

Histone deacetylase inhibitor (HDACI) PCI-24781 potentiates cytotoxic effects of doxorubicin in bone sarcoma cells

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Abstract

Purpose To better understand the mechanisms of cytotoxicity and cell death induced by HDACI PCI-24781 in bone sarcoma cells.

Methods Four bone sarcoma cell lines were treated with PCI-24781, and the cytotoxicity was investigated. Further, accumulation of acetylated histones, p21, and PARP cleavage were evaluated in PCI-24781-treated cells. The synergistic effect of PCI-24781 to doxorubicin and its mechanism was investigated in bone sarcoma cells.

Results MTT assay demonstrated that the growth of bone sarcoma cells was inhibited after treatment with PCI-24781. Accumulation of acetylated histones, p21, and PARP cleavage were found in PCI-24781-treated cells. Expression of DNA

repair protein RAD51 was inhibited, and the expression of apoptosis protein GADD45 α was induced by PCI-24781 in bone sarcoma cells. Bone sarcoma cells treated with PCI-24781 become more sensitive to doxorubicin. The caspase-3/7 activity was increased with doxorubicin and PCI-24781 treatment in these cells.

Conclusions HDACI PCI-24781 has a synergistic effect on doxorubicin-induced apoptosis in bone sarcoma cells.

Keywords Histone deacetylase inhibitor · Chemotherapy · Bone sarcoma · Apoptosis

Introduction

Sarcomas are a diverse group of malignancies of mesenchymal origin [32]. In the United States, there will be approximately 13,000 new cases of sarcomas diagnosed each year, leading to approximately 5,000 deaths [14]. Available therapies for advanced sarcomas include chemotherapy, surgery, and radiotherapy. Bone sarcomas are treated with doxorubicin, ifosfamide, cisplatin, and methotrexate [24, 25]. Unfortunately, these drugs carry substantial toxicity and resistance tends to arise quickly. Complete responses to the agents are rare. After failure of these agents, the therapeutic options are limited, making bone sarcoma an important disease the search for alternative treatment approaches. The advent of molecularly targeted therapies has raised interest in their possible use in the treatment of sarcomas [32]. Unfortunately, the majority of bone sarcomas possess complex karyotypes without characteristic genetic changes [13]. To date, with the notable exception of gastrointestinal stromal tumors (GISTs), the implementation of targeted treatment systems in bone sarcoma has delivered mixed results.

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The acetylation and deacetylation of histones of nucleosomes by histone acetylases and histone deacetylases (HDACs) in chromatin play an important role in the regulation of gene expression. HDACs have been shown to be aberrantly expressed or regulated in cancers [29]. In addition to histones, HDACs also target dozens of non-histone proteins, including many transcription factors, tubulin, heat shock protein 90, and various signaling proteins, which play important roles in cell proliferation, cell death, and gene expression [11, 33, 35]. Altered HDAC activity leads to the inhibition of tumor suppressor genes and promotes the malignant phenotype. Accordingly, by inhibiting deacetylation of histones and allowing acetyl groups to remain on histones, histone deacetylase inhibitors (HDACIs) increase the open chromatin structure that allows gene transcription and may promote cancer cell apoptosis [9]. HDACIs demonstrated potent anticancer activity in many pre-clinical models, and several agents are currently in clinical trials. These drugs mediate multiple molecular changes and in turn can induce cell cycle arrest, apoptosis, or differentiation of cancer cells while displaying limited toxicity in normal cells [7, 9, 31].

Despite HDACIs showing promise as single agents, several recent studies have suggested that the optimal use of HDACIs is likely to be in combination with other chemotherapeutic agents. Many HDACIs, including vorinostat, depsipeptide, MS-275, and TSA, synergize with a large set of other chemotherapeutic drugs, such as gemcitabine, paclitaxel, cisplatin, etoposide, VP-16, and doxorubicin, which exert cytotoxic effects through a variety of mechanisms [2, 8, 10, 16, 27]. Multiple mechanisms have been proposed to explain the potent anticancer activity elicited by this drug combination. In all these cases, at least in vitro, these drugs cooperate with HDACIs to enhance apoptosis or differentiation of cancer cells [23, 28, 37]. In addition, it appears that the principle of synergism between HDACIs and other compounds can be recapitulated in a single hybrid compound [34].

PCI-24781 is a broad spectrum hydroxamic acid-based inhibitor of histone deacetylase that shows antitumor activity in vitro and in vivo pre-clinically and is under evaluation in phase I clinical trials for cancer [6]. PCI-24781 exhibited significant anticancer activity in soft tissue sarcoma [17]. However, information about the efficacy of PCI-24781 on bone sarcoma cells is limited. In the present study, we investigated the mechanisms of cytotoxicity and cell death induced by PCI-24781 on bone sarcoma cell lines. Our study demonstrated that through caspase and growth arrest- and DNA damage-induced gene 45 α (GADD45 α)-dependent mechanisms, PCI-24781 could induce apoptosis and inhibit growth of bone sarcoma cells. Furthermore, PCI-24781 significantly enhanced the cytotoxic effect of doxorubicin in bone sarcoma cells.

