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Histone Deacetylase Inhibitors Restore Radioiodide Uptake and Retention in Poorly Differentiated and Anaplastic Thyroid Cancer Cells by Expression of the Sodium/Iodide Symporter Thyroperoxidase and Thyroglobulin

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Iodide uptake by the thyroid is mediated by the sodium/iodide symporter. Upon iodide uptake, thyroperoxidase catalyzes iodination of tyrosine residues in thyroglobulin, retaining iodide within thyroid follicles. Dedifferentiation-induced loss of these functions in cancers, rendering them unresponsive to radioiodide, occurs with most poorly differentiated and anaplastic tumors. We focused on the histone deacetylase (HDAC) inhibitors (HDACI) as a way to induce differentiation of thyroid cancer cells. We assessed re-expression of thyroid-specific genes mRNA induced by HDACI using quantitative RT-PCR and immunostaining in poorly differentiated papillary and anaplastic thyroid cancer cells. HDACI induced expression of thyroid-specific gene mRNAs and proteins, and accumulation of radioiodide through iodination of generic cellular proteins were detected. HDACI-treated tumors could specifically accumulate ^{125}I as revealed by imaging experiments and radioiodide concentration *in vivo*. In an attempt to determine the mechanism by which these gene expressions occurred, we detected the inhibition of protein synthesis by cycloheximide, which up-regulated the expression of thyroperoxidase and thyroglobulin mRNA in HDACI-treated cells and down-regulated that of sodium/iodide symporter mRNA. Together, our results suggest that HDACI-induced expression of thyroid-specific genes, some of which is mediated by some protein synthesis, may contribute to development of novel strategy against thyroid cancer. (*Endocrinology* 145: 2865–2875, 2004)

THE THYROID GLAND, a relatively common site for development of malignant neoplasms, gives rise to 90% of all endocrine cancers (1). In general, thyroid cancer is a disease with a good prognosis. However, anaplastic thyroid carcinoma constitutes about 5–14% of all thyroid carcinomas and is highly malignant, with a median survival of 2–6 months, rapidly invading adjacent structures, and metastasizing throughout the body, especially to the lung (2, 3). Because no effective therapy is available for patients with these aggressive types of thyroid carcinoma, development of novel therapeutic strategies, including gene therapy, is an urgent priority.

A unique property of thyroid follicular cells is the ability to trap and concentrate iodide, which depends on expression of the sodium/iodide symporter (NIS), thyroglobulin (Tg), and thyroperoxidase (TPO) (4). Several reports indicated that, because of ability for iodide trapping and concentration, radioiodide is an effective therapy in the treatment of differentiated thyroid carcinomas (5–7). NIS protein mediates iodide uptake in normal and well-differentiated neoplastic thyroid cells. TPO iodinates Tg, which leads to iodide retention within thyroid follicles. Concentration of these thyroid-specific functions suggests that restoring lost expression of NIS, TPO, and Tg could provide a basis for radioiodide treatment of thyroid carcinomas. Loss of thyroid-specific functions associated with dedifferentiation in poorly differentiated and anaplastic thyroid cancer cells ordinarily renders them unresponsive to radioiodide therapy. We reported that $Na^{131}I$ administration did not decrease volumes of experimental tumors formed by malignantly transformed rat thyroid cells Tc-rNIS that were stably transfected with rat NIS cDNA but possessed no TPO or Tg expression (8). Concomitant re-expression of NIS, TPO, and Tg therefore would seem necessary for the iodide uptake and retention that could permit effective radioiodide therapy.

Histone acetylation is known to induce changes in nucleosomal conformation that make DNA more accessible to transcription factors (9). Acetylation of histone lysine residues reduces the overall positive charge of the histone, and thus decreases its affinity for the negatively charged DNA molecule (10). Recent reports have shown that histone deacetylase (HDAC) inhibitors (HDACI) can induce expression of silenced genes in cancer cells (11). In bladder carcinoma cells, for example, the HDACI, suberoylanilide hydroxamine acid, induces an increase in $p21^{WAF1}$ mRNA and

Abbreviations: DMSO, Dimethylsulfoxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HBSS, Hanks' balanced salt solution; HDAC, histone deacetylase(s); HDACI, HDAC inhibitor(s); MMI, methimazole; NIS, sodium/iodide symporter; Tg, thyroglobulin; TPO, thyroperoxidase; TSA, trichostatin A; TSH-R, TSH receptor; TTF, thyroid transcription factor.

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protein. Thus, HDACI-induced restoration of tumor suppression- or apoptosis-inducing genes in poorly differentiated cancer, including thyroid cancer, might offer a novel therapeutic strategy.

To develop a way to sensitize anaplastic thyroid carcinoma to radioiodide therapy, we investigated re-expression of thyroid-specific genes, NIS, TPO, and Tg, induced by HDACI. We then examined radioiodide uptake and organification to confirm the function of the re-expressed thyroid-specific genes *in vitro* and *in vivo* models.

Expression of thyroid-specific genes is controlled by the interaction of a complement of thyroid-specific transcription factors with their respective promoters (12–22). At least three transcription factors have been identified: thyroid transcription factor (TTF)-1 (12), TTF-2 (20), and Pax-8 (23). TTF-1, a homeodomain-containing transcription factor expressed in thyroid and lung (12, 24), interacts with all thyroid-specific genes important for thyroid function (12, 13, 16–19). Pax-8, a member of the family of paired box-containing genes, is expressed in the thyroid and kidney (16, 23) and binds to promoter and enhancer sequences in thyroid-specific genes (15, 21, 22, 25). We recently demonstrated that overexpression of TTF-1 could restore Tg promoter activity in Tg-nonproducing poorly differentiated thyroid cancer cell lines, which suggested that TTF-1 is important in the restoration of thyroid-specific gene expression in thyroid cancer cells (24). In an attempt to determine the mechanism by which HDACI induce restoration of thyroid-specific gene expression, we characterized the effect of HDACI on expression of thyroid-specific transcription factors.

Materials and Methods

Reagents

Depsipeptide (FR901228, FK228) was a gift from Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan). A 1-mg/ml solution of depsipeptide was prepared dimethylsulfoxide (DMSO).

Cell culture

BHP18–21v cells, derived from human papillary thyroid cancer, were provided by Dr. J. M. Hershman (Endocrine Research Laboratory, West Los Angeles Veterans Affairs Medical Center, Los Angeles, CA). BHP18–21v cells expressing Pax-8, but neither TTF-1 nor Tg genes, were isolated from BHP18–21 (26). The BHPcell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. A human anaplastic thyroid carcinoma cell line, ARO (27), that lacks Pax-8, Tg, and NIS and also shows decreased expression of TTF-1, was donated by Dr. H. Namba (Department of Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan) and incubated with RPMI 1640 containing 10% fetal calf serum.

FRTL-Tc cells (28) were grown in Coon's modified Ham's F-12 medium supplemented with 5% calf serum containing a mixture of five hormones: insulin (10 μg/ml), cortisol (0.4 ng/ml), transferrin (5 μg/ml), glycyl-L-histidyl-L-lysine acetate (10 ng/ml), and somatostatin (10 ng/ml). Rat NIS cDNA (−29 to 1975 bp, with A in ATG designated as +1) (29) was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA) and introduced into FRTL-Tc cells by electroporation. Stably transfected cells were selected using G418 and then cloned by limited dilution. The cloned cell line (Tc-rNIS) (8) that exhibited the highest iodide uptake activity was used for the subsequent experiments.

Determination of mRNA levels using real-time kinetic quantitative RT-PCR in vitro and in vivo

In an *in vitro* and *in vivo* study, total RNA was isolated from cultured cells using RNeasy Mini kit (QIAGEN, Hilden, Germany). After quantification by spectrophotometry, 5 μg total RNA was reverse-transcribed into cDNA with 160 μM deoxynucleotide triphosphate, 50 ng random hexamer primers, and 200 U Superscript II, per the manufacturer's recommendations (Invitrogen Corp., Carlsbad, CA). Oligonucleotide primers and TaqMan probes for NIS (30), Tg (31), TPO, and TSH receptor (TSH-R) (32) genes are shown in Table 1. These primers were designed to be intron spanning. The monitoring of negative control for each target showed an absence of carryover. One of each type of amplicon, corresponding to each target, was migrated on agarose gel electrophoresis and showed a unique band at the expected size. The primers and TaqMan probes of TPO gene were designed using the computer program Primer Express (PerkinElmer Corp., PE Applied Biosystems, Foster City, CA).

