Effects of Carbon-ion Radiotherapy combined with a Novel Histone Deacetylase Inhibitor, Cyclic Hydroxamic-acid-containing Peptide 31 in Human Esophageal Squamous Cell Carcinoma

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Abstract. *Background: Carbon-ion radiotherapy has several potential advantages over X-rays. This therapy has been applied for various solid tumors including esophageal squamous cell carcinoma (SCC). However, some patients have shown resistance to this treatment. A new effective combined treatment strategy is required for improving the therapeutic effects. Histone deacetylase inhibitors (HDACIs) are new therapeutic candidates for cancer treatment. Several studies have evaluated the combination of X-rays and HDACIs, but, to date, no study has evaluated carbon-ion radiotherapy combined with HDACIs. Materials and Methods: Radio-sensitization to carbon-ion radiotherapy when combined with a novel HDACI cyclic hydroxamic-acid-containing peptide 31(CHAP31) was assessed in human esophageal SCC both in vitro and in vivo. Changes of expression of genes related to DNA repair, by CHAP31 were assessed by quantitative real-time reverse transcriptional PCR analysis. Results: CHAP31 induced sensitization to carbon-ion radiotherapy in vitro and tumor growth was significantly suppressed by the combination of carbon-ion radiotherapy with CHAP31 in comparison to either agent alone in in vivo experiments. CHAP31 inhibited the expression of genes related to DNA repair. Conclusion: CHAP31 sensitizes SCC cells to carbon-ion radiotherapy and this combinatory treatment may be a potentially useful therapeutic strategy for esophageal SCC.*

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Esophageal cancer is a highly malignant disease, in which progression of the disease is observed in most patients at the initial presentation (1). Neoadjuvant cytoreduction treatments are frequently used for tumor downstaging, increasing the resection rate and possibly improving survival (2). Although the combined treatment of radiation and anticancer drugs is a widely used therapeutic strategy, no satisfactory treatment regimen has yet been devised due to severe side-effects and the acquisition of resistance (3-5).

The biological effects of high linear energy transfer (LET) radiotherapy used with carbon-ion irradiation are more pronounced than low-LET conventional radiotherapy, such as X-rays or gamma rays (6). High LET radiotherapy is suitable for the local control of tumors because of its high relative biological effectiveness (RBE) and reduced oxygen enhancement ratio. Another advantage of carbon-ion radiotherapy is its superior dose distribution (7). The sharp Bragg peak of the carbon-ion enables the localization of energy to the target volume and improves the toxicity in conventional radiotherapy. However, severe late complications have been observed in patients who received high dose levels for esophageal cancer (8). Radiation pneumonitis, bone marrow suppression and other toxicities caused by carbon-ion irradiation are less frequent than those of X-ray irradiation in esophageal cancer patients. However, severe esophagitis, stenosis of the esophagus or penetration to either the trachea or aorta have been observed. Conversely, a few cases have also shown resistance to the treatment. Therefore, a suitable treatment strategy combined carbon-ion radiotherapy and other agents for cancer (9, 10) is required to resolve these problems and enhances the anti-tumor effect is needed.

The chief biological effects of radiotherapy are thought to be mediated by DNA damage, which affects cancer cell survival. The repair of DNA damage caused by carbon-ion

irradiation is presumed to be either less or occurs more slowly than that associated with X-rays. However the existence of DNA repair of damage caused by carbon-ion irradiation has been observed in some reports (11, 12) and it could be correlated with resistance to cancer treatment. If DNA repair were to be suppressed following irradiation by charged carbon-ion particles, it could increase the anticancer effect.

Recently, histone deacetylase inhibitors (HDACIs) have been suggested as new therapeutic candidates for molecular targeted therapy for cancer (13-16). Clinical trials have demonstrated the efficacy of HDACIs for several malignant diseases (17-20). The anticancer activity by HDACIs is presumably associated with alterations in gene expression. Some authors have reported that HDACIs inhibit gene expression related to DNA repair and sensitize cells to conventional radiotherapy (21-24). Cyclic hydroxamic-acid-containing peptide 31(CHAP31) has been examined in detail for anti-cancer activity and cellular functions (25). Therefore, the current study analyzed the expression of genes related to DNA repair after CHAP31 administration and tested whether CHAP31 affects the sensitivity of human esophageal SCC cells to carbon-ion radiotherapy both *in vitro* and *in vivo*.