Materials and methods

Human sarcoma cell culture

Human osteosarcoma cell line U-2 OS was obtained from the American Type Tissue Collection (Rockville, MD) [36]. Dr. Efstathios S. Gonos (National Hellenic Research Foundation, Athens, Greece) kindly provided the human osteosarcoma cell line KH OS [18]. Dr. Katia Scotlandi (Institute Orthopedics Rizzoli, Italy) kindly provided the human Ewing's sarcoma cell line TC-71 [20]. The human chondrosarcoma cell line CS-1 was established in our laboratory [30]. All cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin (all obtained from invitrogen, Carlsbad, CA.). HDACI PCI-24781 was synthesized and provided by Pharmacyclics, Inc (Sunnyvale, California). Doxorubicin was obtained as unused residual clinical material at the Massachusetts General Hospital.

Western blot analysis

Protein lysates from cells were generated through lysis with 1 $\times$ RIPA Lysis Buffer (Upstate Biotechnology, Charlottesville, VA). The concentration of the protein was determined by Protein Assay Reagents (Bio-Rad, Hercules, CA) and spectrophotometer (Beckman DU-640, Beckman Instruments, Inc., Columbia, MD). Twenty-five micrograms of total protein was processed on Nu-Page 4–12% Bis-Tris Gel (Invitrogen) and transferred to a pure nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). Antibodies directed against acetylated histone H3 were obtained from Millipore Corporate (Billerica, MA). Antibodies directed against Poly (ADP-ribose) Polymerase (PARP) were obtained from Cell Signaling Technologies (Cambridge, MA). Antibodies directed against p21 were obtained from BD Biosciences (San Jose, CA). Antibodies directed against RAD51, GADD45 α , and actin were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Primary antibodies were incubated at 1:1,000 dilution in Tris-buffered saline, pH 7.4, with 0.1% Tween 20 and overnight at 4°C. Signal was generated through incubation with horseradish peroxidase-conjugated secondary antibodies (Bio-Rad, Hercules, CA) incubated in Tris-buffered saline, pH 7.4, with 5% non-fat milk, and 0.1% Tween 20 at 1:2,000 dilution for 1 h at room temperature. Positive immunoreactions were detected by using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL).

Combination of PCI-24781 with doxorubicin in bone sarcoma cells

To investigate the synergistic effect of PCI-24781 and doxorubicin on the growth of bone sarcoma cells, the cells

were treated with PCI-24781 for 4 h at first, then doxorubicin was added. After treatment for 7 days, the growth of bone sarcoma cells was evaluated by MTT. The bone sarcoma cells were also treated with PCI-24781 or doxorubicin alone for 7 days and evaluated by MTT as controls. To investigate the synergistic effect of PCI-24781 and doxorubicin on inducing apoptosis in bone sarcoma cells, the cells were treated with PCI-24781 for 4 h at first, then doxorubicin was added. After treatment for 48 h, the apoptosis was measured by caspase 3/7 activity assay. The bone sarcoma cells were also treated with PCI-24781 or doxorubicin alone for 48 h and evaluated by caspase 3/7 activity assay.

Cytotoxicity assay

The in vitro cytotoxicity assays were performed by MTT assay as previously described [36]. MTT was obtained from Sigma (St. Louis, MO). Then, 1.5×10^3 cells per well were plated in 96-well plates of DMEM medium containing PCI-24781 and/or doxorubicin. After culture in PCI-24781 and/or doxorubicin for 7 days, 10 μ l of MTT (5 mg/ml in PBS) was added to each well, and the plates were incubated for 4 h. The resulting formazan product was dissolved with acid-isopropanol, and the absorbance at a wavelength of 490 nm (A_{490}) was read on SPECTRAmax[®] Microplate Spectrophotometer (Molecular Devices, Sunnyvale, CA).

Caspase-3/7 activity assay

Caspase activity was measured with the Apo-One Homogeneous Caspase 3/7 assay kit (Promega Corporation) according to the manufacturer's instructions. The induction of apoptosis and associated activation of caspases-3/7 were measured by enzymatic cleavage of the profluorescent substrate rhodamine 110, bis-N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide (Z-DEVD-R110), with release of the intensely fluorescent rhodamine 110-cleaving group. Cells were seeded at a density of 1×10^5 /ml and incubated in a 96-well plate in the presence or absence of drug for 48 h. Hundred microliters homogeneous caspase-3/7 reagent was added to each well, and reaction mixture was incubated for 2 h at room temperature, and then fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. Results are expressed as relative fluorescence unit (RFU).

Data analysis

Values shown are representative of triplicate determinations in two or more experiments. Treatment effects were evaluated using a two-sided Student's *t* test (GraphPad

PRISM[®] 4 software, GraphPad Software, San Diego, CA). Errors are SD of averaged results, and $P < 0.05$ values were accepted as a significant difference between means.