To normalize differences in the amount of cDNA added to the reactions, amplification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed as an endogenous control. Primers and probes for GAPDH were purchased from PerkinElmer Corp., PE Applied Biosystems. Quantitative real-time PCR was achieved in 96 sample tubes using the cDNA equivalent of 100 ng with 1× TaqMan Universal PCR Master Mix, 900 nM of each primer, and 250 nM TaqMan probe. The cycling conditions included an initial phase of 2 min at 50 C, followed by 10 min at 95 C required for optimal uracil-*N*-glycosylase enzyme activity, 45 cycles of 15 sec at 95 C, and 1 min at 60 C. Polymerization reactions were performed in a GeneAmp 5700 Sequence Detection System (PerkinElmer Corp., PE Applied Biosystems).

To validate the quantitative PCR method, standard curves for NIS, TPO, Tg, TSH-R, and GAPDH were constructed from cDNA fragments. The efficiency of each standard curve, as determined by its slope, allowed us to quantify the threshold cycle method according to the manufacturer's instructions. The amount of targeted mRNA was determined by standard curve. For each sample, the targeted mRNA amount was divided by GAPDH to determine a normalized ratio. The crossing points were all less than 30 cycles that are in the linear range of amplification.

TABLE 1. Sequences of primers and TaqMan probes

Genes	Gene bank accession no.			Primer and probe
NIS	U66088	Sense	2089–2109	CCATCCTGGATGACAACTTGG
		Antisense	2166–2187	AAAAACAGACGATCCTCATTG
		Probe		FAM-AGAACTCCCCACTGGAAACAAGAAGCCC-TAMRA
TPO	M17755	Sense	2680–2698	ACCTCGACGGTGATTTGCA
		Antisense	2723–2740	CCGCCTGTCTCCGAGATG
		Probe		FAM-TGGACACGCACTGGCACTAAATCCA-TAMRA
Tg	X05615	Sense	262–280	GTGCCCAACGGCAGTGAAGT
		Antisense	326–348	TCTGCTGTTTCTGTAGCTGACAAA
		Probe		FAM-ACAGACAAGCCACAGGCCGTCCT-TAMRA
TSH-R	M32215	Sense	186–203	CCCAGCTTACCGCCCAGT
		Antisense	238–264	TAGAAAATGCATGACTTGGAATAGTTC
		Probe		FAM-CGCAGACTCTGAAGCTTATTGAGACTCACCTG-TAMRA

Detection of TTF-1 and Pax-8 gene expression using RT-PCR

To determine expression of human TTF-1 and Pax-8 in BHP18–21v and ARO cells, 5 μg total RNA was reverse-transcribed as described above. The cDNAs were amplified using the following intron-flanking primers: human TTF-1 5′ (sense),^{512}AACCTGGGCAACATGAGCGAGCT^{534}; TTF-1 3′ (antisense),^{771}AGCTCGTACACCTGCGCCTGCGAGAAGA^{734}; human Pax-8 5′ (sense),^{10}GATGCCTCACAACTCCATCAGATC^{33}; and Pax-8 3′ (antisense)^{603}TCATCCATTTTCCTCTTGTCGCTG^{586}. The amplification reaction was carried out for 30 cycles, and considered at 94 C for 1 min, 60 C for 1 min, and 72 C for 2 min, followed by a final 10 min elongation at 72 C. All PCR products were analyzed by electrophoresis through 2% agarose gels followed by ethidium bromide staining to ensure amplification of the appropriately sized product.

Immunoperoxidase staining of cells

Immunostaining of thyroid cancer cells treated with HDACI was performed to confirm expression of the NIS, TPO, and Tg proteins in response to HDACI. After treatment of the cells, they were fixed for 15 min in −10 C methanol, air-dried, and then washed three times with PBS. The fixed cells were incubated with an anti-Tg monoclonal (NeoMarkers, Fremont, CA), anti-TPO monoclonal (HyTest Ltd, Eurocity, Turku, Finland), or anti-NIS polyclonal antibody (33) for 30 min and washed with three changes of PBS. Cells then were incubated with biotinylated secondary antibody, washed, and exposed to avidin-biotinylated horseradish peroxidase enzyme reagent and peroxidase substrate (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Then, nuclei were stained with Mayer-hematoxilin (Wako, Osaka, Japan). Cells were examined for staining after rinsing with H_2O.

Radioiodide uptake assay

Cells were grown in six-well plates, washed with Hanks' balanced salt solution (HBSS), and incubated for 1 h at 37 C with 1000 μl HBSS containing 0.2 μCi carrier-free $Na^{125}I$ (Amersham Pharmacia Biotech, Piscataway, NJ) and 10 μM NaI with or without 300 μM $NaClO_4$ (34). The medium containing ^{125}I was removed, and the cell monolayer was washed twice with 1 ml HBSS. Cell-associated radioactivity of each sample was measured with a γ-counter after extraction with ethanol.

Radioiodide organification assay in vitro

After the BHP18–21v cells were incubated with 3 ng/ml depsipeptide, one of HDACI, for 72 h, 300 μM methimazole (MMI), a TPO-specific inhibitor, was added 24 h before the assay. Depsipeptide-treated BHP18–21v cells were then exposed to ^{125}I (0.2 μCi/well in six-well plates) in HBSS containing 10 μM NaI at 37 C for 1 h. Medium containing ^{125}I was removed and washed with fresh medium. Cell lysates were prepared with 1 ml of 0.1-N NaOH, and the radioiodide accumulation of the cells was measured with a γ-counter. Proteins in the cell lysates were precipitated by the addition of 1 ml TCA (final concentration, 20%). Assay tubes were centrifuged at 3300 × *g* for 30 min, and supernatants containing free radioiodide were removed. The precipitated proteins were washed again with 20% TCA, and radioactivity in the pellets were measured with a γ-counter. To measure the Tg- and TPO-independent nonspecific incorporation of ^{125}I into the protein fraction, iodination was measured in NIS-transfected FRTL-Tc, which expresses neither Tg nor TPO.

To investigate the molecular size of radioiodinated intracellular protein substrates, BHP18–21v cells, which were preincubated with or without 3 ng/ml depsipeptide for 48 h in a 10-cm culture dish, were exposed to ^{125}I (20 μCi/dish) in HBSS at 37 C for 1 h. Medium containing ^{125}I was removed and washed with fresh medium. Cell lysates were prepared with 1 ml of 0.1 N NaOH, and proteins in the cell lysates were precipitated by the addition of 1 ml TCA (final concentration, 20%), and pellets were washed again with 20% TCA. The cell pellets were dissolved with 100 μl of 10-mM HEPES/NaOH (pH 7.9), 0.1 mM EDTA, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 1.5 mM phenylmethylsulfonylfluoride, and 10 μg/ml leupeptin. Thirty micrograms of protein extracts of lysate were resolved by SDS-PAGE (35) and processed for analysis by autoradiography. Radioactivity was analyzed using a BAS 2500 image analyzer (Fuji Film Co., Tokyo, Japan).

Analysis of iodide accumulation in vivo

A total of 1×10^7 BHP18–21v cells were transplanted in abdominal sc tissue of 8-wk-old male nude mice (BALB/C *nu/nu* mice). These mice were fed a low-iodide diet (Oriental Yeast, Tokyo, Japan) and were given 100 μg/kg·d L-T_4 (Wako) in their drinking water before the formation of tumors (8). Three weeks after the tumors reached approximately 1 cm in diameter, 5 μg/g·d depsipeptide or the vehicle, 0.1% DMSO (Sigma-Aldrich Corp., St. Louis, MO) in 100 μl PBS, was injected ip for 4 d. A total of 10 μCi $Na^{125}I$ (Amersham Pharmacia Biotech) in PBS was injected ip.

