Nonradioactive Iodide Effectively Induces Apoptosis in Genetically Modified Lung Cancer Cells

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ABSTRACT

We assessed a nonradioactive approach to induce apoptosis in non-small cell lung cancer by a novel iodide uptake and retention mechanism. To enhance tumor apoptosis, we transduced non-small cell lung cancer cells with retroviral vectors containing the sodium iodide symporter (NIS) and thyroperoxidase (TPO) genes. Expression of NIS and TPO facilitated concentration of iodide in tumors. As a consequence of the marked increase in intracellular levels of iodide, apoptosis was seen in >95% of NIS/TPO-modified lung cancer cells. Intraperitoneal injection of potassium iodide resulted in significant tumor volume reduction in NIS/TPO-modified tumor xenografts without apparent adverse effects in SCID mice. Iodide induced an increase in the level of reactive oxygen species. Iodide-induced apoptosis is sensitive to N-acetylcysteine inhibition, suggesting an important role by reactive oxygen species in this apoptotic process. In addition, iodide-induced apoptosis is associated with overexpression of 

INTRODUCTION

Lung cancer is the leading cause of cancer death in both men and women in the United States (1–3). Currently, there is no reliable and effective therapy for patients with inoperable NSCLC, and the prognosis remains very poor. Therefore, development of new therapeutic strategies for the treatment of lung cancer is clearly needed.

The ability to concentrate iodide is a fundamental property of the normally functioning thyroid gland and represents the first step in the synthesis of thyroid hormones. NIS plays a key role in thyroid hormone production by efficiently accumulating iodide from the circulation into the thyrocytes against an electrochemical gradient (4–8). The NIS gene, expressed by the thyroid cells, is also found in salivary gland, choroid plexus, gastric parietal cells, and lactating breast (5, 7, 9). TPO catalyzes iodination of proteins and promotes iodide accumulation within thyrocytes (10, 11). We reported previously that genetic modification of NSCLC cells with NIS and TPO genes led to concentration of radioiodide and enhanced tumor cell apoptosis (12). Our rationale for choosing the NIS and TPO genes is based on the fact that intracellular iodide retention in the normal thyroid is enhanced by TPO-mediated incorporation of iodide into protein. Although this TPO-enhanced NIS-based gene therapy (12) produces concentration of radioiodide in cancer cells, radiation-induced toxicity to normal tissues could limit its clinical application.

Previous studies have shown that excess iodide induces apoptosis in thyroid cells (13). To improve the iodide concentrating capacity of lung cancer cells, we transduced tumor cells with retroviral vectors containing functional NIS and TPO genes to take advantage of the effects of nonradioactive iodide. The iodide concentrating capacity in the transduced lung cancer cell clones was markedly increased, up to 320-fold as determined by 125I uptake assays. We demonstrate here for the first time that nonradioactive iodide effectively induces apoptosis in the NIS/TPO-modified lung cancer cells.

MATERIALS AND METHODS

Cell Culture. Human lung undifferentiated large cell carcinoma cell line H1299 was obtained from American Type Culture Collection (Rockville, MD). The cells were grown at 37°C under an atmosphere of 5% CO2 in air as monolayers in 25-cm2 tissue culture flasks containing 5.0 ml of RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin/streptomycin solution and 2 mM glutamine (JRH Biosciences, Lenexa, KS). Mice. Pathogen-free 6–8-week-old female SCID/beige CB17 mice (T-, B-, natural killer-cell deficient) were obtained from University of California Los Angeles and maintained in cages housed in laminar flow hoods under pathogen-free conditions in the West Los Angeles VA Animal Research Facility. All of the studies were approved by the Institutional Animal Research Committee.