Results

PCI-24781 induces acetylation of histone and inhibits growth in bone sarcoma cell lines

In order to determine whether HDAC inhibition by PCI-24781 affects the proliferation of bone sarcoma cells, a panel of human sarcoma cell lines, including osteosarcoma cell lines U-2 OS and KH OS, chondrosarcoma cell line CS-1 and Ewing's sarcoma cell line TC-71, were exposed in vitro to increasing concentrations (0.05–5 μ M) of PCI-24781 for 48 h. The accumulation of various mechanistic biomarkers proposed to be involved in the cytotoxic activity of HDAC inhibitors was analyzed by Western blot after treatment with PCI-24781. The growth of bone sarcoma cells was evaluated by MTT after treatment with PCI-24781. Western blot analysis demonstrated the dose-dependent accumulation of acetylated histone H3 in four sarcoma cell lines after treatment with PCI-24781 (Fig. 1). Those data suggest that HDAC enzymes are inhibited in these cells. In addition, PCI-24781-induced expression of the cyclin-dependent kinase inhibitor, p21, a protein postulated to play a role in the antitumor effect of HDAC inhibition. MTT assay demonstrated that the growth of all four bone sarcoma cell lines was inhibited after treatment with PCI-24781 (Fig. 2). The effect of PCI-24781 on induction of apoptosis was investigated by Western blot for PARP cleavage, an apoptotic marker associated with apoptosis biochemical event. PARP cleavage was detected in all four bone sarcoma cell lines after treatment with PCI-24781 (Fig. 1).

PCI-24781 synergizes with doxorubicin to inhibit the growth of bone sarcoma cells

To evaluate if PCI-24781 enhances the anticancer activity of conventional chemotherapeutic drugs in bone sarcoma cells, the bone sarcoma cell lines were treated with non-lethal doses of PCI-24781 and various concentrations of doxorubicin. Cytotoxicity assay demonstrated that the bone sarcoma cells, U-2 OS, KH OS, CS-1, and TC-71, when co-treated with PCI-24781, became more sensitive to doxorubicin than when treated with doxorubicin alone (Fig. 3). The IC₅₀ value for doxorubicin in parental U-2 OS cells (IC₅₀ = 0.042) is 3.2 greater than PCI-24781-treated U-2 OS cells (IC₅₀ = 0.013). The IC₅₀ value for doxorubicin in parental KH OS cells (IC₅₀ = 0.433) is 5.1 greater than PCI-24781-treated KH OS cells (IC₅₀ = 0.084). The IC₅₀

Fig. 1 Western blot analysis demonstrated that treating bone sarcoma cells with PCI-24781 resulted in the dose-dependent accumulation of acetylated histone H3 and p21. PARP cleavage was also detected in bone sarcoma cells following treatment with PCI-24781