Three, 12, and 48 h later, the mice were killed, and radioactivity was analyzed with a BAS2500 image analyzer (Fuji Film Corp.). The mice were exposed to the imaging plates for 5 min. To investigate the ratio of tumor radioactivity to liver radioactivity, their tumors and organs were removed, and their weight and radioactivity were measured.

Three hours after injection of $Na^{125}I$, tumors were removed. Part of the tumor tissue was homogenated. After preparation of tissue lysate with 300 μl of 1-N NaOH, insoluble material was removed from the lysate by centrifugation at 3300 × *g* for 10 min at 4 C. Protein in the tissue lysates were precipitated by the addition of 600 μl of 40% TCA. The precipitated proteins were collected by centrifugation at 3300 × *g* for 20 min, and washed twice. Radioactivity in the pellets was measured with a γ-counter. Soluble protein was quantified by the Bradford method using RCDC Protein Assay (Bio-Rad Co., Hercules, CA).

Our institutional committee on animal care approved these studies.

Cytotoxic assay with depsipeptide treatment in vitro

Viabilities of cells treated with depsipeptide were measured with a nonradioactive cell proliferation assay, Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). One day after plating 4×10^3 cells/well in triplicate wells of 96-well culture plates, cells were treated with 1, 3, and 10 ng/ml depsipeptide. Cell viability was assayed 48 h after incubation. Survival of cells is presented as a percentage of the absorbance with depsipeptide-treated cells divided by that with cells not exposed to depsipeptide.

Statistical analysis

All data are expressed as mean ± SEM. Differences between groups were examined for statistical significance using Student's *t* test and a *P* value < 0.05 was considered significant unless otherwise indicated.

Results

HDACI induced re-expression of NIS, TPO, and Tg mRNA in poorly differentiated and anaplastic thyroid cancer cells

To investigate the effects of the HDACI, the cancer cells were incubated with or without an HDACI for 24 h; then total RNA was isolated. Specifically, cells were incubated with 1, 3, and 10 ng/ml depsipeptide, strong HDACI (36), and expression of thyroid-specific genes was analyzed by quantitative real-time RT-PCR performed as described in *Materials and Methods* (Fig. 1A). In untreated BHP18–21v and ARO cells, we could observe neither expression of NIS, TPO, Tg, nor TSH-R. It should be additionally noted that faint expression of TPO and Tg mRNA in untreated ARO cells, and Tg mRNA in untreated BHP18–21v cells were detected, when the crossing points were set bellow 38 cycles. Addition of 3 and 10 ng/ml depsipeptide induced the expression of NIS, TPO, and Tg mRNA in BHP18–21v cells (Fig. 1A). In ARO cells, 3 and 10 ng/ml depsipeptide induced re-expression of NIS mRNA (Fig. 1A). TPO and Tg gene expression were induced by 1 ng/ml depsipeptide.

Additionally, BHP18–21v and ARO cells treated with at

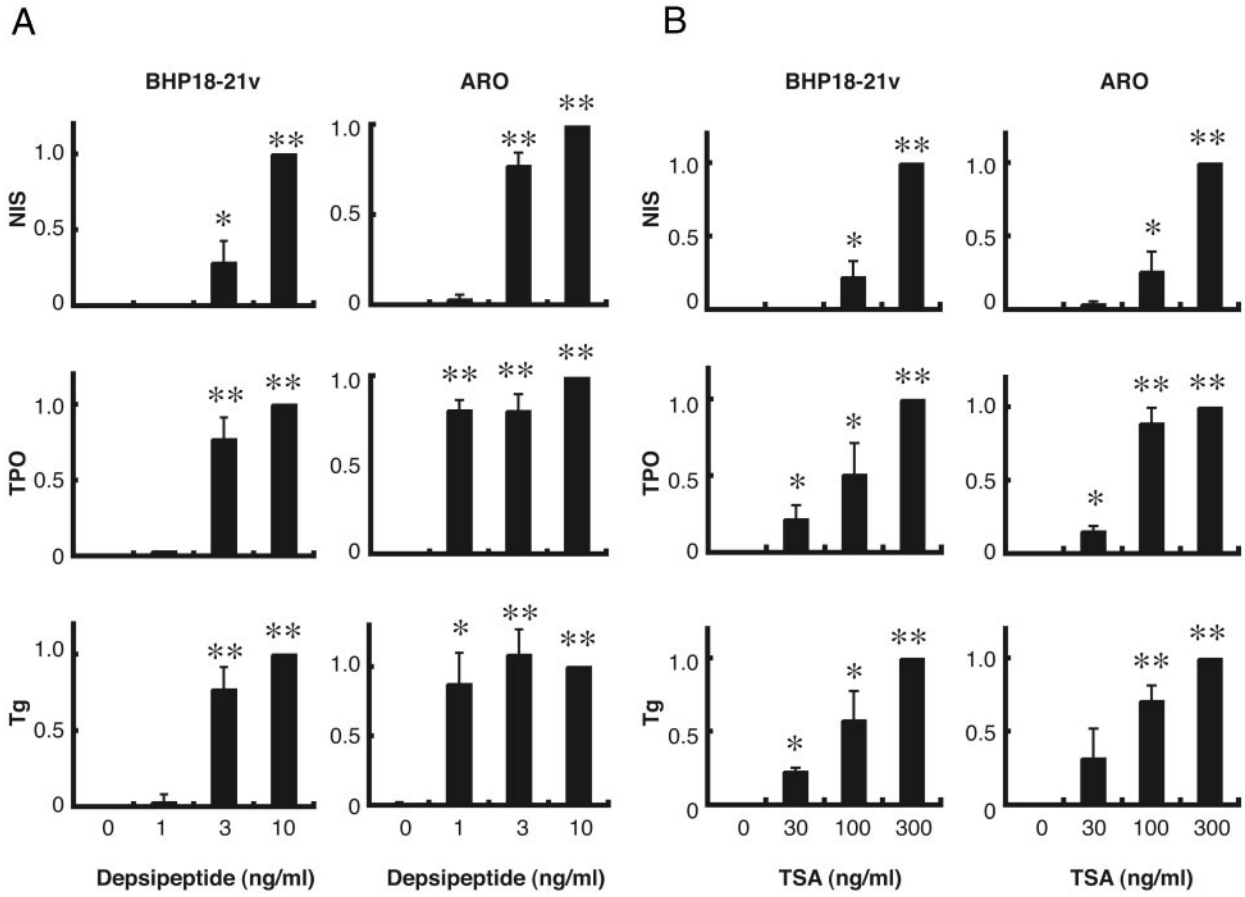


FIG. 1. The re-expression of NIS, TPO, and Tg mRNA induced by HDACI. A, Three thyroid-specific genes mRNA levels were assayed in BHP18–21v and ARO cells were incubated with 1, 3, or 10 ng/ml depsipeptide for 24 h. B, Three thyroid-specific genes mRNA levels were assayed in BHP18–21v, and ARO cells were incubated with 30, 100, or 300 ng/ml TSA for 72 h. From four independent samples, total RNA was isolated. Amounts of NIS, TPO, and Tg mRNA were determined by quantitative real-time RT-PCR with 100 ng cDNA in triplicate. Relative quantification of target cDNA was determined by arbitrarily setting the control value from maximum expression samples to 1. All data are expressed as the mean ± SEM. *, $P < 0.05$; **, $P < 0.001$, compared with untreated cells.

least 30 ng/ml trichostatin A (TSA) (Wako), which was also HDACI (37), expressed NIS, TPO, and Tg mRNA (Fig. 1B). These results indicate that HDACI induced the restoration of thyroid-specific gene expression that had been lost in the process of oncogenesis.

We also investigated time dependence of the HDACI-induced expression of thyroid-specific genes. Amounts of thyroid-specific genes mRNA reached a maximum 48 h after addition of 3 ng/ml depsipeptide to BHP18–21v cells. In ARO cells, re-expression of NIS and TPO reached a maximum after 12 h, whereas Tg gene gradually increased and peaked 48 h after the incubation with 3 ng/ml of depsipeptide (Fig. 2).

