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Okada, Takamitsu
Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University

Handa, Masuo
Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University

Nakashima, Yasuharu
Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University

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Original Article

The Selective Induction of Programed Cell Death in Osteosarcoma Cells by Histone Deacetylase Inhibitor Suberoylanylide Hydroxamic Acid

Takamitsu Okada[1)], Masuo Hanada[1)] and Yasuharu Nakashima[1)]

[1)] *Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.*

Abstract

Despite improvements in multimodal therapies for osteosarcoma (OS), the prognosis of relapsed cases is still very poor because of the resistance to chemotherapy and radiation therapy. Using doxorubicin, we established the drug-resistant (ADR) clone of OS, which also exhibited resistance to apoptosis by radiation.

Histone deacetylase inhibitors (HDACIs) are novel anti-tumor agents against various cancer cells. Here, we describe the antitumor effects of a HDACI, suberoylanylide hydroxamic acid (SAHA), on parental and ADR OS clones, by induction of the different types of cell death.

SAHA increased the expression level of cleaved-PARP and the cell population in Sub-G1 fraction in the parental clone. On the other hand, SAHA induced the expression of both LC3-I and LC3-II but not that of beclin1, the formation of autophagosomes and G2/M arrest in ADR clone. Furthermore, 3-MA, an inhibitor of autophagy, reduced SAHA-induced cell death in ADR clone. These findings indicated that SAHA induced apoptosis in parental clone and autophagic cell death in ADR clone, suggesting that SAHA could select the types of cell death in the tumor cells according to their characteristics of resistance to the cell death.

Key words : histone deacetylase inhibitor, suberoylanylide hydroxamic acid, osteosarcoma, autophgic cell death, apoptosis

Introduction

Osteosarcoma (OS) is the most frequent primary malignant bone tumors in childhood and adolescence. Despite dramatic improvements in the multimodal therapies and outcome, systemic relapses are observed in approximately 30～40% of cases and prognosis of the relapsed cases is still very poor[1)~4)]. It has been reported that there was no significant difference in overall survival between cases with or without second-line chemotherapy after first disease recurrence in OS[5)6)]. These results strongly suggest that the recurrent OS become resistant to drugs used in the chemotherapy. To overcome resistance to the therapy is critical for the improvements of prognosis of the patients with advanced OS.

One of the causes of multidrug resistance of the tumors is attributed to the expression of efflux pumps that reduce the intracellular concentration of the drugs administrated. The efflux pumps are identified as ATP-binding cassette (ABC) trans-

Abbreviations
HDAC, histone deacetylase ; SAHA, suberoylanylide hydroxamic acid, ; PARP, poly-ADP-ribose polymelase ; P-gp, P-glycoprotein ; MRP1, multidrug resistance-associated protein 1 ; OS, osteosarcoma ; EFTs ; Ewing's family tumors, Dox, doxorubicin ; ADR, adriamycin-resistant.

Corresponding author : Takamitsu Okada
Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
Tel : + 81-92-642-5488 Fax : + 81-92-642-5507
E-mail : t-okada@ortho.med.kyushu-u.ac.jp

porters characterized with their homologous ATP-binding domains, including a MDR1 gene product P-glycoprotein (Pgp) and multidrug resistance-associated protein 1 (MRP1)[7)8)]. Since the key drugs used in chemotherapy for OS, including adriamycin (Dox)[9)10)], are the substrates of Pgp and MRP1[11)], the drug-resistance in OS might also be related to these efflux pumps.

Another cause of the multidrug-resistance is the acquired resistance of the tumors to apoptosis signals induced by anti-tumor therapies. The killing effects for tumor cells by most anticancer strategies including chemotherapy, gamma-irradiation, suicide gene therapy or immunotherapy have been linked to the activation of apoptosis pathways in the cells[12)]. Therefore, the tumors resistant to the anticancer therapies might have acquired the apoptosis resistance. Previously, we established a multidrug resistant OS cell line MNNG/ADR by the long-term cultivation of the parental MNNG cells in the presence of increasing concentrations of Dox[13)]. We found that the drug-resistant MNNG/ADR cells not only express multi-drug resistant factors, P-gp and MRP1, but also have cross-resistance to radiation, suggesting the apoptosis-resistance of MNNG/ADR. We thus thought that MNNG/ADR might be useful model of poor-prognostic OS which has resistance to anticancer drugs and radiation.