Retroviral Vector Construction and the Transduction of H1299 Cells. Human NIS and TPO plasmid vectors were described previously (12). Retroviral vectors pLHCX containing a hygromycin-selection marker and pLNCX containing a neomycin-selection marker were obtained from Clontech Laboratories, Inc. (Palo Alto, CA). The retroviral NIS and TPO vectors were constructed by the insertion of the NIS gene into the pLHCX and the TPO gene into the pLNCK vectors. After transfection to the PT-67 retroviral packaging cell line by a liposomal-mediated method (Efectene method; Qiagen Inc., Valencia, CA), the supernatants containing high-titer of retroviruses expressing NIS or TPO were collected to transduce H1299 cells. Single clones of genetically modified H1299 cells were selected with appropriate antibiotic resistant markers. The clones were additionally characterized to confirm the presence of functional NIS and TPO genes by RT-PCR and 125I uptake assays.

Detection of NIS and TPO by RT-PCR. We used the following primer pairs for the detection of human NIS and TPO by RT-PCR: 5’-GCT GAG GAC TTC TTC ACC GGG GGC CGG-3’ (NIS sense primer); 5’-GTC AGG GTT AAA GTC CAT GAT GGT G-3’ (NIS antisense primer); 5’-CAT GTA CGC CAC GAT GCA AGA A’-3’ (TPO sense primer); and 5’-CTG TGG TGT GAA CGC GAT GTC G-3’ (TPO antisense primer). The NIS primer pairs correspond to the coding region 349–374 and 930–906, respectively, and the TPO 199–218 and 778–757, respectively. The amplified products are 558 bp in length for NIS and 579 bp for TPO. Total RNA was isolated with TRIzol reagents (Invitrogen Corp., Carlsbad, CA). The RT-PCR was performed for 35 cycles of amplification using a PTC-100–60 thermal cycler (MJ Research, Inc., Watertown, MA). The PCR products from each reaction were analyzed by 1.5% agarose/ethidium bromide gel electrophoresis.
125I Uptake and Retention Assays. The 125I was obtained from Amersham Pharmacia Biotech (Piscataway, NJ). The methods for 125I uptake and retention assays were described previously (12). To determine 125I uptake in vivo, SCID mice bearing genetically modified tumors were given 1 µCi of 125I per mouse by intraperitoneal injection. Three h after administration of 125I (1 µCi/mouse) by i.p. injection, tumors from each group of mice were removed and weighed, and the tumor-associated radioactivity was determined as 125I uptake/g of tumor weight by a gamma counter. There were no statistically significant differences in tumor size seen in different groups of animals before 125I was administered.

Apoptosis Assays. Apoptosis was quantified by measuring the levels of cellular dye retention within apoptotic cells using an APOPercentage apoptosis assay kit (Accurate Chemical & Scientific Corporation, New York, NY). The APOPercentage method uses a specially designed dye that is selectively imported and accumulated by cells that are undergoing apoptosis (14). The Klenow-FragEL DNA fragmentation method (OncoGene Research Products, Cambridge, MA) allows for the detection of free 3'-OH groups at the ends of DNA fragments generated by apoptotic endonucleases (15). The TUNEL Apoptosis Detection kit was obtained from Upstate Biotechnology Inc. (Waltham, MA). The TUNEL assay is a well-documented method for the detection of DNA fragmentation (16). NMPs ELISA kit was obtained from Oncogene Research Products (Cambridge, MA). NMPs are released from apoptotic cells (12). In this assay, the amount of released NMPs is a function of the number of apoptotic cells. Apoptosis of tumor xenografts was assessed by microscopic examination of Klenow-FragEL-stained sections with a calibrated graticule (a 1-cm2 grid subdivided into 100 1 mm2 squares). A grid square stained in brown occupying >50% of its area was scored as positive, and the total number of positive squares was determined as described previously (17). Twenty separate fields from the histological sections of the tumors were examined under high-power (×200 objective). GEArray Q series human stress and toxicity pathway finder and GEArray Q series human apoptosis kits were obtained from SuperArray Inc. (Bethesda, MD). Specific monoclonal antibodies to CDKN1A and survivin protein levels were determined by ELISA as described previously (17).

