Antitumor histone deacetylase inhibitors suppress cutaneous radiation syndrome: Implications for increasing therapeutic gain in cancer radiotherapy

Yih-Lin Chung,1 Ae-June Wang,2 and Lin-Fen Yao2

1Department of Radiation Oncology, Koo Foundation Sun Yat-Sen Cancer Center, and School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China, and 2Drug Delivery Department, Biochemical Engineering Center, Industrial Technology Research Institute, Hsinchu, Taiwan, Republic of China

Abstract

Radiotherapy is an effective treatment for head and neck, skin, anogenital, and breast cancers. However, radiation-induced skin morbidity limits the therapeutic benefit. A low-toxicity approach to selectively reduce skin morbidity without compromising tumor killing by radiotherapy is needed. We found that the antitumor agents known as histone deacetylase (HDAC) inhibitors (phenylbutyrate, trichostatin A, and valproic acid) could suppress cutaneous radiation syndrome. The effects of HDAC inhibitors in promoting the healing of wounds caused by radiation and in decreasing later skin fibrosis and tumorogenesis were correlated with suppression of the aberrant expression of radiation-induced transforming growth factor β and tumor necrosis factor α. Our findings implicate that the inhibition of HDAC may provide a novel strategy to increase the therapeutic gain in cancer radiotherapy by not only inhibiting tumor growth but also protecting normal tissues. [Mol Cancer Ther. 2004;3(3):317–325]

Introduction

Radiotherapy is an effective modality for head and neck, breast, skin, and anogenital cancers. However, its therapeutic benefit is limited by radiation-induced skin injuries or cutaneous radiation syndrome (CRS), which includes acute reactions of swelling, dermatitis, desquamation, and ulceration, and long-term effects of fibrosis, necrosis, and the development of life-threatening sequelae of sarcoma, squamous, and basal cell carcinoma (1, 2). In fact, the skin is affected in every form of the external radiotherapy of internal organs. The topical application of steroidal or nonsteroidal anti-inflammatories is the most common treatment for CRS, yet the results are unsatisfactory. An approach to selectively reduce skin morbidity without compromising the tumor-killing effects of radiotherapy is a long-sought goal in radiation oncology.

After radiation injury, the release of cytokines [such as tumor necrosis factor (TNF-α)] and growth factors [such as transforming growth factor (TGF-β)] in irritated tissues perpetuates and augments the inflammatory response, while promoting fibroblast recruitment and proliferation but inhibiting epithelial cell growth (3–10). The amplified injury response to radiation by the persistent secretion of TNF-α and TGF-β from epithelial, endothelial, and connective tissue cells, which is possibly caused by a modification in the genetic programming of cell differentiation and proliferation, leads to the histological modifications that characterize CRS (11–14). The chronic activation of TGF-β pathway also stimulates late tumorogenesis (15, 16). Thus, CRS and radiation-induced carcinogenesis could be regarded as a genetic disorder of the wound healing process after radiation exposure. This prompted us to propose whether there is a gene modulator that can simultaneously both reverse the skewed expression of TNF-α and TGF-β in irradiated skin and modulate the oncogenes or tumor suppressors in tumor cells.

A class compound of gene modulators, histone deacetylase (HDAC) inhibitors, such as short-chain fatty acids (phenylbutyrate and valproic acid) and hydroxamic acids (trichostatin A), activates and represses a subset of genes by remodeling the chromatin structure via the altered status in histone acetylation (17–27). HDAC inhibitors have exhibited properties in inducing cell-cycle arrest, cell differentiation, and apoptotic cell death in tumor cells and in decreasing inflammation and fibrosis in inflammatory diseases (17–32). Although the effects of HDAC inhibitors induce bulk histone acetylation, they result in apoptotic cell death, terminal differentiation, and growth arrest in tumor cells but no toxicity in normal cells (17, 21, 22, 33). In addition, the modulation of chromatin conformation by HDAC inhibitors can further radiosensitize tumors, the cells of which are intrinsically radioresistant (34–36).