Histone deacetylase inhibitors (HDACIs) are novel and promising anti-tumor agents. It has been reported that HDACIs can activate transcription of specific genes via the accumulation of histone acetylation and subsequently cause a variety of phenotypic changes, including cell cycle arrest, morphological reversion of transformed cells, differentiation and apoptosis[14)~16)]. A number of HDACIs exert anti-tumor effects on several cancers and are under clinical trials. A recent study demonstrated that an HDACI FK228 exhibited potent anti-tumor effects on OS[17)]. However, we have demonstrated that cyclic tetrapeptide family of HDACIs including FK228 showed cross-resistance to the Dox-resistant clones of OS expressing P-gp and MRP1, and that P-gp and MRP1 played a crucial role in the resistance to FK228[18)]. Thus, we concluded that another HDACIs which are not the substrates of the efflux pumps should be needed for the application of HDACIs to the advanced OS.

Suberoylanilide hydroxamic acid (SAHA) is in the different structural class of HDACIs from the cyclic tetrapeptide. SAHA is under phase I/II clinical trials for relapsed or refractory cases of leukemia and advanced or metastatic malignant tumors. In the present study, we demonstrated that SAHA exhibited the induction of cell death both in the parental MNNG and drug-and radio-resistant MNNG/ADR cells. In MNNG, the treatment with SAHA induced apoptosis with the increased expression of cleaved-PARP and cell population in Sub-G1 fraction those were not observed in MNNG/ADR cells. Since MNNG/ADR was resistant to the apoptotic signals induced by anti-cancer drugs and gamma-irradiation, we further analysed the mechanisms by which SAHA mediated cell death other than apoptosis in the cells. The data demonstrated that SAHA induced the expression of LC3 protein and formation autophagosomes, indicating the induction of autophagic cell death in the drug-resistant OS cells. This is the first report showing the potential clinical utility of SAHA for the novel therapy against the drug- and apoptosis-resistant OS.

Materials and Methods

Reagents and radiation

Adriamycin/Doxorubicin (Dox), FK228 and SAHA were obtained from Kyowa Hakko (Tokyo, Japan), Fujisawa (Osaka, Japan) and Merck (Gibbstown, NJ) respectively. Apicidin was purchased from Calbiochem (San Diego, CA), and 3-methyladenine was from Sigma Chemical (St. Louis, MO). Cells were irradiated using an X-ray irradiation system (Hitachi Medico Technology Corporation, Chiba, Japan), operated at 150kV and 20mA, yielding a dose rate of 3.9Gy/min, after

filtration of the beam by 1.0 mm Al.

Cell lines, culture conditions and establishment of the drug-resistant OS clones

Human OS cell lines MNNG obtained from the American Type Culture Collection (Manassas, VA) was cultured in Dulbecco's modified Eagle's medium (DMEM) (Nissui Pharmaceuticals Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 100 μ g/ml penicillin and 100 μg/ml streptomycin.

The multidrug resistant clone of MNNG, MNNG/ADR, was established and characterized in our laboratory[13]. MNNG/ADR was isolated after multiple steps of selection in the presence of increasing concentrations of Dox. Dox concentration was increased from 2.5 to 100 ng/ml (2.5, 5, 10, 25, 50, 100 ng/ml).

Chemo-sensitivity assay

For the chemo sensitivity assay, 3×10^3 cells were seeded in 96-well plates. After 24 h incubation, various concentrations of Dox, FK228 and apicidin were added to the media. SAHA was added to the media in the presence or absence of 250 μM 3-MA. The cells were incubated for 48 h and the number of viable cells in each well was measured using the CellTiter-Glo^{TM} Luminescent Cell Viability Assay (Promega, Madison, WI), according to the manufacturer's protocol. Before the use of MNNG/ADR cells in this assay, the cells were cultured in the media without Dox for 10 days. The chemo-sensitivity assay was carried out in triplicate and repeated at least three times.

Flow cytometry

Cells treated with FK228, apicidin and SAHA were harvested and fixed with 70% ethanol for 30 min at 4℃. Then, cells were centrifuged, and resuspended in PBTB (PBS containing 0.1% Tween 20 and 0.05% bovine serum albumin) with 10 μg/ml RNase A and 50 μg/ml propidium iodide. Alterations in cell cycle distribution were analyzed using an Epics-XL flow cytometer (Beckman Coulter). Cell proportions were analyzed using the EXPO32 Software (Beckman Coulter). For each sample, 10,000 events were scored.