Materials and Methods

Cell lines and culture. The human esophageal squamous cell carcinoma (SCC) cell lines (T.Tn, TE-2) were obtained from the Japan Cell Research Bank (26, 27). A single point mutation at codon 258 (G→T: termination) in exon 7 of the p53 gene occurs on one allele of the T.Tn cells. The TE-2 line was established from a poorly differentiated human esophageal SCC containing the wild-type p53 gene. Each of these cells can form solid tumors after injection of 5×10^6 cells in the back of an athymic nude mouse. We validated the p53 genetic status by genomic sequencing (data not shown). Both the tumor cell lines were maintained as monolayer cultures in Dulbecco's modified Eagle medium (DMEM)/F-12 (DMEM: F-12=1: 1) containing 10% heat-inactivated fetal bovine serum, 0.1% L-glutamine and 0.05% penicillin-streptomycin. The media and sera were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Histone deacetylase inhibitor; cyclic hydroxamic-acid-containing peptide 31 (CHAP31). The CHAP31 was generously provided by Dr. Minoru Yoshida (Riken Advanced Science Institute, Saitama, Japan). The synthesis and purification of CHAP31 has been previously described in detail (25).

Carbon-ion beam irradiation. The cells and tumors were irradiated with carbon-ions that were accelerated ≤290 MeV/n by a HIMAC (heavy-ion medical accelerator in Chiba) synchrotron at the National Institute of Radiological Science. The irradiation system and biophysical characteristics of carbon-ion irradiation have been described elsewhere (28, 29). Briefly, the initial energy of the accelerated carbon-ion was 290 MeV/n. High-LET beams obtained by using polymethyl methacrylate absorbers with various thickness were used to change the energy of the beam (9). The LET values in all the *in vitro* and *in vivo* experiments were calculated to be 50 keV/μm. The accelerated carbon-ion irradiation, carried out at room temperature, was given 24 hr after the administration of CHAP31. The dose rate of the beam was approximately 3 Gy/min.

In vitro experiments. The cells were maintained in 25-cm^2 plastic flasks and were incubated in a 5% CO_2 incubator at 37˚C. The cells were treated with CHAP31 (0, 5, 15 nM) for 0-24 h. Irradiation (0, 0.5, 1, 2, 3Gy) was carried out after CHAP31 exposure. After irradiation and/or CHAP31 exposure, the cells were removed from the culture by trypsinization in 0.05% trypsin in a 1 mM EDTA solution. The cells were plated onto 60-mm-diameter plastic dishes for the colony formation assay, using 400-30,000 cells after single treatment; 1×10^3 to 6×10^4 cells after combination treatment; 150-300 cells for the controls. The colonies were fixed and stained with a 0.2% crystal violet solution in 20% methanol after 14 days of incubation. The colonies with >50 cells were counted as survival colonies. The survival fractions were calculated as the ratio of survival colonies per number of plated cells. All the experiments were carried out in triplicate (9). Cell survival rates in comparison to the number of survival colonies without irradiation are presented.

Real-time quantitative reverse transcription-PCR (qRT-PCR). The expression changes of DNA repair related (Rad50, Mre11A, Nbs1) and cell cycle related ($p21^{WAF1}$) genes and were examined by qRT-PCR using the LightCycler technique (Roche DiagnosticsGmbH, Mannheim, Germany). Cells were seeded into a 75 cm^2 flask and incubated for 24 hours, then treated with or without an 15 nM concentration of CHAP31 before carbon-ion irradiation (2Gy) and harvested at 24 h after irradiation. Cells were washed with PBS and processed for RNA extraction with RNeasy kit (Qiagen, Inc., Chatsworth, CA). The cDNA templates for real-time PCR were synthesized from 1 μg of total RNA using SuperScript II reverse transcriptase and an oligo-dT primer. The β-actin gene served as internal control. The PCR reaction mixture consisted of DNA Master SYBR Green I mix (LightCycler-FastStart DNA Master SYBR Green I kit, Roche Diagnostics; containing Taq DNA polymerase, deoxynucleotide triphosphate, 3 mmol/L $MgCl_2$, and SYBR green dye), 0.5 μmol/L of each primer and cDNA. The PCR processes were as follows: initial denaturation at 95˚C for 10 min, followed by 45 cycles of denaturation at 95˚C for 15 sec, annealing at 57˚C for 10 sec and elongation at 72˚C for 8 to 18 sec. The Fit Points method provided by the LightCycler software was used to estimate the concentration of each sample. The expression value was calculated as follows: expression level of each mRNA/expression level of β-actin. The experiments were conducted in triplicate. The primers were chosen using Primer3 (available at http://wwwgenome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The following primer sequences were used: Rad50, 5'-CTACCGAGTGGTGATGCTGA-3' and 5'-CCACAGTTGAGGCAGAACG-3'; Mre11A, 5'-AGTAGTGACATT TCGG-GGGGAAGG-3' and 5'-AGTAGTGACATTTCG GGAAGG-3'; Nbs1, 5'-ATGGA-GGCCATATTTCCAGAC-3' and 5'-CAAGCAGCCAGAACTTGGAAG-3'; $p21^{WAF1}$, 5'-ACTTCGACT TTGTCACCGAGA-3' and 5'-CAAGACAGTGACA GGT-CCACAT-3'; and β-actin, 5'-GAGAAAATCTGGCACCACAC-3'; and 5'-TACCCCT-CGTAGATGGGCAC-3'.