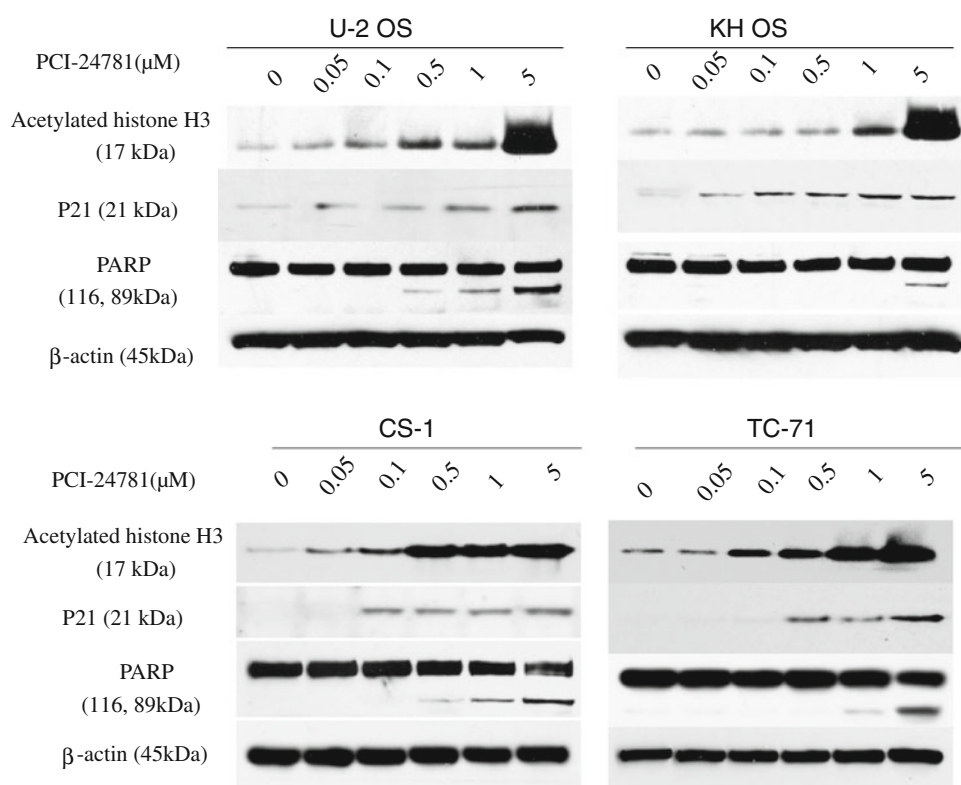
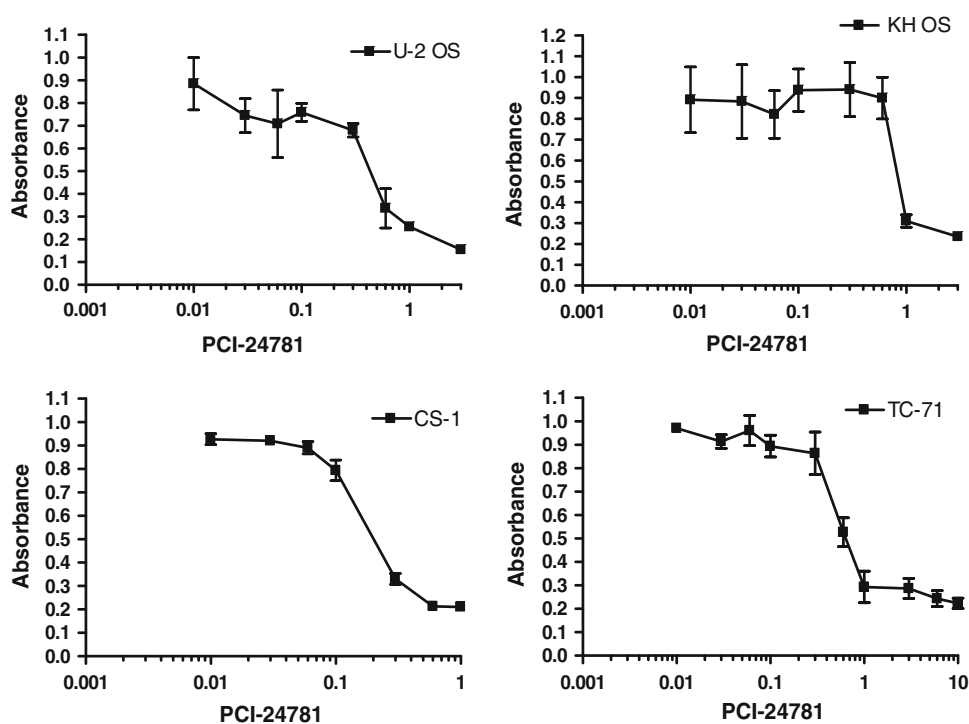


Fig. 2 MTT assay demonstrated that the growth of all four bone sarcoma cell lines was inhibited after treatment with PCI-24781



value for doxorubicin in parental CS-1 cells ($IC_{50} = 0.09$) is 2.2 greater than PCI-24781-treated CS-1 cells ($IC_{50} = 0.04$). The IC_{50} value for doxorubicin in parental TC-71 cells ($IC_{50} = 0.053$) is 3.5 greater than PCI-24781-treated TC-71 cells ($IC_{50} = 0.015$).

PCI-24781 synergizes doxorubicin to induce apoptosis in bone sarcoma cells

To investigate the mechanism underlying the synergy of PCI-24781 with doxorubicin in bone sarcoma cell line, cells

Fig. 3 Cytotoxicity assay demonstrated that the bone sarcoma cells treated with PCI-24781 become more sensitive to doxorubicin than the naive bone sarcoma cells