Western blot analysis

Western blot analyses were carried out as described[20] with several modifications. Cells were harvested and solubilized in a Nonidet P-40-based lysis buffer (20 mM Tris (pH 7.4), 250 mM NaCl, 1.0% Nonidet P-40, 1 mM EDTA, 50 mg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). After incubation on ice for 5 min, cell lysates were clarified by centrifugation at 14,000 rpm for 30 min at 4 ℃. The protein quantity was determined using Bradford protein assays (Bio-Rad) and fractionated on pre-cast 4～12% gradient MOPS polyacrylamide gels (NOVEX, San Diego, CA). After transfer to nitrocellulose membranes, membranes were pretreated with TBS containing 5 % dry milk and 0.05% Triton X-100 (TBST) for 1 h at room temperature and then incubated with antibodies to acetylated histone H3 (Upstate Biotechnology, Inc., Lake Placid, NY), cleaved-PARP (Promega, Madison, WI), actin (BD Pharmingen, San Diego, CA), LC-3 (kindly gifted from Tamotsu Yoshimori, Department of Cell Genetics, National Institute of Genetics, Yata 1111 Mishima, Shizuoka-ken 411-8540, Japan) for 1 h at room temperature. After several washes in TBST, the filters were treated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz, Santa Cruz, CA) at room temperature for 1 h. After a final wash with TBST, immunoreactivity of the blots was detected using an enhanced chemiluminescence (ECL) detection system (Amersham, Buckinghampshire, UK).

Statistical analysis

For data on chemo-sensitivity assay (% cell death), the results were represented as means± SD from three independent experiments of triplicate wells. Data were analyzed by repeated measure ANOVA with Scheffe post-hoc test. Statistical analyses were performed using the StatView J-5.0 software (SAS Institute Inc, Cary, NC). P values less than 0.01 were considered to be significant.

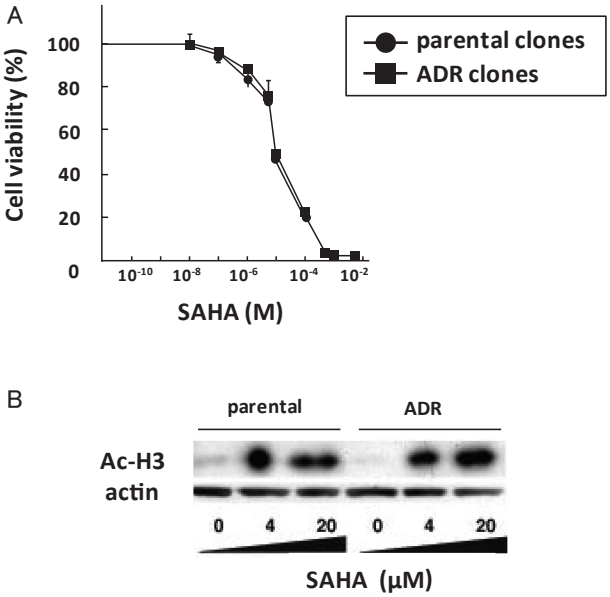

Fig. 1 (**A**) MNNG/ADR did not show cross-resistance to SAHA. The parental and ADR clones of MNNG (●, parental clone ; ■, ADR clone) were treated with various doses of SAHA for 48 h. The percentages of viable cells were determined by the CellTiter-Glo™ Luminescent Cell Viability Assay. The data represents the means of three separate experiments performed in triplicate ; bars represent SD. (**B**) Effect of SAHA on acetylation of histone H3 in MNNG and MNNG/ADR clones. Cells were incubated with various concentrations of SAHA for 24 h. The whole cell lysates were isolated, and the accumulation of acetylated histone H3 was examined by western blot analysis using antibodies against the acetylated histone H3 (17 kD). The actin blot was performed as loading control.

Transmission electron microscopy

Cells were harvested, pelleted, and fixed in 2.5% glutaraldehyde/2 % paraformaldehyde in cacodylate buffer. After rinse with cacodylate buffer, the samples were postfixed in 2% osmium tetroxide for 1 h. The samples were then rinsed with water, followed by dehydration in a graded series of alcohol (50%, 75%, and 95～100% alcohol) followed by propylene oxide, and kept overnight in 1 : 1 propylene oxide/poly Bed 812. The samples were embedded in Poly Bed 812 and cured in a 60℃ oven. Ultrathin sections were obtained with a Reichert Ultracut S microtome. Sections were stained with uranyl acetate and lead citrate and photographed by using a Jeol 1200 EX 11 transmission electron microscope.