Evaluation of the effects on tumor growth. Balb/c nu/nu athymic male mice (6-8 weeks old; CLEA Japan, Inc. Tokyo, Japan) were used. The animal experiments were performed at the Laboratory Animal Center of the National Institute of Radiological Science. The guidelines from the National Institute of Radiological Sciences Safety and Health Regulations for Handling Experimental Animals

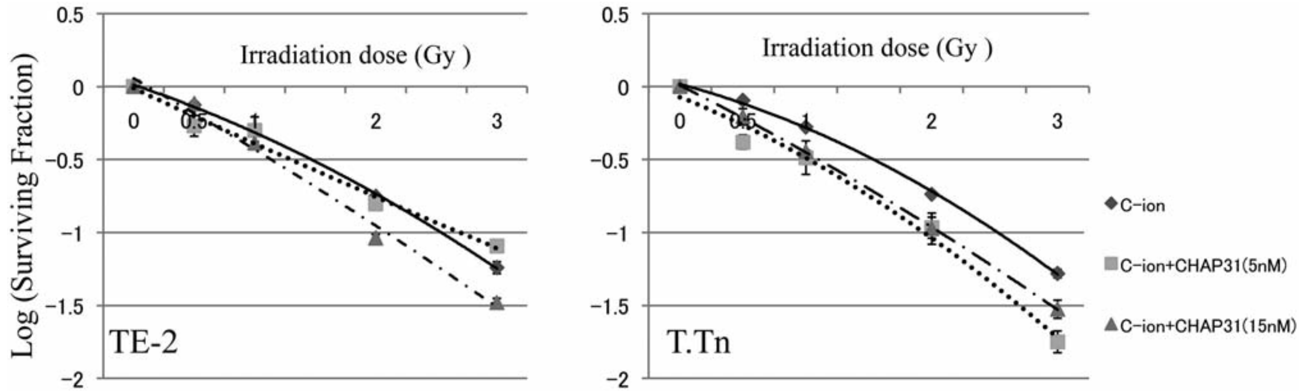

Figure 1. *In vitro growth suppression of human esophageal squamous cell carcinoma (TE-2, T.Tn) cells irradiated with carbon-ion beam with or without 24h CHAP31 exposure before irradiation. Curves are normalized to CHAP31 treatment alone. C-ion; Carbon-ion irradiation.*

(2001) were followed. The mice with 5×10^6 T.Tn or TE-2 cells were inoculated subcutaneously in 0.2 ml culture medium and randomly divided on day 0 of treatment into 4 groups as follows: i) control group; ii) CHAP31 only injection group; iii) carbon-ion irradiation only group and iv) CHAP31 injection before and after carbon-ion irradiation group to adjust mean tumor volume of each group. CHAP31 was administered to the tumor-bearing mice by intravenous (*i.v.*) injections with a 27-gauge hypodermic needle at a dose of 5 mg/kg on day 1, 4, and 7. On day 2, 24 h after the first CHAP31 injection, the tumor was irradiated with a single dose of 3 Gy. The mice were anesthetized by intraperitoneal injections of pentabarbiturate prior to irradiation. The tumor size was measured with calipers every 5 days over a period of 35 days. The tumor volume was calculated according to the (length × width^2)/2 and presented as the mean (± standard deviation (SD)) mm^3.