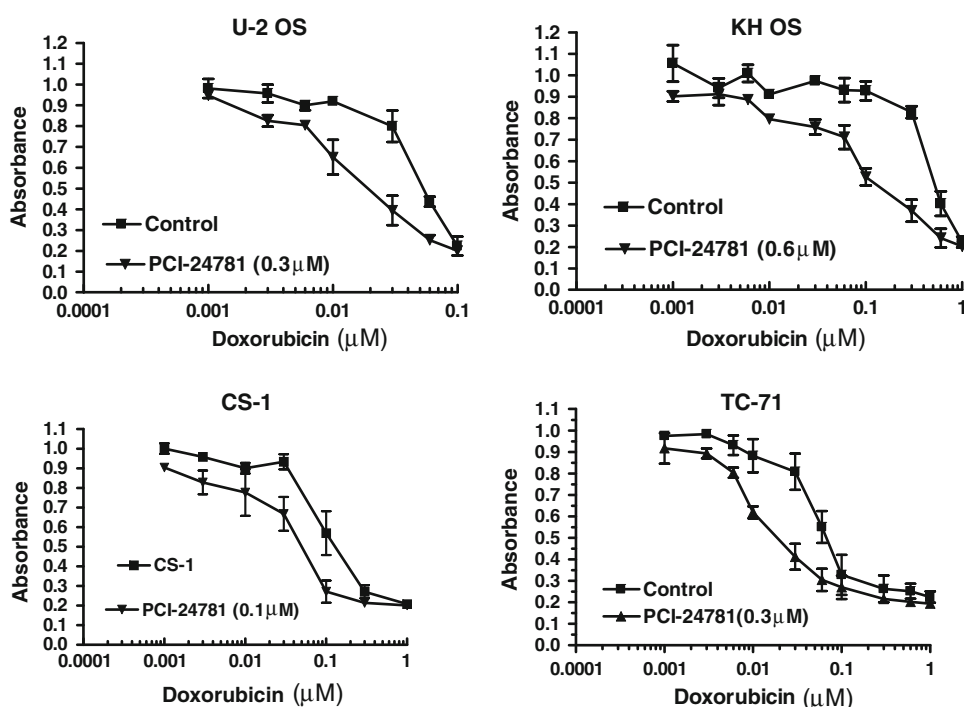
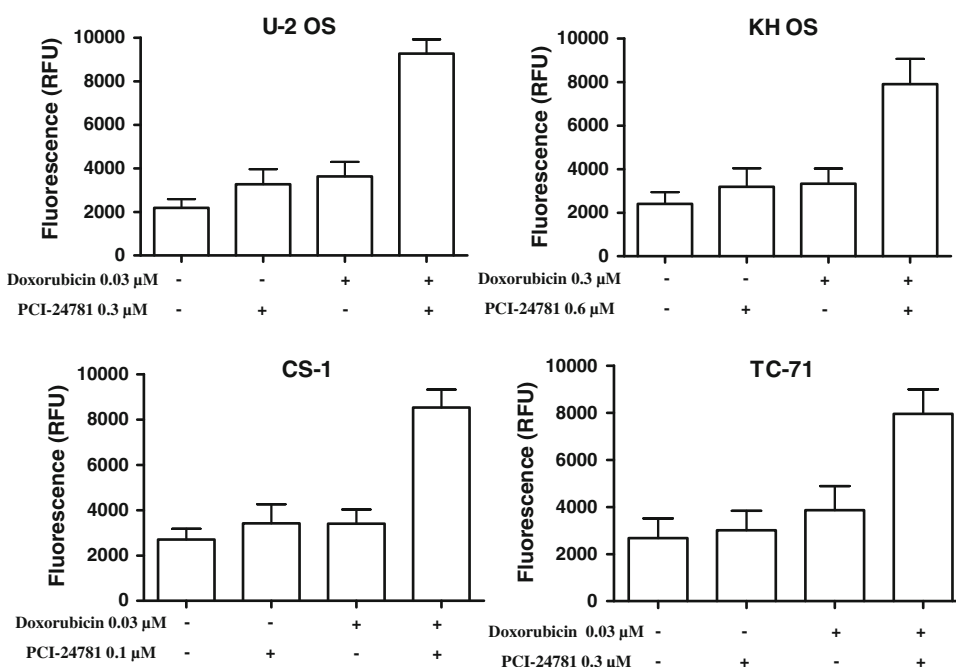


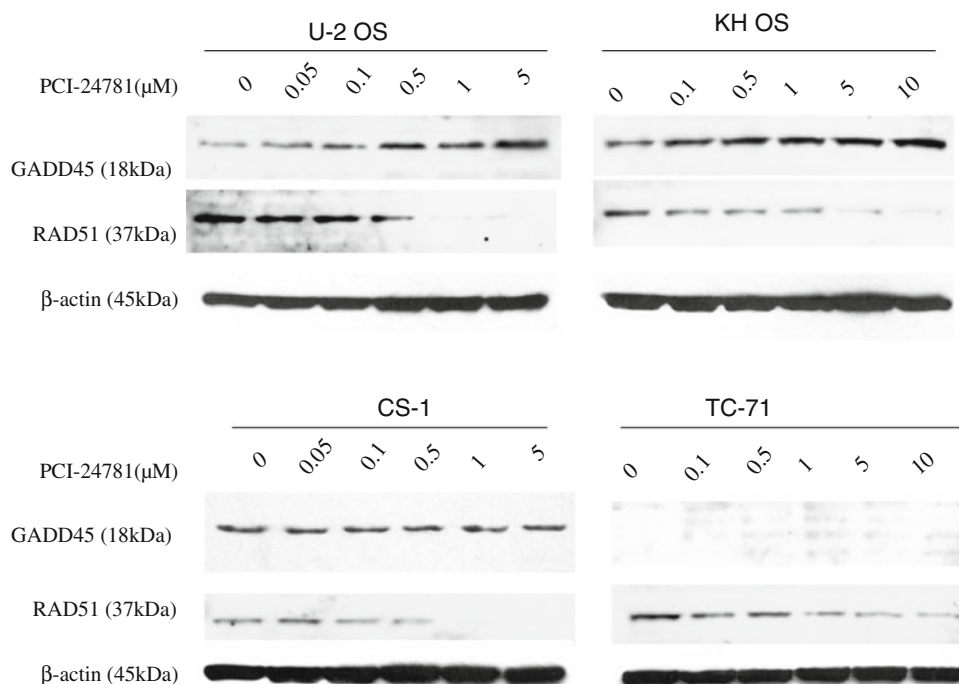
Fig. 4 The caspase-3/7 activity assay demonstrated increase in caspase-3/7 activity in bone sarcoma cells after treatment with combination of PCI-24781 and doxorubicin compared with the bone sarcoma cells treated with PCI-24781 or doxorubicin alone ($P < 0.0001$)