Results

SAHA could reduce the viability of MNNG/ADR as well as the parental MNNG

We initially investigated whether SAHA could reduce the viability of MNNG/ADR clone as well as the parental MNNG clone. Since we have found that MNNG/ADR expressed P-gp and MRP1 and that cyclic-tetrapeptide families of HDACIs including FK228 and apicidin were the substrates of these efflux pumps[19], we used Dox, FK228 and apicidin as controls in the chemosenseitivity assay. After ADR and the parental clones of MNNG were treated with media containing various concentrations of Dox, FK228, apicidin and SAHA for 48 h, the number of viable cells was

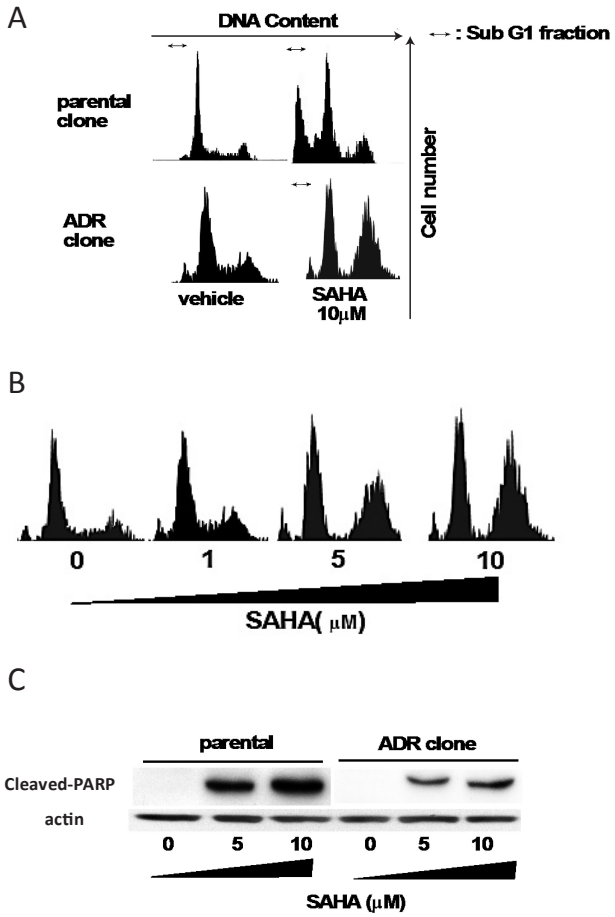

Fig. 2 SAHA induced less apoptosis in MNNG/ADR than in MNNG. (**A**) Cells were incubated with 10 μM SAHA for 24 h and fixed in 70% ethanol. After staining with PI, the apoptotic DNA content was analyzed by flow cytometry. (**B**) Cells were incubated with various doses of SAHA for 24 h and fixed in 70 % ethanol. After staining with PI, the apoptotic DNA content was analyzed by flow cytometry. (**C**) After the cells were incubated with various concentrations of SAHA for 24 h, the whole cell lysates from the cells were subjected to western blot analysis using anti cleaved-PARP and actin antibodies.

analyzed[19]. The chemo-sensitivity assay revealed that SAHA suppressed the growth of MNNG/ADR clone as well as that of MNNG (Fig. 1A).

We next examined the status of acetylated histone H3 (Ac-H3) to investigate whether SAHA would show the same inhibitory effects on HDACI activities in MNNG/ADR as in MNNG. After the treatment with SAHA for 24 h, whole cell lysates from the cells were extracted and subjected to western blot analysis. The results indicated that SAHA could increase the level of Ac-H3 in MNNG/ADR clone as same in the parental clone (Fig. 1B). These data support the notion that SAHA might not be the substrates of P-gp and MRP1, unlike FK228 and apicidin.