Results

In vitro cytotoxic effects of CHAP31 and carbon-ion irradiation. The *in vitro* survival curves of the T.Tn and TE-2 cells treated for 24 h with CHAP31 before carbon-ion irradiation of are shown in Figure 1. The combined therapy was stronger than either single therapy used alone in regard to its anticancer efficacy. In addition, the relationship between CHAP31 administration and carbon-ion irradiation was statistically significant analyzed using two-way ANOVA for each cell line ($p<0.001$).

Effect of CHAP31 on expression of genes related to DNA repair and cell cycle. The gene expression levels of Rad50, Mre11A and Nbs1 decreased after 24 h CHAP31 administration (Figure 2a, 2b). The gene expression levels of p21^{WAF1} increased. The gene expression of Rad50 and Mre11A in the TE-2 cells of Mre11A in the T.Tn cells was increased after carbon-ion irradiation. CHAP31 inhibited the gene expressions increased by carbon-ion irradiation (Figure 2c, 2d).

In vivo effects of carbon-ion irradiation and CHAP31 administration. The growth suppression effects evaluated by the tumor volumes are shown in Figure 3. At day 35, the mean tumor volume of the combined treatment group was significantly smaller than the tumor volume in either single therapy group ($p<0.05$, Mann-Whitney *U*-test, respectively).

Discussion

The mechanism by which HDACIs sensitize cells to conventional radiotherapy is thought to be due to cell cycle G2/M arrest, inhibition of DNA repair and increases in DNA double strand breaks. HDACIs are thought to alter gene expression by the hyperacetylation of histone tails.

Recent studies also reported that HDACIs exert cell cycle arrest by inducing p21^{WAF1} in some other cell lines (16, 30-32).

The Mre11-Rad50-Nbs1 (MRN) complex, consisting of proteins encoded by the genes Mre11, Rad50 and Nbs1, plays a crucial role in DNA double-strand break (DSB) repair (33) (Figure 4). The complex has many biological functions, including the formation of double strand breaks during meiosis, homologous recombination, telomere maintenance, S-phase checkpoint and genome stability during replication. The current results showed that CHAP31 suppressed the expression of this complex. This suggests that HDACs could inhibit the function of the MRN complex.

In the present study, the administration of CHAP31 and carbon-ion beam irradiation efficiently enhanced the anti-tumor effect on human esophageal SCC in both *in vitro* and *in vivo* experiments. The *in vitro* experiments showed a significant synergistic effect as suggested by the two-way ANOVA of each cell line, between carbon-ion irradiation and CHAP31 administration for esophageal SCC. CHAP31 sensitized the esophageal SCC cells *in vitro* (data not shown).