were treated with combination of PCI-24781 and doxorubicin, and the activity of caspase 3/7 was evaluated. The caspase-3/7 activity assay demonstrated increase in caspase-3/7 activity in bone sarcoma cell lines after treatment with combination of PCI-24781 and doxorubicin compared with the bone sarcoma cells treated with PCI-24781 or doxorubicin alone ($P < 0.0001$; Fig. 4). The expression of RAD51 and GADD45 α was evaluated by Western blot after treatment with various concentrations of PCI-24781. Western blot

demonstrated that the expression of RAD51 was inhibited by PCI-24781 in all four bone sarcoma cell lines (Fig. 5). The expression of GADD45 α was induced by PCI-24781 in osteosarcoma cell lines U-2 OS and KH OS (Fig. 5). The expression of GADD45 α was detected in chondrosarcoma cell CS-1. However, the expression of GADD45 α was not increased after treatment with PCI-24781 in CS-1 cells. The induction of GADD45 α was not found in Ewing's sarcoma cell TC-71 after treatment with PCI-24781.

Fig. 5 Western blot demonstrated that the expression of RAD51 was inhibited by PCI-24781 in bone sarcoma cells. The expression of GADD45 was induced by PCI-24781 in osteosarcoma cells U-2 OS and KH OS



Discussion

HDACIs currently in clinical development show some degree of pre-clinical activity against malignant cells proliferating in culture. Numerous animal model studies have demonstrated significant antitumor efficacy of HDACIs from virtually every structural class [9]. The rationale for the antitumor activity of HDACIs is characterized as inducing either cytostasis (cell cycle arrest), differentiation, or apoptosis. Increases in DNA accessibility caused by changes in acetylation may also enhance DNA damage and repair more directly [1, 3]. However, the HDAC-dependent mechanisms accounting for the observed and rather selective modulation of gene expression, as well as specific patterns of antitumor activity, remain poorly understood [7, 9, 31]. Treatment of cultured tumor cell lines grown in vitro with PCI-24781 resulted in the accumulation of acetylated histone and acetylated tubulin, resulting in an inhibition of tumor cell growth and the induction of apoptosis. Furthermore, tumor cells are thought to be more sensitive than normal cells to both the growth inhibiting and apoptosis promoting effects of most HDACIs. The induction of apoptosis by PCI-24781 was more effectively and rapidly occurring in tumor cells when compared to non-neoplastic cells [6]. PCI-24781 administered to mice harboring tumor xenografts also resulted in a statistically significant reduction in tumor growth at doses that were well tolerated as measured by body weight [6]. Lopez et al. [17] have shown that HDACI could induce S phase depletion, G2/M cell cycle arrest, and increase apoptosis on soft tissue sarcoma cells. The current study demonstrates that PCI-24781

can induce apoptosis and inhibit the growth of bone sarcoma cells. The accumulation of various HDACI mechanistic biomarkers, including acetylated histones and p21, was proposed to be involved in the antitumor activity of PCI-24781 in these bone tumor cells.

The optimal use of HDACIs is likely to be in combination with other anticancer therapies. Several studies have shown that HDACIs can synergistically enhance the anticancer activity of a large set of structurally and functionally diverse chemical compounds and biologically active polypeptides [2, 7, 8, 10, 16, 27]. It is possible that HDACIs will find their greatest utility not as monotherapies but as components of combination drug regimes. Thus, identifying relevant drug combinations and responsive cancer types and elucidating the mechanisms underlying the ability of HDACIs to enhance the efficacy of the chemotherapeutic agents is an important task. In the present study, we found caspase 3 and 7, two caspases that are responsible for the majority of intracellular caspase-induced cleavages and cell death were significantly induced by combination of PCI-24781 and doxorubicin in these bone sarcoma cell lines. This indicates that PCI-24781 has synergistic effect with doxorubicin to induce apoptosis in bone sarcoma cell lines. In addition, the expression of RAD51, which is a homologous recombination (HR) and DNA repair protein, was found to be inhibited in all four bone sarcoma cell lines after treatment with PCI-24781. RAD51 has been found to be increased in expression in a wide range of human tumors and multiple primary tumor cell types, which could alter recombination pathways to contribute to the chromosomal

rearrangements found in these cells [4, 5, 12, 21, 26]. Elevated amounts of RAD51 have been linked to tumor resistance to radiation and chemotherapy, and inhibiting the expression of RAD51 could reduce the appearance of DNA damage-induced nuclear foci and also increase sensitivity to chemotherapeutic drugs [26]. Our data indicate that inhibiting the expression of RAD51 by PCI-24781, results in a decrease in homology-directed repair of double-strand breaks (DSBs), which may play a role in the mechanisms underlying the anticancer synergy between PCI-24781 and doxorubicin in bone sarcoma cells. Lopez et al. [17] has also shown that PCI-24781 can synergize with doxorubicin in sarcomas, both in cells and in animal models. Maiso et al. [19] reported that the combination of doxorubicin and panobinostat, another HDACI, could provoke a strong activation of a DNA damage response in acute myeloid leukemia.