Thus, on the basis of the potential possibility in simultaneously, coordinately, selectively, and epigenetically manipulating the expression of tumor suppressors, oncogenes, proinflammatory cytokines (TNF-α), and fibrogenic growth factors (TGF-β) by differentially remodeling the chromatin in normal and tumor cells, the use of HDAC...
inhibitors as topical agents to prevent systemic reactions warrants study, to test whether they can decrease radiation-induced skin damage and still exhibit antitumor effects. The present study may provide a novel strategy to maximize the therapeutic effectiveness of cancer radiotherapy.

Materials and Methods

Topical Drug Formulations and Preparation
The preparation of the topical HDAC inhibitors has been described elsewhere (37). In brief, different ratios of various amounts of sodium phenylbutyrate (Triple Crown America, Perkasie, PA), valproic acid (Sigma Chemical Co., St. Louis, MO) or trichostatin A (Sigma), white petrolatum, cetyl alcohol, paraffin, tefose, superpolystate, methylparaben, propylparaben, deionized water, Coster 5000, and Myriyol (all from Merck & Co., Whitehouse Station, NJ) were mixed, stirred at 400 rpm for 5 min at 70°C to form a paste, and then cooled at room temperature. Tests for skin permeation (using skin purchased from Ohio Valley Tissue & Skin Center, Cincinnati, OH), drug stability, skin irritation, and shelf life were performed at the Drug Delivery Department, Biochemical Engineering Center, Industrial Technology Research Institute, Hsinchu, Taiwan. Only the formulations with good stability, high skin penetration, low skin irritation, and long shelf life were selected for the study.

Generation of CRS, Treatment, and Evaluation of Skin Reactions
Skin over the gluteal area as large as 2 cm × 2 cm of adult female Sprague-Dawley rats weighing 150–175 g were irradiated by an electron beam with 6 MeV of energy on day 0 at 4 Gy/min up to 40 Gy to the prepared area after the rats were anesthetized. Vehicle, Vaseline (a negative control), madecassol (a positive control), or different HDAC inhibitors were applied topically at a dose of 0.5, 1.0, or 2.0 cm of adult rats in each group were calculated. The average levels of cytokine/growth factor mRNA from three skin samples in each group were normalized to the internal control GAPDH and expressed as a ratio to the average level in time-matched control groups. The Mann-Whitney test (Stata Statistical Software, College Station, TX) was used to determine statistical significance at the P < 0.05 level for differences in average skin scores and in average mRNA levels, respectively, between treated and control rats.

Histological, Immunohistochemical, and Immunofluorescence Tests
The samples were fixed in 10% formalin-buffered solution and embedded in paraffin wax. Serial 3-μm sections were cut, dewaxed, and stained with H&E-safranin. For immunohistochemical analyses, the paraffin sections were deparaffinized by xylene and rehydrated by sequential concentrated alcohols. The slides were subjected to microwave antigen retrieval (800 W, twice for 5 min each) in 0.01 M sodium citrate buffer (pH 6.0). The endogenous hydrogen peroxidase activity was blocked by 3% H2O2 for 10 min. The slides were incubated with a protein-blocking agent (Dako, Glostrup, Denmark) for 20 min and then treated with the rabbit polyclonal anti-TNF-α antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature, followed by the secondary anti-rabbit antibody incubation for 30 min, and then they were washed with PBS three times. The peroxidase reaction was visualized using AEC (3% 3-amino-9-ethylcarbazole in N,N-dimethylformamide) as chromagen for 3–10 min, and then the slides were counterstained with Mayer’s hematoxylin and mounted. Because inflamed skin tends to bind antibodies nonspecifically, we also used an isotype-matched normal antibody as a control, to confirm positive TNF-α staining in the skin wound. For immunofluorescence analyses, the paraffin sections were treated using the same protocol as those for immunohistochemical testing, except for the use of primary goat anti-acetylated histone H3 and rabbit anti-TGF-β1 and TGF-β2 antibodies (all from Santa Cruz Biotechnology), and the secondary rhodamine-conjugated (red) anti-goat (Jackson ImmunoResearch, West Grove, PA) and FITC-labeled (green) anti-rabbit antibodies (Dako).