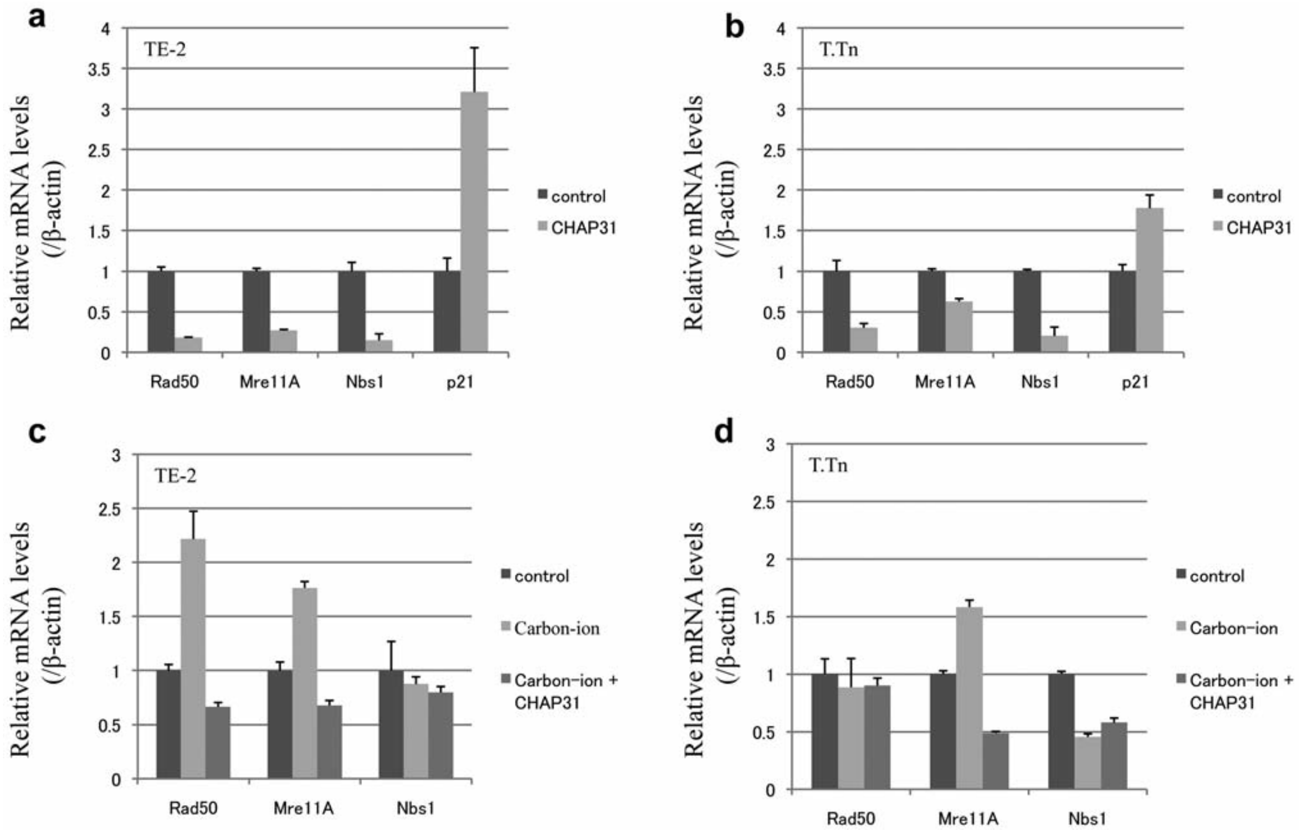

Figure 2. *The changes of gene expression of Rad50, Mre11A, Nbs1 and p21^{WAF1} after CHAP31 administration by quantitative real-time reverse transcription-PCR (qRT-PCR) analysis. Values shown are means ±standard deviation of 3 independent experiments. Control: untreated cells.*

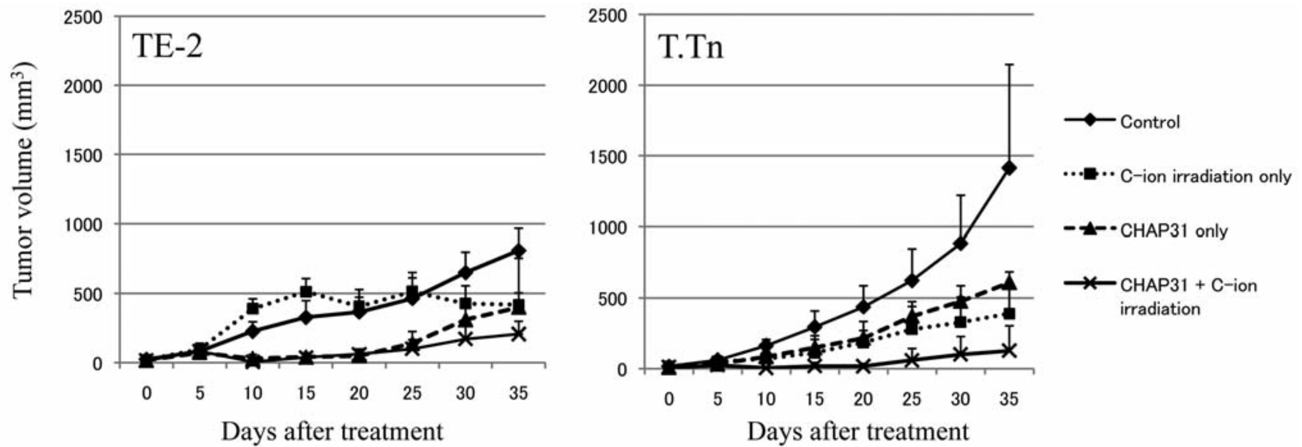

Figure 3. *The effect of carbon-ion 3 Gy irradiation and/or CHAP31 (5mg/kg i.v.) on in vivo tumor growth of esophageal squamous cell carcinoma (TE-2, T.Tn). Values represent the mean and standard deviation of 5 mice. Control: untreated.*

In the *in vivo* experiments, CHAP31 was administered to mice by *i.v.* injections. This method was considered an appropriate preclinical model. The combination therapy showed a statistically significant growth suppression in comparison to each of the single treatment groups. However, there was no significant effect of the combination of CHAP31 administration and carbon-ion irradiation based on two-way ANOVA. The *in vivo* tumor growth might be affected by