GADD45 α plays an important role in cell cycle control, survival, and apoptosis. The up-regulation of GADD45 α is an essential step for apoptosis induction in cancer cells by a variety of proapoptotic agents [15, 22]. It was reported that the deficiency of GADD45 α expression resulted in multi-drug resistance in osteosarcoma cells, and transfection with GADD45 α could make the osteosarcoma cells become more sensitive to chemotherapeutic drugs [36]. We found treatment of U-2 OS and KH OS by PCI-24781-induced GADD45 α expression (Fig. 5). These data suggest PCI-24781 synergizes with doxorubicin in osteosarcoma cells by activating GADD45 α . This will result in down-regulating the threshold of apoptosis for osteosarcoma cells to undergo apoptotic cell death triggered by doxorubicin. On the other hand, the GADD45 α gene expression was not increased in the chondrosarcoma cell line CS-1 and Ewing's sarcoma cell line TC-71 by PCI-24781, suggesting a GADD45 α -independent mechanism of PCI-24781-induced apoptosis in these cell lines. This indicates that the ability of PCI-24781 to synergize with doxorubicin has different mechanisms on different bone sarcoma cell lines.

In conclusion, the present study demonstrates that HDACI PCI-24781 has antitumor activity on bone sarcoma cell lines. It has synergistic effect on doxorubicin-induced apoptosis in bone sarcoma cells.

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References

- Adimoolam S, Sirisawad M, Chen J, Thiemann P, Ford JM, Buggy JJ (2007) HDAC inhibitor PCI-24781 decreases RAD51 expression and inhibits homologous recombination. *Proc Natl Acad Sci USA* 104:19482–19487
- Arnold NB, Arkus N, Gunn J, Korc M (2007) The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances gemcitabine-induced cell death in pancreatic cancer. *Clin Cancer Res* 13:18–26
- Banuelos CA, Banath JP, MacPhail SH, Zhao J, Reitsem T, Olive PL (2007) Radiosensitization by the histone deacetylase inhibitor PCI-24781. *Clin Cancer Res* 13:6816–6826
- Baumann P, Benson FE, West SC (1996) Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell* 87:757–766
- Benson FE, Stasiak A, West SC (1994) Purification and characterization of the human Rad51 protein, an analogue of *E. coli* RecA. *EMBO J* 13:5764–5771
- Buggy JJ, Cao ZA, Bass KE, Verner E, Balasubramanian S, Liu L, Schultz BE, Young PR, Dalrymple SA (2006) CRA-024781: a novel synthetic inhibitor of histone deacetylase enzymes with anti-tumor activity in vitro and in vivo. *Mol Cancer Ther* 5:1309–1317
- Carew JS, Giles FJ, Nawrocki ST (2008) Histone deacetylase inhibitors: mechanisms of cell death and promise in combination cancer therapy. *Cancer Lett* 269:7–17
- Dowdy SC, Jiang S, Zhou XC, Hou X, Jin F, Podratz KC, Jiang SW (2006) Histone deacetylase inhibitors and paclitaxel cause synergistic effects on apoptosis and microtubule stabilization in papillary serous endometrial cancer cells. *Mol Cancer Ther* 5:2767–2776
- Drummond DC, Noble CO, Kirpotin DB, Guo Z, Scott GK, Benz CC (2005) Clinical development of histone deacetylase inhibitors as anticancer agents. *Annu Rev Pharmacol Toxicol* 45:495–528
- Fuino L, Bali P, Wittmann S, Donapaty S, Guo F, Yamaguchi H, Wang HG, Atadja P, Bhalla K (2003) Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. *Mol Cancer Ther* 2:971–984
- Glozak MA, Sengupta N, Zhang X, Seto E (2005) Acetylation and deacetylation of non-histone proteins. *Gene* 363:15–23
- Gupta RC, Bazemore LR, Golub EI, Radding CM (1997) Activities of human recombination protein Rad51. *Proc Natl Acad Sci USA* 94:463–468
- Helman LJ, Meltzer P (2003) Mechanisms of sarcoma development. *Nat Rev Cancer* 3:685–694
- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008) Cancer statistics, 2008. *CA Cancer J Clin* 58:71–96
- Jiang T, Soprano DR, Soprano KJ (2007) GADD45A is a mediator of CD437 induced apoptosis in ovarian carcinoma cells. *J Cell Physiol* 212:771–779
- Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F (2003) Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Cancer Res* 63:7291–7300
- Lopez G, Liu J, Ren W, Wei W, Wang S, Lahat G, Zhu QS, Bornmann WG, McConkey DJ, Pollock RE, Lev DC (2009) Combining PCI-24781, a novel histone deacetylase inhibitor, with chemotherapy for the treatment of soft tissue sarcoma. *Clin Cancer Res* 15:3472–3483
- Lourda M, Trougakos IP, Gonos ES (2007) Development of resistance to chemotherapeutic drugs in human osteosarcoma cell lines largely depends on up-regulation of Clusterin/Apolipoprotein J. *Int J Cancer* 120:611–622
- Maiso P, Colado E, Ocio EM, Garayoa M, Martin J, Atadja P, Pandiella A, San-Miguel JF (2009) The synergy of panobinostat plus doxorubicin in acute myeloid leukemia suggests a role for HDAC inhibitors in the control of DNA repair. *Leukemia* 23(12):2265–2274
- Manara MC, Perdichizzi S, Serra M, Pierini R, Benini S, Hattinger CM, Astolfi A, Bagnati R, D'Incalci M, Picci P, Scotlandi K