Northern Blot Assay
Total RNA was isolated from frozen skin samples using Trigent (Molecular Research Center Inc., Cincinnati, OH). Total RNA (30 μg) was electrophoresed in a
denaturing formaldehyde-agarose gel, blotted onto Hybond N (Amersham, Amersham, United Kingdom), and fixed by UV irradiation. The membrane was incubated with \(^{32}P\)-labeled probes, as described below, in Rapid-hyb buffer (Amersham). To prepare probes for rat TGF-\(\beta\)1 and TGF-\(\beta\)2, their full-length coding sequences were amplified by reverse-transcription PCR using specific forward (TGF-\(\beta\)1, 5'-CGGGTGGCCAGGCAAGCGC-3' and TGF-\(\beta\)2, 5'-CATGCACTACTGTTGCT-3') and reverse (TGF-\(\beta\)1, 5'-GGAATTGTGCTATATTTCTGC-3' and TGF-\(\beta\)2, 5'-CCAGGACTTTAGCTGCA-3') primers. A template set of TNF-\(\alpha\) and GAPDH from the RNase protection assay kit (Riboquant; PharMingen) was used to generate \(^{32}P\)-labeled antisense RNA probes that hybridized with the mRNA for TNF-\(\alpha\) and GAPDH.

**Western Blot Assay**

For the analysis of acetylated histones, collected irradiated skin, treated with or without phenylbutyrate were digested with 5 mg/ml collagenase (Sigma) and 1.5 mg/ml DNase (Sigma) and were passed through a wire mesh to prepare isolated cells. Nuclei were then isolated by lysis of the cells in a buffer that contained 10 mM Tris-HCl (pH 6.5), 50 mM sodium bisulfite, 1% Triton X-100, 10 mM MgCl\(_2\), and 8.6% sucrose and a Dounce homogenizer was used. Histones were isolated by means of acid extraction, as described elsewhere (39). Isolated histones (5 \(\mu\)g) were then separated in 15% polyacrylamide-0.1% SDS, 100 mM Tris-HCl, and 8.6% sucrose and treated with enhanced chemiluminescent reagent (Amersham), and exposed to film.

**Generation of Cutaneous Tumor Models and Treatment**

The syngeneic carcinoma cells 1MEA7R.1 and CT-26 (purchased from American Type Culture Collection, Manassas, VA) were s.c. injected into the flank areas of female BALB/c mice. The tumor was allowed to a maximum dimension of 0.5 cm. Topical HDAC inhibitors or vehicle were applied at a dose of 200 mg/mouse to cover the whole tumor surface and surrounding skin twice per day for 4 weeks. The tumor size was calculated weekly with a caliper according to the formula \(a^2+b^2/2\), where \(a\) and \(b\) are the larger and smaller diameters (in centimeters), respectively.

**Results**

**Topical Phenylbutyrate Treatment Induces Histone Hyperacetylation**

Among the known HDAC inhibitors, phenylbutyrate, which is a natural low-toxic aromatic fatty acid purified from mammalian urine and plasma, has been approved by the US Food and Drug Administration for the treatment of inherited genetic diseases and malignancies (40–44). We prepared several topical formulations of phenylbutyrate to increase local concentrations, because a plasma level of phenylbutyrate above 0.5 mm (the minimal dose to induce histone hyperacetylation and antitumor effects) is difficult to maintain via the oral or i.v. routes (37, 45, 46). Among the different formulations, the phenylbutyrate cream (Tri-c-02-3), which showed good stability, low rates of skin irritation, a long shelf life, and a high rate of skin penetration, was selected for the present study (37) (Fig. 1A).

To determine what dosage of topical phenylbutyrate was suitable to treat irradiated skin, we used the amount of histone hyperacetylation in the nucleus as a marker to demonstrate the extent of drug penetration and to indicate whether the local drug concentration was enough to exert biological effects. Western blot analysis for acetylated histones in the irradiated skin 6 h after irradiation (40 Gy single fraction) showed that the acetylated form of histone H3 was mildly increased in the control and vehicle-treated groups but was markedly increased with the topical treatment of 1% phenylbutyrate cream at a dose of 200 mg/irradiated skin surface given immediately after irradiation (Fig. 1B). Immunofluorescence staining further demonstrated that histone hyperacetylation in irradiated skin was visually evident deep in the s.c. layer at 6 h after drug treatment (i.e., coincident with the peak of the drug concentration in the skin test) (Fig. 1, A and C).