SAHA induced less apoptosis in ADR clone than in the parental clone

A number of studies have demonstrated that HDACIs cause a variety of phenotypic changes, such as cell cycle arrest and apoptosis[14)~16)]. Therefore, we next performed flow cytometric

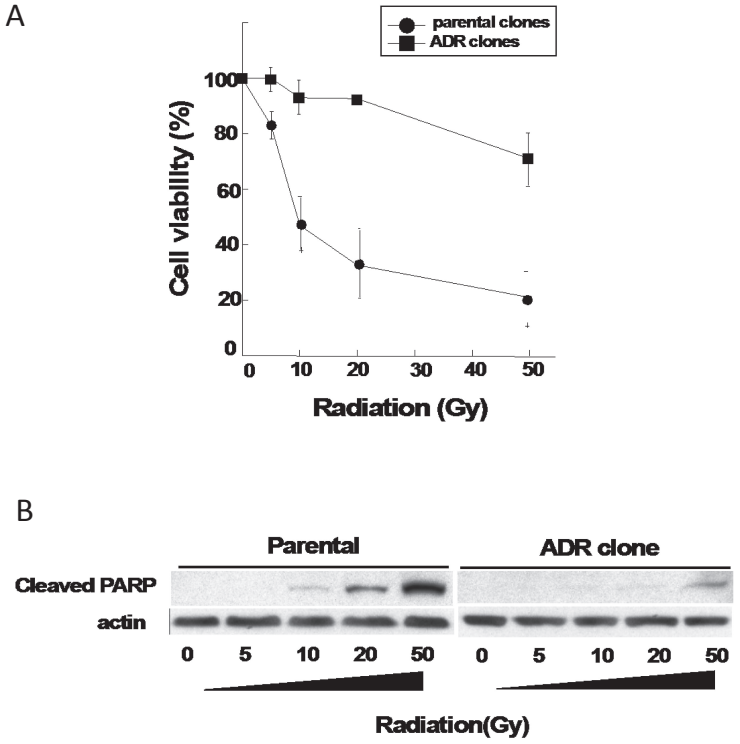

Fig. 3 MNNG/ADR showed cross-resistance to the radiation. (**A**) After the exposure to various strength of the radiation, the cells were incubated for 48 h, and the number of viable cells was counted. The data represents the means of three separate experiments performed in triplicate ; bars represent SD. (**B**) After the irradiation in various strength, the cells were incubated for 24 h, then the cell lysates from these cells were subjected to western blot analysis using cleaved-PARP and actin antibodies.

analysis to define cell cycle profiles of MNNG and MNNG/ADR treated with SAHA. The treatment with 4 μM SAHA for 24 h caused the accumulation of populations in the sub-G1 fraction in the parental MNNG cells (Fig. 2A). However, the increase in percentage of sub-G1 fraction induced by 4 μM SAHA in MNNG/ADR (1.9%) was less than that in MNNG (30.1%). When the concentration of SAHA was increased, the accumulation of the cell population of MNNG/ADR in G2/M fraction in the cell cycle was observed (Fig. 2B).

We further examined the proteolytic cleavage of poly (ADP-ribose) polymerase (PARP) as another marker of apoptosis in ADR and parental clones. The 116 kDa PARP is specifically cleaved to produce an 85 kDa fragment during apoptosis. Western blot analysis using the antibody specific to 85 kDa fragment of the cleaved PARP demonstrated that SAHA induced PARP cleavage dose-dependently in both MNNG and MNNG/ ADR cells. However, the induction level of the cleaved PARP by SAHA in MNNG/ADR was apparently lower than that in MNNG (Fig. 2C). These data indicate that although SAHA could induce apoptosis in both the parental and MNNG/ADR clones, the induction of apoptosis was much less in MNNG/ADR than in the parental MNNG cells.

MNNG/ADR cells showed cross-resistance to the radiation-induced apoptosis

SAHA could reduce the viability of MNNG and MNNG/ADR cells to the same extent, however, there was a clear difference between the extent of SAHA-induced apoptosis in MNNG and MNNG/ADR. To evaluate whether MNNG/ADR would exhibit the unresponsiveness to apoptotic signals, we performed a cell-viability assay with irradia-

tion. The ADR and parental clones of MNNG were exposed to the various doses of radiation. 24h later, the number of viable cells was counted. We found that although the radiation suppressed the cell viability of MNNG/ADR, the extent of the inhibition of the viability of MNNG/ADR was less than that of MNNG (Fig. 3A), indicating that MNNG/ADR clone exhibited cross-resistance against the radiation. We further examined the expression of the cleaved PARP in ADR and parental clones of MNNG after the irradiation. Western blot analysis demonstrated that radiation dose-dependently induced the cleavage of PARP in both MNNG and MNNG/ADR, however, the induction level of PARP cleavage in MNNG/ADR clone was much less than that in MNNG (Fig. 3B). These results indicated that the apoptosis was less induced in MNNG/ADR than in MNNG by radiation, suggesting that MNNG/ADR might have an anti-apoptotic phenotype in addition to the overexpression of ABC transporters. Taken together, the results suggest that SAHA might induce cell death via certain mechanism other than apoptosis in MNNG/ADR cells.