- (2005) The molecular mechanisms responsible for resistance to ET-743 (Trabectedin; Yondelis) in the Ewing's sarcoma cell line, TC-71. *Int J Oncol* 27:1605–1616
21. Mohrenweiser HW, Wilson DM 3rd, Jones IM (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res* 526:93–125
 22. Mullan PB, Quinn JE, Gilmore PM, McWilliams S, Andrews H, Gervin C, McCabe N, McKenna S, White P, Song YH, Maheswaran S, Liu E, Haber DA, Johnston PG, Harkin DP (2001) BRCA1 and GADD45 mediated G2/M cell cycle arrest in response to antimicrotubule agents. *Oncogene* 20:6123–6131
 23. Nawrocki ST, Carew JS, Douglas L, Cleveland JL, Humphreys R, Houghton JA (2007) Histone deacetylase inhibitors enhance let-atumumab-induced apoptosis via a p21Cip1-dependent decrease in survivin levels. *Cancer Res* 67:6987–6994
 24. Patel SR, Vadhan-Raj S, Papadopolous N, Plager C, Burgess MA, Hays C, Benjamin RS (1997) High-dose ifosfamide in bone and soft tissue sarcomas: results of phase II and pilot studies—dose-response and schedule dependence. *J Clin Oncol* 15:2378–2384
 25. Picci P, Bacci G, Ferrari S, Mercuri M (1997) Neoadjuvant chemotherapy in malignant fibrous histiocytoma of bone and in osteosarcoma located in the extremities: analogies and differences between the two tumors. *Ann Oncol* 8:1107–1115
 26. Richardson C (2005) RAD51, genomic stability, and tumorigenesis. *Cancer Lett* 218:127–139
 27. Rikiishi H, Shinohara F, Sato T, Sato Y, Suzuki M, Echigo S (2007) Chemosensitization of oral squamous cell carcinoma cells to cisplatin by histone deacetylase inhibitor, suberoylanilide hydroxamic acid. *Int J Oncol* 30:1181–1188
 28. Ruefli AA, Ausserlechner MJ, Bernhard D, Sutton VR, Tainton KM, Kofler R, Smyth MJ, Johnstone RW (2001) The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species. *Proc Natl Acad Sci USA* 98:10833–10838
 29. Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem* 76:75–100
 30. Shao L, Kasanov J, Hornicek FJ, Morii T, Fondren G, Weissbach L (2003) Ecteinascidin-743 drug resistance in sarcoma cells: transcriptional and cellular alterations. *Biochem Pharmacol* 66:2381–2395
 31. Smith KT, Workman JL (2009) Histone deacetylase inhibitors: anticancer compounds. *Int J Biochem Cell Biol* 41:21–25
 32. Steinert DM, Blakely LJ, Salganick J, Trent JC (2003) Molecular targets in therapy for human soft-tissue and bone sarcomas. *Curr Oncol Rep* 5:295–303
 33. Sterner DE, Berger SL (2000) Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* 64:435–459
 34. Tavera-Mendoza LE, Quach TD, Dabbas B, Hudon J, Liao X, Palijan A, Gleason JL, White JH (2008) Incorporation of histone deacetylase inhibition into the structure of a nuclear receptor agonist. *Proc Natl Acad Sci USA* 105:8250–8255
 35. Walkinshaw DR, Yang XJ (2008) Histone deacetylase inhibitors as novel anticancer therapeutics. *Curr Oncol* 15:237–243
 36. Yang C, Yang S, Wood KB, Hornicek FJ, Schwab JH, Fondren G, Mankin H, Duan Z (2009) Multidrug resistant osteosarcoma cell lines exhibit deficiency of GADD45alpha expression. *Apoptosis* 14:124–133
 37. Zhao Y, Tan J, Zhuang L, Jiang X, Liu ET, Yu Q (2005) Inhibitors of histone deacetylases target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic protein Bim. *Proc Natl Acad Sci USA* 102:16090–16095