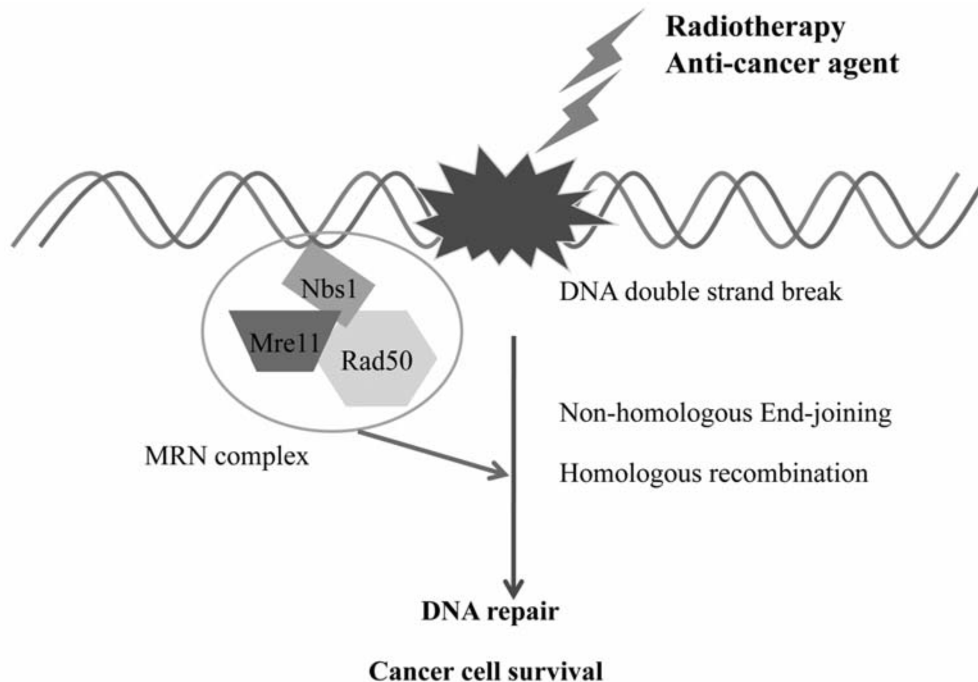

Figure 4. *The Mre11-Rad50-Nbs1 (MRN) complex plays a crucial role in DNA double-strand break repair.*

other elements such as the tumor stroma, paracrine factors, tumor vessels, or drug delivery and so on. Carbon-ion radiotherapy itself is an effective therapeutic modality for esophageal SCC. In this study, CHAP31 treatment was therefore shown to enhance the antitumor effects of carbon-ion irradiation on a human esophageal SCC cell line. Molecular targeted drugs such as HDACIs have demonstrated low toxicity and some agents are already used clinically. The use in combination with a carbon-ion localized radiation field may therefore be a less toxic and more effective therapeutic combination modality for esophageal cancer.

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References

1 Isono K and Ochiai T: Recent advances of treatment of cancer of the esophagus. Ann Cancer Res Ther *1*: 9-16, 1992.

2 Ancona E, Ruol A, Castoro C, Chiarion-Sileni V, Merigliano S, Santi S, Bonavina L and Peracchia A: First-line chemotherapy improves the resection rate and long-term survival of locally advanced (T4, any N, M0) squamous cell carcinoma of the thoracic esophagus: Final report on 163 consecutive patients with 5-year follow-up. Ann Surg *226*: 714-723, 1997.

3 Houghton JA, Weiss KD, Williams LG, Torrance PM and Houghton PJ: Relationship between 5-fluoro-2'-deoxyuridylate, 2'-deoxyuridylate, and thymidylate synthase activity subsequent to 5-fluorouracil administration, in xenografts of human colon adenocarcinomas. Biochem Pharmacol *35*: 1351-1358, 1986.

4 Shimada H, Hoshino T, Okazumi S, Matsubara H, Funami Y, Nabeya Y, Hayashi H, Takeda A, Shiratori T, Uno T, Ito H and Ochiai T: Expression of angiogenic factors predicts response to chemoradiotherapy and prognosis of oesophageal squamous cell carcinoma. Br J Cancer *86*: 552-557, 2002.

