M, Yao HR, Zeng YJ and Su FX: Ubc9 expression predicts chemoresistance in breast cancer. *Chin J Cancer* 30: 638-644, 2011.
- Mo YY, Yu Y, Shen Z and Beck WT: Nucleolar delocalization of human topoisomerase I in response to topotecan correlates with sumoylation of the protein. *J Biol Chem* 277: 2958-2964, 2002.
- Han JY, Lee GK, Yoo SY, Yoon SJ, Cho EY, Kim HT and Lee JS: Association of SUMO1 and Ubc9 genotypes with tumor response in non-small-cell lung cancer treated with irinotecan-based chemotherapy. *Pharmacogenomics* 10: 86-93, 2010.
- Masson M, Menissier-de Murcia J, Mattei MG, de Murcia G and Niedergang CP: Poly(ADP-ribose) polymerase interacts with a novel human ubiquitin conjugating enzyme: hUbc9. *Gene* 190: 287-296, 1997.
- Koldamova RP, Lefterov IM, DiSabella MT and Lazo JS: An evolutionarily conserved cysteine protease, human bleomycin hydrolase, binds to the human homologue of ubiquitin-conjugating enzyme 9. *Mol Pharmacol* 54: 954-961, 1998.
- Schwartz DR, Homanics GE, Hoyt DG, Klein E, Abernethy J and Lazo JS: The neutral cysteine protease bleomycin hydrolase is essential for epidermal integrity and bleomycin resistance. *Proc Natl Acad Sci USA* 96: 4680-4685, 1999.
- Chen J, Chen Y and He Q: Action of bleomycin is affected by bleomycin hydrolase but not by caveolin-1. *Int J Oncol* 41: 2245-2252, 2012.
- Pellegrino S and Altmeyer M: Interplay between ubiquitin, SUMO, and poly(ADP-Ribose) in the cellular response to genotoxic stress. *Front Genet* 7: 63, 2016.
- Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott C, Meier W, Shapira-Frommer R, Safra T, *et al*: Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med* 366: 1382-1392, 2012.
- Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott CL, Meier W, Shapira-Frommer R, Safra T, *et al*: Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: A preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol* 15: 852-861, 2014.
- Chen Y, Xu R, Chen J, Li X and He Q: Cleavage of bleomycin hydrolase by caspase-3 during apoptosis. *Oncol Rep* 30: 939-944, 2013.
- Dieckhoff P, Bolte M, Sancak Y, Braus GH and Irniger S: Smt3/SUMO and Ubc9 are required for efficient APC/C-mediated proteolysis in budding yeast. *Mol Microbiol* 51: 1375-1387, 2004.
- Tomasi ML, Tomasi I, Ramani K, Pascale RM, Xu J, Giordano P, Mato JM and Lu SC: S-adenosyl methionine regulates ubiquitin-conjugating enzyme 9 protein expression and sumoylation in murine liver and human cancers. *Hepatology* 56: 982-993, 2012.
- Gao N, Shang B, Zhang X, Shen C, Xu R, Xu H, Chen R and He Q: Potent antitumor actions of the new antibiotic boningmycin through induction of apoptosis and cellular senescence. *Anticancer Drugs* 22: 166-175, 2011.
- Huang RY, Kowalski D, Minderman H, Gandhi N and Johnson ES: Small ubiquitin-related modifier pathway is a major determinant of doxorubicin cytotoxicity in *Saccharomyces cerevisiae*. *Cancer Res* 67: 765-772, 2007.
- Hu Y and Parvin JD: Small ubiquitin-like modifier (SUMO) isoforms and conjugation-independent function in DNA double-strand break repair pathways. *J Biol Chem* 289: 21289-21295, 2014.
- Towne CF, York IA, Watkin LB, Lazo JS and Rock KL: Analysis of the role of bleomycin hydrolase in antigen presentation and the generation of CD8 T cell responses. *J Immunol* 178: 6923-6930, 2007.
- Suszynska J, Tisonczyk J, Lee HG, Smith MA and Jakubowski H: Reduced homocysteine-thiolactonase activity in Alzheimer's disease. *J Alzheimers Dis* 19: 1177-1183, 2010.
- Son ED, Kim Y, Joo KM, Kim HJ, Lee E, Nam GW, Cho EG, Noh M, Chung JH, Byun SY, *et al*: Skin dryness in apparently healthy human skin is associated with decreased expression of bleomycin hydrolase in the stratum corneum. *Clin Exp Dermatol* 40: 247-253, 2015.
- Zimny J, Sikora M, Guranowski A and Jakubowski H: Protective mechanisms against homocysteine toxicity: The role of bleomycin hydrolase. *J Biol Chem* 281: 22485-22492, 2006.
- Borowczyk K, Tisończyk J and Jakubowski H: Metabolism and neurotoxicity of homocysteine thiolactone in mice: Protective role of bleomycin hydrolase. *Amino Acids* 43: 1339-1348, 2012.
- Messner S, Schuermann D, Altmeyer M, Kassner I, Schmidt D, Schär P, Müller S and Hottiger MO: Sumoylation of poly(ADP-ribose) polymerase 1 inhibits its acetylation and restrains transcriptional coactivator function. *FASEB J* 23: 3978-3989, 2009.
- Zilio N, Williamson CT, Eustermann S, Shah R, West SC, Neuhaus D and Ulrich HD: DNA-dependent SUMO modification of PARP-1. *DNA Repair (Amst)* 12: 761-773, 2013.
- Scott CL, Swisher EM and Kaufmann SH: Poly (ADP-ribose) polymerase inhibitors: Recent advances and future development. *J Clin Oncol* 33: 1397-1406, 2015.

42. Yu B, Swatkoski S, Holly A, Lee LC, Giroux V, Lee CS, Hsu D, Smith JL, Yuen G, Yue J, *et al*: Oncogenesis driven by the Ras/Raf pathway requires the SUMO E2 ligase Ubc9. *Proc Natl Acad Sci USA* 112: E1724-E1733, 2015.
43. Lin CH, Liu SY and Lee EH: SUMO modification of Akt regulates global SUMOylation and substrate SUMOylation specificity through Akt phosphorylation of Ubc9 and SUMO1. *Oncogene* 35: 595-607, 2016.
44. Ying S, Dünneber T, Si J and Hamann U: Estrogen receptor alpha and nuclear factor Y coordinately regulate the transcription of the SUMO-conjugating UBC9 gene in MCF-7 breast cancer cells. *PLoS One* 8: e75695, 2013.
45. Hirohama M, Kumar A, Fukuda I, Matsuoka S, Igarashi Y, Saitoh H, Takagi M, Shin-ya K, Honda K, Kondoh Y, *et al*: Spectomycin B1 as a novel SUMOylation inhibitor that directly binds to SUMO E2. *ACS Chem Biol* 8: 2635-2642, 2013.