**Topical Phenylbutyrate Treatment Suppresses CRS**

The acute skin reaction after irradiation was scored to grade toxicity in five animals in each group receiving vehicle, phenylbutyrate, madecassol (a steroid-positive control), or Vaseline (a negative control) from Day 1 to Day 90 (Fig. 2). The skin score increased with a more severe skin reaction. The skin reactions in the phenylbutyrate group tended to be less marked than those in other groups. Transient skin erythema was noted within hours to the first 2 days in all groups. Depilation of the epidermis appeared earlier (at Day 5) in the phenylbutyrate group, but the scores in the Vaseline and vehicle control groups were higher within 11 days. By Day 21, the Vaseline and vehicle groups had progressed to wet desquamation in most areas, whereas in the madecassol and phenylbutyrate groups, wound healing in the epithelium had begun quickly.

After 90 days, the long-term effects of radiation in the skin were evaluated by histological testing. At Day 180, skin samples taken from the phenylbutyrate group showed...
soft characteristics and less capillary bleeding, whereas skin samples taken from the vehicle group exhibited rigid characters and easily oozing. Histological sections were examined to provide a qualitative description of group differences in reepithelialization after irradiation and in the development of dermal fibrosis (Fig. 3). The histology in the phenylbutyrate group at Day 180 showed a thicker epidermis with 10–30 cell layers, less subepithelial swelling, a thinner dermis with less collagen deposition, and few skin appendages, compared with the histological results in the vehicle group at Day 180 and with the control groups of normal skin and acute reaction at Day 7. These observations suggest that phenylbutyrate promotes epithelial healing in early radiation-induced skin damage and inhibits late, radiation-related proliferative dermal fibrosis.

Changes in Radiation-Induced Histology after Topical Phenylbutyrate Treatment Correlate with Suppression in Inflammatory and Fibrogenic Cytokine Expressions

Because the development of CRS has been attributed to radiation-induced temporal changes and the persistent up-regulation of proinflammatory cytokines such as TNF-α and fibrogenic growth factors such as TGF-β1 and β2(3–16), we next studied whether the suppression of CRS development by the topical phenylbutyrate treatment is correlated with the suppression of TNF-α and TGF-β.

The timing of the peak appearance of TGF-β1, TGF-β2, and TNF-α expression levels induced by radiation correlated with the progression in CRS development in all experimental groups (Figs. 2–Figs. 4). In the phenylbutyrate group, the highest surge of TGF-β1, TGF-β2, and TNF-α appeared at 6 h after irradiation, but levels were subsequently suppressed after Day 14. The suppression still persisted at 12 months, even when topical phenylbutyrate treatment was discontinued at Day 90. In the Vaseline and vehicle control groups, mRNA levels of TGF-β1, TGF-β2, and TNF-α in the irradiated skin increased and fluctuated above the nonirradiated control levels over a period of 1 year and reached the first peak of 2- and 3-fold above the nonirradiated control levels at 6 h after irradiation, the second peak of 10.5- to 16-fold around 14–28 days after irradiation, and the third peak of 13- to 14-fold at 9 months after irradiation; levels then declined to 2- to 3-fold normal levels by 12 months after irradiation. Although the mRNA levels of TGF-β1, TGF-β2, and TNF-α at the first peak at 6 h in the phenylbutyrate group were higher than those in the Vaseline and vehicle control groups, they decreased to the levels lower than those in the Vaseline and vehicle groups at Day 14 and returned to the nonirradiated control levels at Day 28–35.