HDACI induces radioiodide uptake in BHP18–21v and ARO cells

Radioiodide uptake was measured in an effort to determine whether depsipeptide-induced NIS mRNA yielded a functional protein. After 48 h treatment with depsipeptide, BHP18–21v and ARO cells were incubated with $Na^{125}I$ (0.2 μCi/well) with or without 300 μM sodium perchlorate for 1 h (Fig. 3, A and B). In nontreated BHP18–21v and ARO cells, no difference in intracellular radioactivity was seen in cells with or without addition of sodium perchlorate, specific inhibitor. This result indicated that the cell line had lost its ability to take up iodide via NIS protein in thyroid cancer cells.

BHP18–21v cells incubated with 1 ng/ml depsipeptide showed a small increase in radioiodide uptake. However, there were no significant differences compared with sodium perchlorate-treated cells. Treatment with 3 and 10 ng/ml depsipeptide induced significant radioiodide uptake in BHP18–21v cells (Fig. 3A). This radioiodide uptake was inhibited by sodium perchlorate. In ARO cells, incubation with 1 ng/ml depsipeptide induced no radioiodide accumulation; 3 and 10 ng/ml of depsipeptide increased the count of radioiodide uptake (Fig. 3B). These results showed that the depsipeptide induced significant iodide uptake that was mediated by newly produced NIS protein. These results agreed with the findings in quantitative real-time RT-PCR (Fig. 1) that induction of NIS mRNA was required for at least 3 ng/ml depsipeptide.

To evaluate the effect of TSH, we performed the iodide uptake assay pretreated with or without TSH. There were no

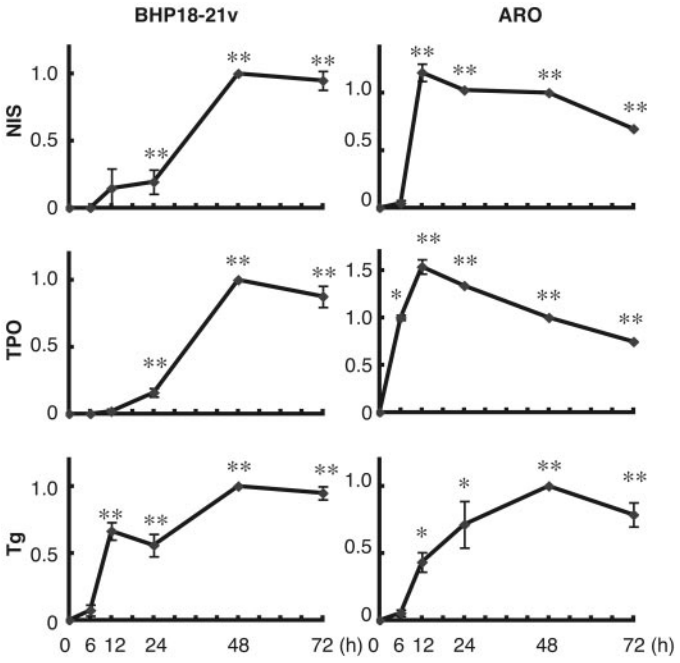


FIG. 2. Time-dependent expression of thyroid-specific genes mRNA. Re-expression of thyroid-specific genes was analyzed in BHP18–21v and ARO cells incubated with 3 ng/ml depsipeptide for 12, 24, 48, or 72 h. Total RNA was isolated in three independent samples. Amounts of thyroid-specific genes mRNA were determined by quantitative real-time RT-PCR with 100 ng cDNA in triplicate. Relative quantification of target cDNA was determined by arbitrarily setting the control value of samples at 48 h incubation to 1. All data are expressed as the mean ± SEM. *, $P < 0.05$, compared with control cells; **, $P < 0.001$, compared with controls cells.

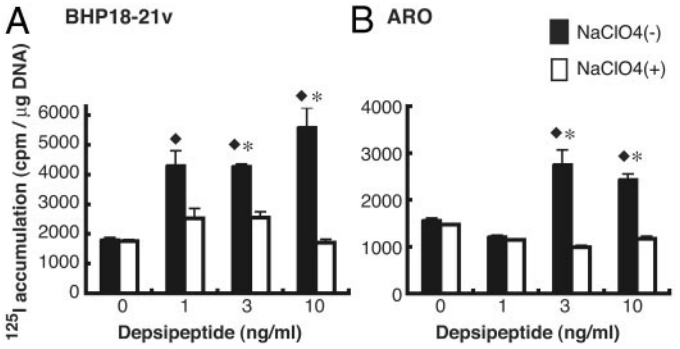


FIG. 3. Characterization of radioiodide uptake in BHP18–21v and ARO cells incubated with depsipeptide. A, BHP18–21v cells were incubated with depsipeptide for 48 h. B, ARO cells were incubated with depsipeptide for 48 h. These cancer cells were incubated in 0.2 μCi $Na^{125}I$-containing medium with or without 300 μM of $NaClO_4$ for 1 h. Cells were washed twice with ice-cold HBSS and exposed to 100% ethanol. Extracted radioactivity was measured with a γ-counter. All data are expressed as the mean ± SEM (n = 9). ◆, $P < 0.01$, compared with untreated cells; *, $P < 0.01$, compared with $NaClO_4$-treated cells.

differences in the amount of depsipeptide-induced iodide uptake between TSH-treated and untreated cells (data not shown). In addition, no expression of TSH-R mRNA was observed in either depsipeptide- or TSA-treated BHP18–21v or ARO cells (data not shown). These results indicate that TSH-R gene expression is hardly influenced by HDACI, and depsipeptide-induced iodide uptake is TSH-independent.

Immunocytochemical demonstration of HDACI-induced NIS, TPO, and Tg proteins expression in BHP18–21v and ARO cells

To analyze whether HDACI-up-regulated NIS, TPO, or Tg mRNAs is translated to corresponding protein product, immunoreactive NIS, TPO, or Tg proteins expressed in BHP18–21v and ARO cells were stained with anti-NIS, anti-TPO, or anti-Tg antibody. The presence of the NIS protein was detected by immunocytochemistry in HDACI-treated BHP18–21v and ARO cells and was observed in the cell membrane and cytosol (Fig. 4, B, C, K, and L). On the contrary, untreated cells were not stained (Fig. 4, A and J). Treatment with anti-TPO antibody did not yield any staining in BHP18–21v or ARO cells untreated with HDACI (Fig. 4, D and M). In contrast, marked staining was observed in both cell lines treated with HDACI (depsipeptide at 3 ng/ml or TSA at 300 ng/ml) for 48 h (Fig. 4, E, F, N, and O). This TPO immunostaining was localized in the region of cell membrane and cytosol. Expressed immunoreactive Tg protein also was stained with anti-Tg monoclonal antibody in BHP18–21v and ARO cells treated with depsipeptide (3 ng/ml) or TSA (300 ng/ml). Tg protein was detected in the area of the cytosol (Fig. 4, H, I, Q, and R). No Tg staining was observed in untreated cells (Fig. 4, G and P). These results clearly show that HDACI induced the expression of NIS, TPO, and Tg proteins, whereas re-expressed NIS and TPO protein were localized in either region of cell membrane and cytosol.