SAHA could induce autophagic cell death

Apoptosis is defined as programmed cell death type I, whereas another types of cell death have been reported[20]. Autophagic cell death is defined as the programmed cell death type II and characterized by the formation of autophagosome which could be detected by transmission electron microscopy[21]. Therefore, we next investigated the ultrastructural morphology of MNNG and MNNG/ADR treated with SAHA. SAHA treatment induced the formation of many double-membrane cytoplasmic engulfing cytoplasm or cytoplasmic organelles, called as autophagosme (Fig. 4A b-d). Fig. 4A-d, a higher-magnified picture, shows the typical autophagosomes formed in MNNG/ADR cells by SAHA. We also counted the number of the autophagosomes in the cells. The treatment with SAHA significantly increased the number of autophagosomes both in MNNG/ADR and MNNG. However, the induction level of the formation of autophagosomes was much higher in MNNG/ADR than that in MNNG (Fig. 4B).

Since autophagy is known to be inhibited by a PI3 kinase inhibitor 3-MA, we tested whether SAHA-induced cell death would be sensitive to the challenge with 3-MA. Strikingly, the administration of 3-MA did not affect the induction of cell death by SAHA in MNNG cells, but reduced that in MNNG/ADR (Fig. 4C). These results demonstrate that the inhibition of autophagy lead to the inhibition of SAHA-induced cell death in MNNG/ADR, suggesting SAHA might induce the autophagic cell death in the ADR cells.

SAHA induced autophagy with the induction of both LC3-I and LC3-II in MNNG/ADR

Microtubule-associated protein 1 light chain (LC3), a homologue of Apg8p essential for autophagy in yeast, is associated to the autophagosme membranes. Two isoforms of LC3, LC3-I and LC3-II, were produced post-translationally in various cells. The 16kD-final form of LC-3, designated LC3-II, associates tightly with the autophagosomal membrane and is the only reliable marker of autophagic activity[23]. Therefore, we also examined the expression of LC3-II as the further marker of autophagy in MNNG/ADR cells treated with SAHA. Western blot analysis using the LC3 antibody demonstrated that SAHA increased the expression of LC3-II both in time- and dose-dependent manners (Fig. 5). It has been reported that LC3-I is cleaved and converted to LC3-II, and that the increase in LC3-II and the decrease in LC3-I during autophagy was observed[22)23]. However, our data interestingly showed that SAHA induced the expression of both LC3-I and LC3-II.

Discussion

HDACIs are novel and promising anti-tumor agents which cause cell cycle arrest, morphological reversion of transformed cells, differentiation and apoptosis by the activation of transcription of