5 Aschele C, Sobrero A, Faderan MA and Bertino JR: Novel mechanisms of resistance to 5-fluorouracil in human colon cancer (HCT-8) subline following exposure to two different clinically relevant dose schedules. Cancer Res *52*: 1855-1864, 1992.

6 Ritter MA, Cleaver JE and Tobias C: High-LET radiations induce a large proportion of non-rejoining DNA breaks. Nature *266*: 653-655, 1977.

7 Chen GT, Castro JR and Quivey JM: Heavy charged particle radiotherapy. Ann Rev Biophys Bioeng *10*: 499-529, 1981.

8 Tsujii H, Mizoe JE, Kamada T, Baba M, Kato S, Kato H, Tsuji H, Yamada S, Yasuda S, Ohno T, Yanagi T, Hasegawa A, Sugawara T, Ezawa H, Kandatsu S, Yoshikawa K, Kishimoto R and Miyamoto T: Overview of clinical experiences on carbon ion radiotherapy at NIRS. Radiother Oncol *73(Suppl 2)*: S41-49, 2004.

9 Oohira G, Yamada S, Ochiai T, Matsubara H, Okazumi S, Ando K, Tsujii H, Hiwasa T and Shimada H: Growth suppression of esophageal squamous cell carcinoma induced by heavy carbon-ion beams combined with p53 gene transfer. Int J Oncol *25*: 563-569, 2004.