HDACI augments radioiodide organification in BHP18–21v cells

As described above, we demonstrated that HDACI induced the re-expression of NIS, TPO, and Tg protein. To confirm the function of the expressed TPO and Tg, we measured HDACI-induced radioiodide organification. BHP18–21v cells were incubated with 1, 3, and 10 ng/ml depsipeptide for 48 h. MMI (300 μM), which inhibits the TPO-induced

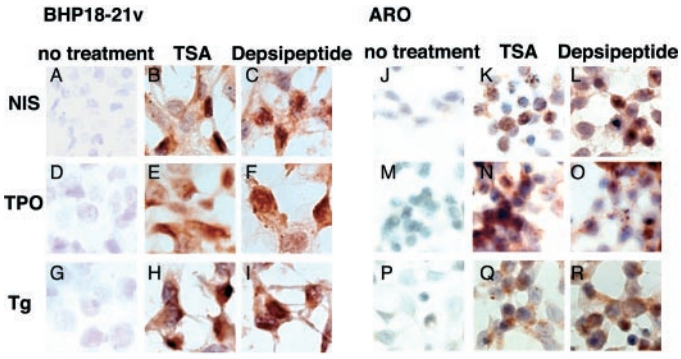


FIG. 4. Detection of immunoreactive NIS, TPO, and Tg proteins in HDACI-treated BHP18–21v and ARO thyroid cancer cells. BHP18–21v and ARO cells were incubated for 48 h with 3 ng/ml depsipeptide or 300 ng/ml TSA. A–C and J–L, The presence of NIS protein was examined by immunostaining using an anti-NIS monoclonal antibody in BHP18–21v and ARO cells treated with or without HDACI. D–F and M–O, The fixed cells were stained with an anti-TPO monoclonal antibody. G–I and P–R, Tg immunostaining was examined in cells incubated with or without HDACI. The fixed cells were incubated with an anti-NIS, anti-TPO, and anti-Tg monoclonal antibody and then a biotinylated secondary antibody, and then stained with avidin-biotinylated horseradish peroxidase.

iodination of Tg, was added 24 h before the assay. After cells were exposed to ^{125}I (0.1 μCi) for 1 h, radioiodide uptake was quantified. Approximately 3.41% of radioiodide taken up was retained in the protein fraction derived from BHP18–21v cells without depsipeptide (Fig. 5A). Tc-rNIS cells that expressed NIS, but not TPO or Tg, retained only about 2.11% of the iodide. This retention of radioiodide in untreated BHP18–21v and Tc-rNIS cells appeared to be nonspecific, because pretreatment of the cells with MMI did not significantly diminish the amount of protein-bound radioiodide. Treatment of BHP18–21v cells with 10 ng/ml depsipeptide significantly increased the amount of radioiodide bound to cellular proteins to almost 7.58%, compared with the cells pretreated with MMI ($P < 0.05$) (Fig. 5A). This organification induced by depsipeptide increased in a dose-dependent manner. Pretreatment of cells with MMI inhibited the increase in radioiodide organification.

In addition, to analyze whether re-expressed TPO could iodinate intracellular protein substrates, the molecular size of radioiodinated intracellular proteins was investigated. After BHP18–21v cells, which were pretreated with or without depsipeptide, were exposed to ^{125}I for 1 h, the intracellular proteins were separated by SDS-PAGE. In 3 ng/ml depsipeptide-treated cells, radiolabeled protein bigger than 210 kDa was detected, whereas it failed to produce radiolabeled protein without depsipeptide (Fig 5B).

These results indicate that TPO induced by HDACI was capable of facilitating iodide organification into protein substrates, in which the HDACI-induced expression of Tg played a crucial role on this effect.

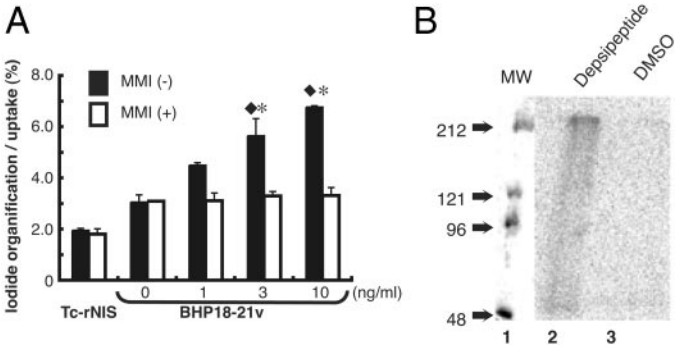


FIG. 5. Depsipeptide-induced iodide organification in BHP18–21v poorly differentiated thyroid cancer cells. A, Tc-rNIS cells were established from FRTL-Tc malignantly transformed thyroid cells transfected with NIS expression vector (8). Because the Tc-rNIS cell line overexpresses the NIS gene but does not express TPO and Tg, it was used as a negative control for iodide organification. BHP18–21v cells that were incubated in depsipeptide-containing medium with or without MMI were exposed to 0.2 μCi/well $Na^{125}I$ for 1 h. The cells were washed in buffer, and radioactivity was counted. The radioactivity of ^{125}I bound to protein was determined by TCA precipitation. All data are expressed as the mean ± SEM (n = 6). ♦, $P < 0.05$, compared with nontreated with depsipeptide; *, $P < 0.05$, compared with MMI-treated cells. B, BHP18–21v cells were pretreated with or without 3 ng/ml depsipeptide for 48 h and exposed to 20 μCi/dish $Na^{125}I$ for 1 h. Each sample (30 μg protein) was electrophoresed and analyzed using a BAS 2500 imaging analyzer (Fuji Film Co.). Lane 1, The molecular weight (MW) of the standard; lane 2, 3ng/ml depsipeptide-treated BHP18–21v cells; lane 3, DMSO-treated BHP18–21v cells.

Depsipeptide leads to efficient iodide accumulation in vivo

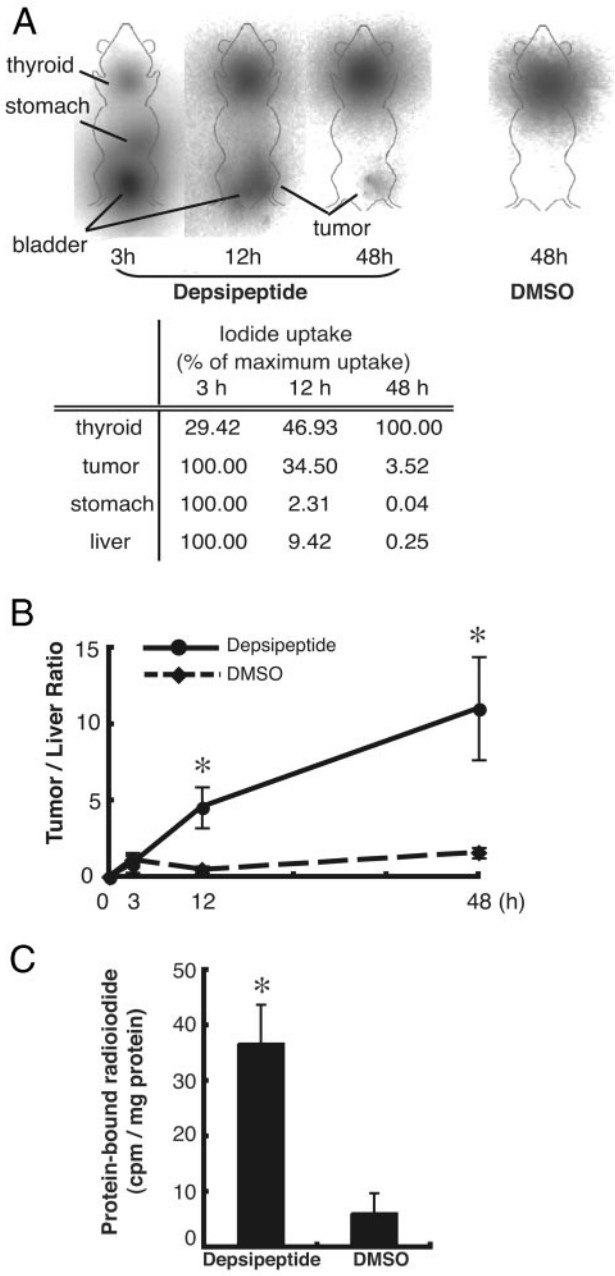
To evaluate the efficiency of depsipeptide *in vivo*, BHP18–21v cells were transplanted on the left flank of nude mice by sc injection of 1×10^7 cells. One week after transplantation, the cells formed small tumors at the injection site. The tumors grew very rapidly and were 1 cm in diameter 3 wk after transplantation. At this point, we injected 5 μg/g·d depsipeptide or DMSO in PBS ip for 4 d. To prevent radioiodide uptake by thyroid gland, mice were treated with L-T_4.