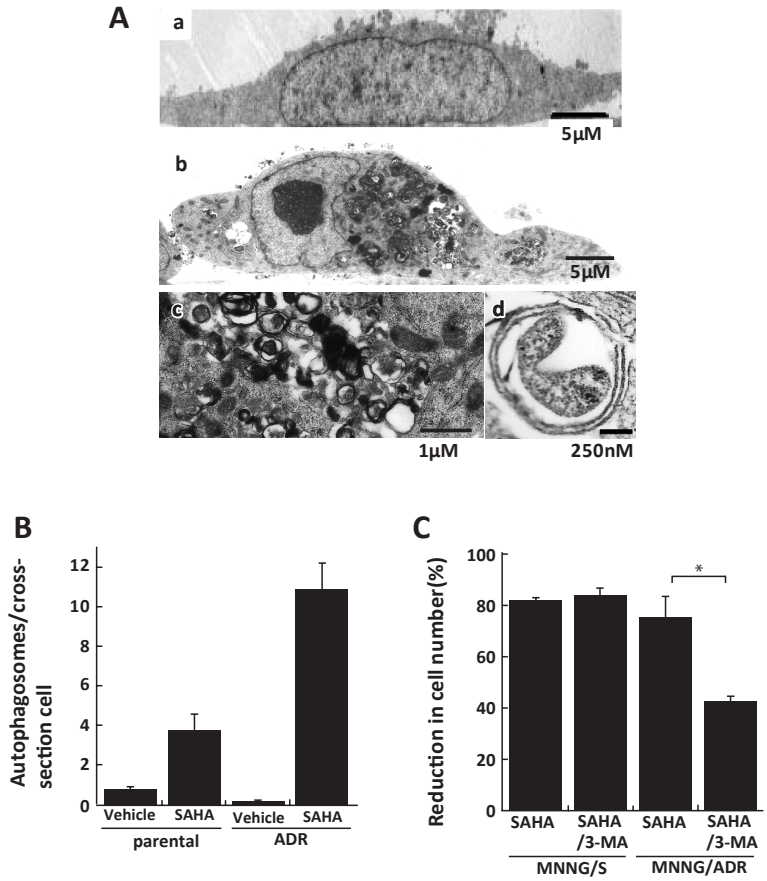

Fig. 4 SAHA induced autophagy in MNNG/ADR. (**A**) MNNG/ADR were treated without (a) or with 4 μM SAHA (b–d) for 24 h, and transmission electron microscopic study was performed as described in Materials and Methods. SAHA induced autophagosome formation in MNNG/ADR (b–d). Higher magnified pictures (c and d) show the clear autophagosome structures. (**B**) Quantitation of the number of autophagosomes per cross-sectioned cells. The actin blot was performed as loading control. (**C**) 3-MA inhibited SAHA-induced cell death. Cells were incubated with various concentrations of SAHA for 24 h in the presence or absence of 250 μM 3-MA.

the specific genes[14)~16)]. In the present study, we clearly demonstrated that SAHA could induce not only apoptosis in the parental OS cells, but also autophagic cell death in the cells resistant to apoptosis. Although previous study have suggested that SAHA induces programmed cell death type I (apoptosis) and type II (autophagic cell death)[24)], only the formation of autophagosomes was shown using the electron microscope. In the present study, we demonstrated that SAHA induced the expression of LC3-II, the marker of autophagy, and that SAHA formed autophagosomes in MNNG/ADR cells. Moreover, 3-MA which is known as the inhibitor of autophagy efficiently inhibited the induction of cell death by SAHA in the apoptosis-resistant OS cells. To our knowledge, this is the first report clearly demonstrating that SAHA could induce the autophagoic cell death in the cells resistant to apoptosis signals.

We also demonstrated two new findings regarding the SAHA-mediated autophagy. The first is the pathway of autophagy induced by SAHA. It has been shown that the expression of beclin1 is upregulated in the induction of autophagy[25)]. It is also reported that LC3-I is

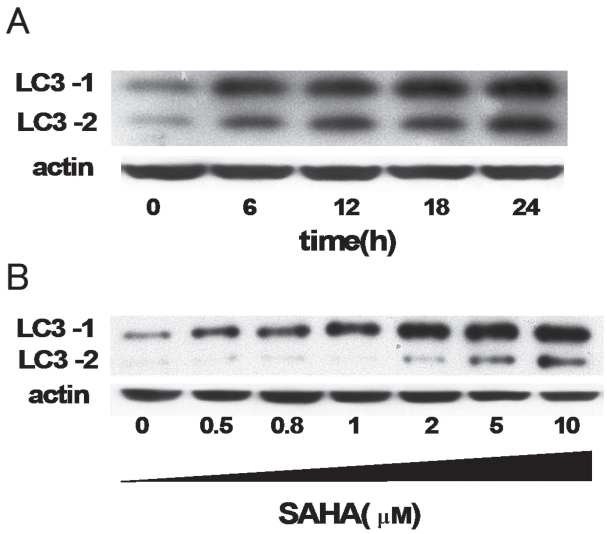

Fig. 5 SAHA induced autophagy with the induction of LC3-I and LC3-II in MNNG/ADR. (**A**) MNNG/ADR was treated with 4 μM SAHA. The induction of LC3-I and LC3-II expression at the indicated time after the treatment with SAHA was examined by western blot analysis using antibody against LC3. The actin blot was performed as loading control. (**B**) MNNG/ADR was treated with various doses of SAHA for 24 h. Whole cell lysates were isolated, and the induction of LC3-I and LC3-II was examined by western blot analysis using the antibody against LC3. The actin blot was performed as loading control.

converted to LC3-II, and the expression of LC3-I decreases whereas that of LC3-II increases during autophagy[23]. However, in the present study, we found that SAHA upregulated the expression of both LC3-I and LC3-II in MNNG/ADR cells. Therefore we next examined the expression level of LC3-I mRNA using quantitative RT-PCR. Although the protein expression of LC3-I was clearly detected as shown in Fig. 5, we failed to detect the expression of LC3-I mRNA (data not shown). In order to clarify the mechanisms of induction of the expression of LC3-I, the further studies are needed.