10 Kitabayashi H, Shimada H, Yamada S, Yasuda S, Kamata T, Ando K, Tsujii H and Ochiai T: Synergistic growth suppression induced in esophageal squamous cell carcinoma cells by combined treatment with docetaxel and heavy carbon-ion beam irradiation. Oncol Rep *15(4)*: 913-918, 2006.
11 Hirayama R, Furusawa Y, Fukawa T and Ando K: Repair kinetics of DNA-DSB induced by X-rays or carbon ions under oxic and hypoxic conditions. J Radiat Res (Tokyo) *46(3)*: 325-332, 2005.
12 Yashiro T, Koyama-Saegusa K, Imai T, Fujisawa T and Miyamoto T: Inhibition of potential lethal damage repair and related gene expression after carbon-ion beam irradiation to human lung cancer grown in nude mice. J Radiat Res (Tokyo) *48(5)*: 377-383, 2007.
13 Walkinshaw DR and Yang XJ: Histone deacetylase inhibitors as novel anticancer therapeutics. Curr Oncol *15(5)*: 237-243, 2008.
14 Lee EJ, Lee BB, Kim SJ, Park YD, Park J and Kim DH: Histone deacetylase inhibitor scriptaid induces cell cycle arrest and epigenetic change in colon cancer cells. Int J Oncol *33(4)*: 767-776, 2008.
15 Law AY, Lai KP, Lui WC, Wan HT and Wong CK: Histone deacetylase inhibitor-induced cellular apoptosis involves stanniocalcin-1 activation. *314(16)*: 2975-2984, 2008.
16 Hoshino I, Matsubara H, Hanari N, Mori M, Nishimori T, Yoneyama Y, Akutsu Y, Sakata H, Matsushita K, Seki N and Ochiai T: Histone deacetylase inhibitor FK228 activates tumor suppressor Prdx1 with apoptosis induction in esophageal cancer cells. Clin Cancer Res *11(21)*: 7945-7952, 2005.
17 Whitehead RP, Rankin C, Hoff PM, Gold PJ, Billingsley KG, Chapman RA, Wong L, Ward JH, Abbruzzese JL and Blanke CD: Phase II trial of romidepsin (NSC-630176) in previously treated colorectal cancer patients with advanced disease: a Southwest Oncology Group study (S0336). Invest New Drugs [Epub ahead of print], 2008.
18 de Bono JS, Kristeleit R, Tolcher A, Fong P, Pacey S, Karavasilis V, Mita M, Shaw H, Workman P, Kaye S, Rowinsky EK, Aherne W, Atadja P, Scott JW and Patnaik A: Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors. Clin Cancer Res *14(20)*: 6663-6673, 2008.
19 Braiteh F, Soriano AO, Garcia-Manero G, Hong D, Johnson MM, Silva Lde P, Yang H, Alexander S, Wolff J and Kurzrock R: Phase I study of epigenetic modulation with 5-azacytidine and valproic acid in patients with advanced cancers. Clin Cancer Res *14(19)*: 6296-6301, 2008.
20 Sharma S, Symanowski J, Wong B, Dino P, Manno P and Vogelzang N: A Phase II Clinical Trial of Oral Valproic Acid in Patients with Castration-Resistant Prostate Cancers Using an Intensive Biomarker Sampling Strategy. Transl Oncol *1(3)*: 141-147, 2008.
21 Chinnaiyan P, Cerna D, Burgan WE, Beam K, Williams ES, Camphausen K and Tofilon PJ: Postradiation sensitization of the histone deacetylase inhibitor valproic acid. Clin Cancer Res *14(17)*: 5410-5415, 2008.
22 Harikrishnan KN, Karagiannis TC, Chow MZ and El-Osta A: Effect of valproic acid on radiation-induced DNA damage in euchromatic and heterochromatic compartments. Cell Cycle *7(4)*: 468-476, 2008.
23 Cuneo KC, Fu A, Osusky K, Huamani J, Hallahan DE and Geng L: Histone deacetylase inhibitor NVP-LAQ824 sensitizes human nonsmall cell lung cancer to the cytotoxic effects of ionizing radiation. Anticancer Drugs *18(7)*: 793-800, 2007.
24 Entin-Meer M, Yang X, VandenBerg SR, Lamborn KR, Nudelman A, Rephaeli A and Haas-Kogan DA: *In vivo* efficacy of a novel histone deacetylase inhibitor in combination with radiation for the treatment of gliomas. Neuro Oncol *9(2)*: 82-88, 2007
25 Komatsu Y, Tomizaki K, Tsukamoto M, Kato T, Nishino N, Sato S, Yamori T, Tsuruo T, Furumai R, Yoshida M, Horinouchi S and Hayashi H: Cyclic Hydroxamic-acid-containing Peptide 31, a Potent Synthetic Histone Deacetylase Inhibitor with Antitumor Activity. Cancer Res *61*: 4459-4466, 2001.
26 Takahashi K, Kanazawa H, Chan H, Hosono T, Takahara M and Sato K: A case of esophageal carcinoma metastatic to the mandible and characterization of two cell lines (T.T. and T.Tn) Jpn. J Oral Maxillofac Surg *36*: 307-316, 1990.
27 Nishihira T, Kasai M, Mori S, Watanabe T, Kuriya Y, Suda M, Kitamura M, Hirayama K, Akaishi T and Sasaki T: Characteristics of two cell lines (TE-1 and TE-2) derived from human squamous cell carcinoma of the esophagus. Gann *70*: 575-584, 1979.
28 Kanai T, Endo M, Minohara S, Miyahara N, Koyama-ito H, Tomura H, Matsufuji N, Futami Y, Fukumura A, Hiraoka T, Furusawa Y, Ando K, Suzuki M, Soga F and Kawachi K: Biophysical characteristics of HIMAC clinical irradiation system for heavy-ion radiation therapy. Int J Radiat Oncol Biol Phys *44*: 201-210, 1999.
29 Ando K, Koike S, Nojima K, Chen YJ, Ohira C, Ando S, Kobayashi N, Ohbuchi T, Shimizu W and Kanai T: Mouse skin reactions following fractionated irradiation with carbon ions. Int J Radiat Biol *74*: 129-138, 1998.
30 Huang L and Pardee AB: Suberoylanilide hydroxamic acid as a potential therapeutic agent for human breast cancer treatment. Mol Med *6*: 849-866, 2000.
31 Sasakawa Y, Naoe Y, Inoue T, Sasakawa T, Matsuo M, Manda T and Mutoh S: Effects of FK228, a novel histone deacetylase inhibitor, on human lymphoma U-937 cells *in vitro* and *in vivo*. Biochem Pharmacol *64*: 1079-1790, 2002.
32 Sowa Y, Orita T, Minamikawa S, Nakano K, Mizuno T, Nomura H and Sakai T: Histone deacetylase inhibitor activates the WAF1/Cip1 gene promoter through the Sp1sites. Biochem Biophys Res Commun *241*: 142-150, 1997.
33 Bartkova J, Tommiska J, Oplustilova L, Aaltonen K, Tamminen A, Heikkinen T, Mistrik M, Aittomäki K, Blomqvist C, Heikkilä P, Lukas J, Nevanlinna H and Bartek J: Aberrations of the MRE11-RAD50-NBS1 DNA damage sensor complex in human breast cancer: MRE11 as a candidate familial cancer-predisposing gene. Mol Oncol *2(4)*: 296-316, 2008.

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