Autoradiographic imaging of the depsipeptide-treated mice displayed the accumulation of radioiodide in the physiological NIS-expressed organs, thyroid, stomach, and bladder. However, 48 h after, no accumulation was observed in stomach and bladder (Fig. 6A). In contrast, depsipeptide-treated tumors could accumulate ^{125}I, as revealed by imaging at 12 and 48 h (Fig. 6A). Radioactivity in the tumors, thyroid, stomach, and liver were measured and were represented as a percent of the maximum uptake (Fig. 6A). The disappearance of radioiodide from the stomach paralleled the decrease in radioactivity from the liver. In contrast, the depsipeptide-treated tumors could reserve approximately 3.52% of maximum uptake 48 h after injection (Fig. 6A). The percentage of injected dose per gram of tissue of depsipeptide-treated tumors at 48 h after injection of ^{125}I was 0.70 ± 0.16%, whereas 0.14 ± 0.04% of radioiodide remained in DMSO-treated tumors ($P < 0.05$). In addition, radioiodide measurement by whole-body scanning showed that values of 5.95 ± 0.39% and 1.49 ± 0.93% of whole-body retention at 48 h were detected in tumors treated with depsipeptide and DMSO, respectively. Radioiodide accumulation in thyroid glands gradually increased and peaked 48 h after injection.

To examine the time-dependent accumulation of ^{125}I in these mice, the tumor/liver ^{125}I concentration ratio was measured at 3, 12, and 48 h after injection of 10 μCi $Na^{125}I$. Three hours after the injection, there was no significant difference between the tumor/liver ratios in depsipeptide- and DMSO-treated mice. At 12 and 48 h, the tumor/liver ^{125}I concentration ratio was 4.56 ± 1.35 and 11.03 ± 3.37 in depsipeptide-treated mice, respectively, whereas that in DMSO-treated mice was 0.48 ± 3.37 and 1.57 ± 0.33, respectively. There was a significant difference in the tumor/liver ^{125}I concentration ratio between the depsipeptide-treated and DMSO-treated mice after 12 h ($P < 0.05$) (Fig. 6B).

To analyze iodide organification *in vivo*, we measured protein-bound radioiodide in xenotransplanted tumors. A total of 10 μCi of $Na^{125}I$ was injected into the mice treated with depsipeptide or DMSO. The removed tumors were disrupted, and intracellular protein was extracted. The protein-bound radioiodide in depsipeptide-treated mice was 36.74 ± 7.0 cpm/mg, whereas that in DMSO-treated mice was 6.16 ± 3.6 cpm/mg ($P < 0.05$) (Fig. 6C).

To investigate the effects of the HDACI on the expression of NIS, TPO, Tg, and TSH-R *in vivo*, we analyzed the mRNA levels of these thyroid-specific genes in depsipeptide- or DMSO-treated tumors using quantitative real-time PCR (Table 2). The mice were treated with 5 μg/g·d depsipeptide or DMSO in PBS for 4 d. In DMSO-treated mice, NIS, TPO, Tg, and TSH-R mRNA were not observed. Re-expression of NIS, TPO, and Tg was detected in depsipeptide-treated tumors,



	Iodide uptake (% of maximum uptake) 3 h	12 h	48 h
thyroid	29.42	46.93	100.00
tumor	100.00	34.50	3.52
stomach	100.00	2.31	0.04
liver	100.00	9.42	0.25

FIG. 6. Induction of intratumoral radioiodide accumulation by depsipeptide *in vivo*. BHP18–21v cells were transplanted on the left flank of nude mice by sc injection. Mice were treated with L-T_4 to avoid massive uptake by the thyroid. A total of 5 μg/g·d depsipeptide was injected ip for 4 d. After the injection of 10 μCi of ^{125}I, the radioactivity from the mice was analyzed using a BAS2500 imaging analyzer. A, Typical images 3, 12, and 48 h after the injection. Thyroid, stomach, and bladder are observed by their physiologic uptake. Radioactivity from the tumors, thyroid, stomach, and liver was measured and represented as a percent of the maximum uptake. B, After calculating the amounts of radioiodide uptake per tissue weight, tumor/liver radioiodide uptake ratio was elevated. C, The intratumoral radioactivity of ^{125}I bound to protein was determined by TCA precipitation. Radioactivity was measured with a γ-counter and normalized by dividing the amounts of proteins weight. All data are expressed as the mean ± SEM (n = 6). *, $P < 0.05$, compared with DMSO-treated tumors.

whereas the induction of TSH-R mRNA was not observed. These results clearly demonstrated that depsipeptide could induce the expression of thyroid-specific genes that is necessary for effective cell processing of iodide, and this depsipeptide-induced radioiodide uptake is TSH-independent.

HDACI stimulates TTF-1 gene expression

Expression of thyroid-specific genes (NIS, TPO, and Tg) is modulated, in part, by the interaction of the TTF-1 and Pax-8, thyroid-specific transcription factors with their respective promoters (16). To analyze the mechanisms by which the HDACI-induced re-expression of thyroid-specific genes took place, we examined changes in the expression of the thyroid-specific transcription factor after treatment of HDACI using RT-PCR. In untreated ARO cells, only faint expression of TTF-1 mRNA was detected (Fig. 7). Depsipeptide treatment in this cell line caused a dose-dependent increase in expression of the TTF-1 gene. Maximum gene expression was obtained at 10 ng/ml depsipeptide. However, depsipeptide were unable to restore the expression of Pax-8 mRNA that was lost in ARO cells (data not shown). In BHP18–21v cells, which express Pax-8 but not TTF-1 mRNA, depsipeptide induced the expression of TTF-1 mRNA in a dose-dependent manner. Maximum TTF-1 gene expression was observed in cells incubated with 10 ng/ml depsipeptide for 24 h (Fig. 7A).

We also analyzed time-dependency of HDACI-induced expression of the TTF-1 gene. After incubation with 3 ng/ml depsipeptide, TTF-1 mRNA levels reached a maximum after 24 h and after 12 h in BHP18–21v and ARO cells, respectively. Conversely, Pax-8 mRNA levels significantly decreased in BHP18–21v cells after incubation with depsipeptide.

Cycloheximide diminishes depsipeptide-induced NIS gene expression and enhances the TPO and Tg gene expression

HDACI directly activates the regulatory machinery of transcription via an increase of acetylated histones (38), but also can modulate expression of certain transcription factors, including TTF-1 (Fig. 7). To investigate whether new protein synthesis was necessary for induction of thyroid-specific genes in depsipeptide-treated BHP18–21v and ARO cells, we measured expression of thyroid-specific genes with or without cycloheximide (Biomol Corp., Plymouth Meeting, PA) treatment. After BHP18–21v and ARO cells were incubated with 3 ng/ml depsipeptide in the presence or absence of 30 μg/ml cycloheximide for 24 h, expression levels of thyroid-specific genes were quantified by real-time RT-PCR. Cycloheximide partly, but significantly, inhibited the depsipeptide-induced up-regulation of NIS mRNA (Fig. 8); approximately 70% of induced NIS gene expression was abolished. Thus, a portion of depsipeptide-induced up-regulation of NIS mRNA required ongoing protein synthesis.

The faint increases in TPO and Tg mRNA levels were observed in the presence of cycloheximide without depsipeptide (Fig. 8). The presence of cycloheximide led to elevated expression levels of TPO mRNA induced by 3 ng/ml depsipeptide in BHP18–21v and ARO cells. In depsipeptide-treated BHP18–21v and ARO cells, cycloheximide similarly induced 30.0-fold and 16.8-fold increases in Tg mRNA levels, respectively. These results suggested that depsipeptide-

TABLE 2. The expression of NIS, TPO, Tg, and TSH-R mRNA in xenotransplanted BHP18-21v cells isolated from DMSO-treated mice and depsipeptide-treated mice

	NIS	TPO	Tg	TSH-R
Depsipeptide	$2.84 \times 10^{-3} \pm 0.001^a$	$1.11 \times 10^{-1} \pm 0.047^a$	$7.67 \times 10^{-1} \pm 0.195^a$	0
DMSO	0	0	0	0

Each group of mice was treated with DMSO or depsipeptide for 4 d. Amounts of NIS, TPO, Tg, and TSH-R mRNA were determined by quantitative real-time RT-PCR with 100 ng cDNA in duplicate. All data are ratio to GAPDH mRNA level and expressed as the mean ± SEM.
[a] $P < 0.05$.