The second is the relationship between autophagy and cell cycle. Although the association of the cell cycle with apoptosis has been well analyzed, little is known about that with autophagy. In this study, we demonstrated that SAHA induced apoptosis and the accumulation of sub-G1 fracrion in the parental MNNG cells, and that SAHA induced autophagy and G2/M arrest in MNNG/ADR clone (Fig. 2). Several studies reported that mild heat shock, temozolomide and plumbagin induced autophagy and G2/M arrest in malignant glioma and breast cancer cells[26)~28)]. Therefore our results were consistent with the previous studies. These observations suggest the possibility that there might be close relationship between autophagy and G2/M arrest. Further examinations should be needed for the elucidation of precise mechanisms of autophagic cell death in the apoptosis-resistant OS cells by SAHA.

MNNG/ADR clone used in this study had the resistance to radiation as well as to antitumor drugs. The mechanisms of the resistance were associated with the overexpression of ABC transporters and the unresponsiveness to apoptotic signals. Since the clinical recurrent and metastatic tumors might also have the similar resistance to various antitumor modalities, novel

therapeutic approaches for the advanced tumors are strongly needed. Our results herein suggested the effectiveness of SAHA in the therapeutic application for OS and further apoptosis-resistant OS. SAHA could induce apoptosis in the parental OS cells (apoptosis-sensitive) and autophagic cell death in multidrug- and radio-resistant OS cells (apoptosis-resistant). Thus, these data indicated the possibility that SAHA might have the dual mechanisms to induce cell death in tumor cells, according to the characteristics of their resistance to apoptosis. SAHA is under clinical trials in advanced cases of several malignant tumors. The present study provides, for the first time, the strong rationale for the clinical application of SAHA to the advanced OS.

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（和文抄録）

ヒストン脱アセチル化酵素阻害剤 Suberoylanilide Hidroxamic Acid は骨肉腫細胞株において，二つのプログラム細胞死を選択的に誘導する

九州大学大学院医学研究院臨床医学部門外科学講座整形外科学分野

岡 田 貴 充，花田麻須大，中 島 康 晴

骨肉腫の治療成績は近年飛躍的に改善されたが，再発・転移例の予後は依然として不良である．再発・転移例は制癌剤や放射線療法に抵抗性を持つことが多く，この問題の克服が骨肉腫の予後向上に重要である．骨肉腫の薬剤耐性には多剤耐性因子である P-glycoprotein（P-gp）や MRP1 の関与が示唆されており，一方放射線耐性機序としては apoptosis 抵抗性の獲得が挙げられる．

我々は以前 P-gp，MRP1 を発現する多剤耐性骨肉腫細胞株 MNNG/ADR を樹立したが，この細胞は P-gp，MRP1 による制癌剤耐性だけでなく，apoptosis 抵抗性により放射線耐性を有しており再発・転移例の良いモデルと考えられた．

ヒストン脱アセチル化酵素阻害剤（HDACI）の suberoylanilide hydroxamic acid（SAHA）は新規抗腫瘍薬として注目されている．薬剤感受性のある骨肉腫細胞株 MNNG では SAHA は cleaved-PARP の発現を上昇させ，細胞周期の sub-G1 分画を上昇させアポトーシスを誘導して抗腫瘍効果を発揮する．アポトーシス抵抗性を持つ多剤耐性骨肉腫細胞株 MNNG/ADR に対しては LC3-Ⅰと LC3-Ⅱの発現を誘導し電子顕微鏡で autophagosome の形成を促進しており，programed cell death type2 に分類されるオートファジー細胞死を誘導して抗腫瘍効果を発揮していることが分かった．この事実は SAHA は腫瘍細胞の持つ細胞死に対する抵抗性によって，誘導する細胞死のタイプを選択できる可能性を示唆している．

現在の化学療法や放射線療法による抗腫瘍治療は，腫瘍細胞に主として apoptosis による細胞死を誘導する．これらの治療に耐性を示す腫瘍細胞は，多剤耐性因子の発現や apoptosis 抵抗性を獲得している可能性が考えられる．アポトーシスと異なる細胞死である「オートファジー細胞死」をも誘導する SAHA は，これらの治療抵抗性の腫瘍に対する効果が期待できる．

キーワード：ヒストン脱アセチル化酵素阻害剤，suberoylanylide hydroxamic acid，骨肉腫，オートファジー細胞死，アポトーシス