TGF-β1 and TGF-β2 have similar cellular effects in inhibiting epithelial cell growth and promoting dermal

Figure 1. Induction of histone hyperacetylation in skin. A, pharmacokinetic studies of delivery of different phenylbutyrate formulations through the skin. Among them, the 1% phenylbutyrate (PB) cream (Tri-c-02-3) was used because of its stability and long shelf life. B, Western blot analysis for acetylated H3 in the irradiated skin (40 Gy single fraction) treated with or without phenylbutyrate cream for 6 h after irradiation. The Coomassie blue-stained gel sections demonstrate the equivalence of protein loading. Lane 1, normal skin without irradiation; lane 2, irradiated skin without any treatment; lane 3, irradiated skin treated with the vehicle; lane 4, irradiated skin treated with 1% phenylbutyrate cream. C, immunofluorescence staining of acetylated H3 in the irradiated skin treated with or without phenylbutyrate cream for 6 h after irradiation. The acetylated H3 in the nuclei was used as marker indicating the extent of drug penetration. Left upper panel, irradiated skin without any treatment; right upper panel, normal skin without irradiation; left lower panel, irradiated skin treated with 1% phenylbutyrate cream; and right lower panel, irradiated skin treated with the vehicle.

Figure 2. Evaluation of acute skin reactions after irradiation. Time course of the average skin score for the acute skin reaction after irradiation from Day 1 to Day 90. The skin score increases with more severe skin reaction. *, P < 0.05; **, P < 0.001, in comparison with the phenylbutyrate (PB)-treated and vehicle groups.
fibroblast proliferation, but TGF-β3 has the opposite effect (4, 10). Thus, we also studied the level change of TGF-β3 mRNA and found that it exhibited a slightly transient increase of 2-fold in all irradiated groups at 14 days then progressively decrease to the nonirradiated control level or lower. No significant differences in TGF-β3 mRNA levels were observed between the irradiated groups treated with Vaseline, vehicle, or phenylbutyrate.

Immunofluorescence staining further confirmed that TGF-β1 and β2 proteins were predominately present in the keratinocytes of the thinner epidermis and in myofibroblasts of the proliferative thicker dermis in the vehicle group, whereas the amount of TGF-β1 and β2 protein was low in both the thicker epidermis and the thinner dermis in the phenylbutyrate group, compared with the staining pattern in normal skin (Fig. 5).

Moreover, at Day 270, three of five rats in the Vaseline group and four of five rats in the vehicle group, compared with zero of five rats in the phenylbutyrate-treated group, showed chronic ulceration, necrosis, bullae formation, and inflammatory cell infiltration. The decrease in late radiation-induced skin damage by topical phenylbutyrate was consistent with the suppression of TNF-α expression (Fig. 6).

**Topical Phenylbutyrate Treatment Both Prevents Late Radiation-Induced Skin Tumor Formation and Exhibits a Direct Antitumor Effect on Cutaneous Tumor Growth**

Many studies have demonstrated that the chronic overexpression of TGF-β stimulates neoplastic growth (8, 15). Thus, the decrease of TGF-β expression caused by phenylbutyrate treatment might decrease the incidence of late radiation-induced tumorigenesis. We found that newly developed skin or cutaneous tumors increased with time after 50 weeks in the control group that did not receive phenylbutyrate treatment, but no tumors developed in the phenylbutyrate-treated group (Fig. 7). At 90 weeks, a cumulative tumor incidence of 15% (6 of 39) was observed in the irradiated group without phenylbutyrate treatment, compared with 0% (0 of 42) in the irradiated groups with phenylbutyrate treatment. The histology of radiation-induced tumors included fibroma, spindle cell carcinoma, basal cell carcinoma, and squamous cell carcinoma.

The topical phenylbutyrate formulation was further tested to assess whether it retained its antitumor effects. BNL 1MEA7R.1 and CT-26 carcinoma cells, which showed growth inhibition by phenylbutyrate in vitro by the up-regulation of p21Cip1 (Fig. 8A), were inoculated into the back of syngeneic mice. Cutaneous tumors were allowed to grow to the largest dimension 0.5 cm, and phenylbutyrate was topically applied to the tumor surface at 200 mg/mouse twice per day. By 4 weeks, the tumor sizes of 1MEA7R.1 and CT-26 carcinomas in the placebo groups were almost 6- and 1.6-fold larger than those in the phenylbutyrate-treated groups, respectively (Fig. 8B). Cutaneous tumors in the phenylbutyrate-treated groups grew slowly, without skin ulceration, whereas tumors in the control or placebo group grew rapidly and showed a necrotic appearance and skin ulceration (Fig. 8C). After 5 weeks of treatment, withdrawal of topical phenylbutyrate resulted in a loss of tumor growth inhibition, and these tumors then reached the same size as those in the control or placebo groups within 2 weeks.