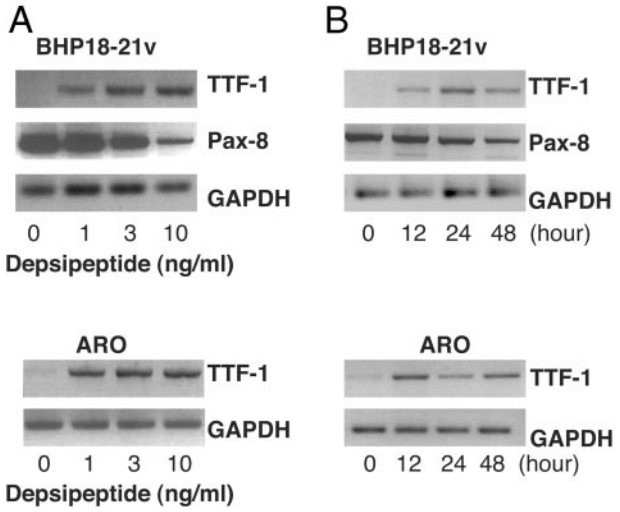


FIG. 7. Effect of depsipeptide on the expression of thyroid-specific transcription factors. Amounts of TTF-1, Pax-8, and GAPDH mRNA were determined by RT-PCR. A, BHP18–21v and ARO cells were incubated with 1, 3, or 10 ng/ml of depsipeptide for 24 h. B, Time-course analysis was performed by RT-PCR. TTF-1 and Pax-8 gene expression was analyzed using BHP18–21v and ARO cells incubated with 3 ng/ml depsipeptide for 12, 24, or 48 h.

induced re-expression of TPO and Tg genes was not mediated by newly produced-transcription factors.

Depsipeptide exhibits cytotoxic effect against BHP18–21v and ARO cells in vitro

We examined the effect of depsipeptide on viabilities of ARO and BHP18–21v cells. In both cell lines, 1 ng/ml depsipeptide exhibited no cytotoxicity. Administration of 3 ng/ml depsipeptide was not cytotoxic in BHP18–21v, whereas 51% of ARO cells resulted in cell death ($P < 0.05$). Almost 90% cell death was observed in ARO and BHP18–21v cells treated with 10 ng/ml depsipeptide ($P < 0.05$) (Fig. 9). In comparison with depsipeptide-induced expression of thyroid-specific genes, higher concentrations of depsipeptide were required for the cytotoxic effect.

Discussion

Radioiodide therapy of thyroid carcinoma with preserved ability of trapping and concentration of iodide is not effective in poorly differentiated or anaplastic thyroid cancers, in which thyroid-specific gene expression is lacking (6, 39). In the present study, we showed that exposure of poorly differentiated papillary thyroid BHP18–21v cells and anaplastic thyroid ARO cells to the HDACI induced the expression of NIS, TPO, and Tg mRNAs. We also demonstrated immunoreactive NIS, TPO, and Tg proteins in HDACI-treated

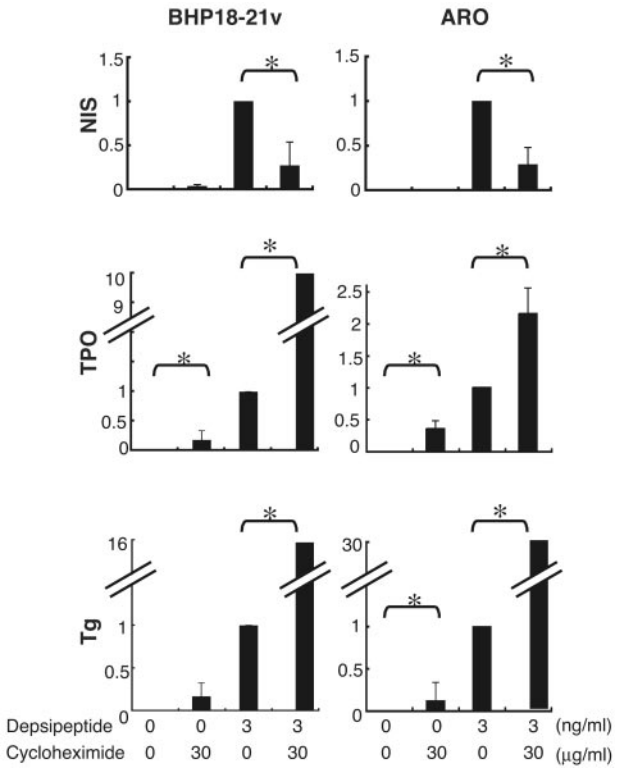


FIG. 8. Effect of cycloheximide on regulation of thyroid-specific genes in depsipeptide-treated BHP18–21v and ARO cells. BHP18–21v and ARO cells were incubated with 1, 3, or 10 ng/ml depsipeptide in the presence or absence of cycloheximide for 24 h. Total RNA was isolated in four independent samples. Amounts of NIS, TPO, and Tg mRNA were determined by quantitative real-time RT-PCR with 100 ng cDNA in triplicate. Relative quantification of target cDNA was determined by arbitrarily setting the control value from 3 ng/ml depsipeptide-incubation samples to 1. All data are expressed as the mean ± SEM. *, $P < 0.05$.

BHP18–21v and ARO cells. We provided further evidence clearly demonstrating that HDACI-treated cancer cells increased their radioiodide uptake and prolonged radioiodide retention in depsipeptide-treated tumors *in vitro* and *in vivo*.

We have reported that, despite radioiodide accumulation, $Na^{131}I$ did not significantly change the volume of experimental tumors formed by rat undifferentiated thyroid cells with or without transfection with NIS (8). In that study, extracorporeal measurement of radioactivity in the tumors revealed that ^{125}I accumulation peaked at 90 min and decreased to 50% 6 h after injection *in vivo*. In contrast, ^{125}I uptake in thyroid glands gradually increased and peaked 48 h after injection. These results demonstrated the differences between NIS-expressed tumor and thyroid glands in time-dependent iodide accumulations may be related to their

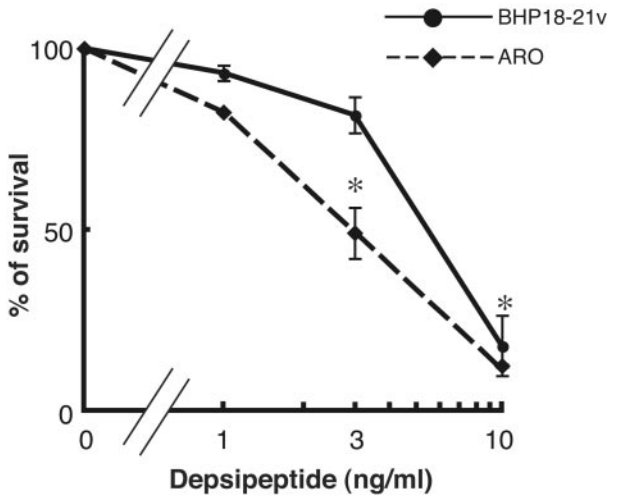


FIG. 9. Effect of depsipeptide on cell viability in BHP18–21v and ARO cells. BHP18–21v and ARO cells were incubated with 1, 3, or 10 ng/ml for 48 h. Cell viability were analyzed using a Cell Counting Kit. Survival of cells is presented as a percentage of the absorbance with depsipeptide-treated cells divided by that with cells not exposed to depsipeptide. All data are expressed as the mean ± SEM. *, $P < 0.05$, compared with untreated cells.

abilities to organify iodide. These results indicate that cytotoxic efficacy of radioiodide may be limited by the radioiodide efflux, and that enhancing the intracellular retention of iodide is necessary for effective radioiodide therapy. Rapid washout of radioiodide from tumors and low binding of radioiodide are probably consequences of the absence of TPO and Tg gene expression.