**Similar Effects of Other Structurally Unrelated Antitumor HDAC Inhibitors in Treating CRS**

In addition to phenylbutyrate, other structurally unrelated antitumor HDAC inhibitors, such as trichostatin A (an antifungal agent) and valproic acid (an antiseizure agent), also ameliorated the development of CRS and decreased the radiation-induced TGF-β1, TGF-β2, and TNF-α expression (Fig. 9, A and B).

**Discussion**

Although previous studies have demonstrated that HDAC inhibitors have effects in modulating multiple genes to
inhibit tumor growth, reducing lipopolysaccharide induced circulating cytokines, affecting cytokine production to attenuate concanavalin A-induced hepatic injury, and reversing skewed cytokine expression in autoimmune inflammatory diseases (17, 18, 24, 26, 27), the present study appears to be the first to examine the cytokine- or gene-modulatory effects of the inhibition of HDAC in CRS and radiation-induced tumorigenesis. By virtue of reducing the levels of radiation-induced cytokines such as TNF-α and TGF-β, we found that HDAC inhibitors could be candidates

![Figure 4.](image1.png)

**Figure 4.** Expression levels of TGF-β1 and TNF-α after irradiation. Temporal variation in mRNA levels of TGF-β1, TGF-β2, TGF-β3, and TNF-α in skin after irradiation, normalized to the internal control GAPDH, and expressed as a ratio to levels in nonirradiated control samples. Points, mean of mRNA levels of five samples in the same group of Vaseline, vehicle, or phenylbutyrate (PB). The arrow indicates that the drug treatment was discontinued after Day 90 (*, P < 0.05; **, P < 0.001, in comparison with the phenylbutyrate-treated and vehicle groups).

![Figure 5.](image2.png)

**Figure 5.** Immunofluorescence. TGF-β expression in the skin after irradiation. The expression level of TGF-β in the irradiated skin was high in the superficial dermis of acute reaction at Day 7 and in the thin epidermis and proliferative dermis in the vehicle group at Day 180 but was low in the hyperplastic epidermis and thinner dermis in the phenylbutyrate (PB) group at Day 180.
for the treatment of CRS and the prevention of late tumorigenesis. Although the down-regulation of TNF-α and TGF-β by the inhibition of HDAC is likely to contribute to the effects observed in the present study, HDAC inhibitors may have another mechanisms, for example, the hyperacetylation of nonhistone proteins such as ribosomal S3 or the Rel-A subunit of NF-κB (47, 48). Moreover, as a consequence of nuclear hyperacetylation, which results in genetic reprogramming, HDAC inhibitors also up-regulate the cell cycle kinase inhibitor p21Cip1, which, in part, accounts for their antitumor properties (21).

TGF-β is a pleiotropic growth factor that inhibits epithelial cell growth but promotes mesenchymal cell proliferation and neoplastic growth (8–10, 15, 16). In

Figure 6. Immunohistochemistry. TNF-α expression in the long-term skin damage at Day 270 after irradiation. The expression levels of TNF-α in the normal skin and phenylbutyrate-treated group were low. In contrast, the high expression levels of TNF-α in the Vaseline and vehicle groups correlated with skin necrosis and heavy inflammatory infiltrates. A, normal skin; B, irradiated skin treated with the topical phenylbutyrate; C, irradiated skin treated with Vaseline (the arrow indicates the necrotic wound); and D, irradiated skin treated with vehicle.

Figure 7. Skin tumorigenesis after irradiation. Cumulative skin tumor incidence after skin irradiation increased with time in the groups without the topical phenylbutyrate (PB) treatment.