Our present study is the first report showing simultaneous induction of NIS, TPO, and Tg gene expression and iodide organification by HDACI in undifferentiated papillary thyroid cancer cells *in vitro*. Furthermore, we demonstrated HDACI-induced radioiodide accumulation in tumors formed with thyroid cancer cells *in vivo*. Certain reagents or transcription factors have been found to induce re-expression of thyroid-specific genes. Fagin *et al.* (40) reported that papillary thyroid cancer cells transfected with wild-type p53 showed re-expression of TPO mRNA and protein. Re-expression of Pax-8 mRNA, which specifically activates the TPO promoter, also was demonstrated. Schmutzler (41) reported that all-trans retinoic acid increased NIS mRNA in human follicular thyroid cancer cells. Venkataraman *et al.* (42) similarly found that 5-azacytidine could restore the expression of NIS mRNA and radioiodide uptake in benign thyroid adenoma cells. Kitazono *et al.* (43, 44) reported that HDACI induced the re-expression of NIS and Tg mRNA in human anaplastic thyroid cancer cells.

TPO is the primary enzyme involved in iodide organification (45), being active at the apical pole of the thyrocytes, where oxidizing H_2O_2 is produced (46). It has been shown that TPO can also be active intracellularly in the presence of H_2O_2, leading to low levels of intracellularly iodinated proteins (47). Recently, Huang *et al.* (48) reported that an increase of iodide organification was observed in NIS- and TPO-cotransfected lung cancer cells. Boland *et al.* (49) reported that the levels of iodide organification obtained were too low to increase the iodide retention time in the thyroid cancer cells cotransfected with NIS and TPO. These reports suggested that expression of Tg was required for the effective iodide retention and organification. In this report, radiolabeled protein greater than 210 kDa was specifically observed, and iodide organification was detected in depsipeptide-treated thyroid cancer cells. These results supported the hypothesis that the induction of iodide organification may require combined expression of NIS, TPO, and Tg, and TPO induced by depsipeptide was capable of facilitating iodide organification into protein substrates, in which the HDACI-induced expression of Tg could play a crucial role on this effect. However, the fact that smear bands appeared suggests a possibility that some other protein substrates might be iodinated in the process of depsipeptide-induced iodide organification.

In the present report, we demonstrated that inhibition of histone deacetylation by HDACI induced the expression of thyroid-specific genes in thyroid cancer cells. Acetylation and deacetylation of histones plays a significant role in regulation of gene transcription in many cell types (38). Histone acetylation alters nucleosomal conformation, which in turn can increase accessibility of transcriptional regulatory proteins to chromatin templates (50). HDAC oppose these events by catalyzing removal of acetyl groups from the amino-terminal lysine residues of core nucleosomal histones (51). Although HDACI act on the general regulatory machinery of transcription, our results demonstrated that HDACI do not do so nonspecifically. For example, HDACI did not increase expression of the GAPDH and Pax-8 genes (Figs. 1 and 7).

TTF-1 and Pax-8 are major transcription factors regulating the Tg and TPO promoter activity positively. We observed that depsipeptide could increase in the expression level of TTF-1. In contrast, the expression levels of Pax-8 mRNA were decreased in depsipeptide-treated BHP18–21v cells, and were not observed in depsipeptide-treated ARO cells. We detected that overexpression of TTF-1 using an adenovirus vector inhibited the expression of Pax-8 mRNA in BHP18–21v cells (12, 13, 16–19). Therefore, we suspect that re-expressed TTF-1 induced by HDACI could influence the reduction of Pax-8 mRNA in BHP18–21v cells. Because depsipeptide exhibited an opposite effect on the expression of TTF-1 and Pax-8, we studied the effect of cycloheximide on Tg and TPO gene expression to determine whether HDACI acts through newly synthesized transcription factors or, instead, directly activates the transcriptional machinery for thyroid-specific genes. Figure 8 shows that the presence of cycloheximide did not decrease the levels of HDACI-induced TPO or Tg expression. These results suggested that HDACI-induced re-expression of TPO or Tg genes was not mediated by newly produced *trans*-activating factors.

Moreover, cycloheximide increased the expression of TPO and Tg mRNA in depsipeptide-treated and untreated cells. We suspect that unknown proteins inhibit TPO and Tg gene expression in cancer cells, and cycloheximide might block the synthesis of these unknown proteins.

In contrast, this study revealed that up-regulation of the NIS gene partly involved newly produced proteins, presumably some transcription factors. In our study, HDACI solely induced expression of TTF-1. Kitazono *et al.* (52) demonstrated that TTF-1 gene was re-expressed in thyroid cancer

cells by simultaneous treatment with depsipeptide and 8-Br-cAMP. In the rat NIS gene, TTF-1 was shown to activate the rat NIS promoter by a direct interaction with its proximal enhancer region (18). Further, Taki *et al.* (22) found a TTF-1-binding site in the human NIS upstream enhancer, but TTF-1 did not act as a *trans*-activating factor on the human NIS upstream element. Schmitt *et al.* (53) also reported that transcription of a TTF-1 expression vector showed no effect on luciferase activity driven by the 9-kb NIS promoter. We presently suspect that an unidentified protein activated by HDACI was involved in NIS gene induction. It is also possible that newly synthesized TTF-1 is involved in the induction of NIS gene expression via interactions of the NIS gene other than these previously investigated.

HDACI was identified as an antitumor agent (54). In human bladder cancer cells, HDACI induced apoptosis, which might be related to the re-expression of p21, c-myc, and gelsolin genes (11). In human breast cancer cells, increased p21, phosphorylation of Bcl-2, and apoptosis have been observed after treatment with depsipeptide (55). In the case of thyroid cancer, Greenberg *et al.* (56) demonstrated that 500 nM TSA induced apoptosis and cell-cycle arrest in anaplastic thyroid cancer cells. In our study, poorly differentiated and anaplastic thyroid cancer cells treated with 30 ng/ml (99 nM) TSA showed re-expression of the thyroid-specific genes that are required for effective radioiodide therapy (Fig. 1B). HDACI previously have been shown to simultaneously induce differentiation and apoptosis, as well as cell-cycle arrest, in breast cancer, bladder cancer, and leukemia in mice (11, 54, 55, 57, 58). In this study, 10 ng/ml depsipeptide could induce cell-death concomitantly with iodide accumulation in poorly differentiated and anaplastic thyroid cancer cells (Figs. 3 and 9). It is reasonable to speculate that the depsipeptide-induced cell-death can be a beneficial effect, from the standpoint of cancer therapy using radioiodide.

Depsipeptide, a strong inhibitor of histone deacetylase, is currently in phase I trials in the United States. These trials suggest that the maximum tolerated plasma concentration is about 472.6 ng/ml (59). In this study we confirmed that a low-dose depsipeptide concentration (1–10 ng/ml) induced iodide uptake and organification in poorly differentiated thyroid cancer cells. Furthermore, depsipeptide-treated tumors could specifically accumulate radioiodide, as revealed by imaging experiments and radioiodide concentration *in vivo*. Schlumberger *et al.* (60) reported that the presence of ^{131}I uptake that could be detected using whole-body scanning was one of the most important prognostic factors in thyroid cancer patients with metastases. Although the tumoral uptake was dependent on tumor size, it was reported that most of positive scans were above 0.7% of whole-body retention 48 h after administration of radioiodide (61, 62). In the present study, whole-body scanning performed 48 h after injection (Fig. 6A) showed that depsipeptide- and DMSO-treated tumors could accumulate 5.95 ± 0.39% and 1.49 ± 0.93% of whole-body retention, respectively ($P < 0.01$). We therefore speculate that this HDACI-induced iodide retention may confer some therapeutic effects on poorly differentiated and anaplastic thyroid carcinoma.

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