Figure 8. Phenylbutyrate inhibits tumor growth. A, time-course analysis of the up-regulated levels of p21Cip1 protein in BNL 1ME7R.1 and CT-26 carcinoma cells during treatment with 4 mM phenylbutyrate (PB). B, growth inhibition test in vivo. The cutaneous tumors (BNL 1ME7R.1 and CT-26 carcinoma cells), grown to a size of 0.5 cm, were treated with the topical phenylbutyrate or vehicle at a dose of 200 mg/mouse, to cover the whole tumor surface and surrounding skin twice per day for 4 weeks. At the end of this period, the cutaneous tumor sizes of 1ME7R.1 and CT-26 in mice in the placebo groups were almost 6- and 1.6-fold larger than those in the topical phenylbutyrate-treated groups, respectively. C, an initial tumor size of 1ME7R.1 beneath the skin about 0.5 cm in dimension before treatment (left), a placebo- or vehicle-treated tumor at week 4 (middle), and a phenylbutyrate-treated tumor at week 4 (right).
the present study, the sustained high expression levels of TGF-β1 and -β2 in irradiated skin was significant in explaining the manifestation of thin epidermis and proliferative dermis in the vehicle group at Day 180 as well as the occurrence of tumor formation after 50 weeks. In contrast, the appearance of hyperplastic epidermis and a thinner dermis at Day 180, as well as no skin tumor development noted at Week 90 after irradiation in the phenylbutyrate-treated group, correlated with the early suppression of the chronic up-regulation of TGF-β1 and β2 by phenylbutyrate. Although TGF-β is certainly a key growth factor, the development of CRS and the recovery from a perpetual wound might not depend on the modulation of a single factor. The wound healing process after irradiation involves a complex feedback network of interacting cytokines and growth factors, including platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, granulocyte macrophage colony-stimulating factor, connective tissue growth factor, IL-1, IL-4, inhibiting growth factor-1, and TNF-α, and complex interactions among the parenchymal, epithelial, endothelial, and inflammatory cells (3, 12). Among them, TNF-α is implicated in triggering the recruitment of inflammatory cells through the expression of adhesion molecules on the vascular surface, as well as the stimulation of fibroblast proliferation (12, 49, 50). Thus, in addition to the down-regulation of long-term TGF-β1 activation, the simultaneous suppression of radiation-induced TNF-α in mesenchymal cells and macrophages should also contribute to the decrease in acute skin inflammation and the incidence of late skin necrosis and fibrosis seen in the present study.

Drugs that have previously been tested in the management of CRS include antioxidants (vitamin E and superoxide dismutase), anti-inflammatory agents (corticosteroids, colchicines, D-penicillamine, and TNF-α antagonist antibodies), and anti-fibrogenic agents (IFN, TGF-β antagonist, and angiotensin-converting enzyme inhibitors) (7). However, few of these are able to simultaneously ameliorate acute dermatitis, prevent the occurrence of fibrosis, and reduce late tumorigenesis; moreover, toxicities, side effects, tumor protection possibilities, and a lack of antitumor effects are troublesome. Rather than focusing on agents that act only on a few steps in the pathogenesis of CRS, our study provides a novel strategy to exert anti-inflammatory, anti-fibrogenic, and antitumor effects at one time by HDAC inhibitors to remodel chromatin and epigenetically regulate multiple genes involved in radiation-induced skin damage and tumor growth.

To our knowledge, this is the first time that the antitumor HDAC inhibitors have been shown to protect skin from radiation-induced damage and tumorigenesis. Our study warrants further clinical investigations into the combination of therapeutic radiation and HDAC inhibitors in increasing the therapeutic gain of cancer radiotherapy. Although TGF-β and TNF-α could be the targets of HDAC inhibitors, more cellular and molecular studies are needed to identify the precise mechanisms of HDAC inhibitors in both suppressing CRS and preventing tumorigenesis.

References


Antitumor histone deacetylase inhibitors suppress cutaneous radiation syndrome: Implications for increasing therapeutic gain in cancer radiotherapy

Yih Lin Chung, Ae-June Wang and Lin-Fen Yao