Determination of ROS Level by FACScan. DCFH-DA was obtained from Molecular Probes, Inc. (Eugene, OR). DCFH-DA is an oxidation-sensitive fluorescent chemical compound readily taken up by the cells and deacylated to a nonfluorescent form (DCFH; Ref. 13). DCFH is oxidized by ROS to 2,7-dichloro-5(6)-carboxyfluorescein (DCF; Ref. 13). DCF is further oxidized by ROS to fluorescent 2',7'-dichloro-5(6)-carboxyfluorescein (DCF-H2) (Ref. 13). DCF-H2 is fluorescent and can be measured by FACScan. The fluorescent compound is then further oxidized to nonfluorescent 2',7'-dichloro-5(6)-carboxyfluorescein (DCF-DH) (Ref. 13). DCF-DH is then oxidized by ROS to nonfluorescent 2',7'-dichloro-5(6)-carboxyfluorescein (DCF-DH2) (Ref. 13). DCF-DH2 is nonfluorescent and can be measured by FACScan. The fluorescent compound is then further oxidized to nonfluorescent 2',7'-dichloro-5(6)-carboxyfluorescein (DCF-DH2) (Ref. 13).

RESULTS

Genetically Modified Lung Cancer Cells Express the NIS and TPO Genes. We reported previously that a significant increase in 125I uptake could be achieved in NSCLC cell lines after transient transfection with the NIS gene (12). We also found that cotransfection of the TPO gene with NIS increased radiodiode uptake and retention in lung cancer cells, and enhanced radiation-induced apoptosis compared with NIS transfection alone (12). To additionally explore the therapeutic potential of NIS/TPO gene-based therapy for cancer, we made retroviral constructs that express the NIS or TPO genes. To allow for selection and characterization of tumor clones, the NIS construct contained a hygromycin-resistance gene (pLHCX-NIS) and the TPO the neomycin-resistance gene (pLNCX-TPO). The packaging cell line PT-67 was transfected using a liposomal-based method to generate a high titer of retroviruses expressing functional NIS or TPO genes. The retroviruses were harvested and used to transduce the human lung poorly differentiated carcinoma cell line H1299. The successful introduction of the NIS or both NIS and TPO genes into the tumor cells was confirmed by RT-PCR with gene-specific primer pairs. Parental lung cancer cells do not express either NIS or TPO (Fig. 1). In contrast, tumor cells that were transduced with the NIS or both NIS and TPO genes express significant levels of the transduced genes (Fig. 1).

NIS/TPO-modified Lung Cancer Cells Markedly Increase 125I Uptake and Retention. Forty-two NIS-transduced hygromycin-resistant H1299 clones and 63 NIS/TPO cotransduced H1299 clones that were both hygromycin- and G418-resistant were isolated. To assess the effectiveness of the NIS/TPO gene expression from tumor cells we performed 125I uptake (Fig. 2A) and retention assays (Fig. 2B). Four of the 42 (9.5%) NIS-transduced stable clones had 10–50-fold increase in 125I uptake, 4 of 42 clones (9.5%) ranged from 5- to 10-fold, 17 of 42 clones (40%) ranged from 1- to 5-fold, whereas the remainder showed no increase. In contrast, 9 of 63 (14.3%) NIS/TPO cotransduced stable clones had >100-fold increase in 125I uptake with the highest 125I uptake clone up to 320-fold, 9 of 63 (14.3%) clones ranged from 50- to 100-fold, 13 of 63 (20.6%) clones ranged from 10- to 50-fold, 7 of 63 clones (11.1%) ranged from 5- to 10-fold, 11 of 63 clones (15.9%) ranged from 1- to 5-fold, and the remainder had no increase. Consistent with previous results (12), inhibition of NIS with KClO4 completely inhibited the increased uptake in both NIS and NIS/TPO cotransduced clones, indicating that the increased radiodiode uptake was NIS-mediated. Three NIS/TPO cotransduced clones (NIS/TPO1, 2, and 3) in the high 125I uptake category (an increase in 125I uptake up to 320X, 170X, and 164X) and the highest 125I uptake clone transduced by NIS (NIS, an increase in 125I uptake up to 17 times) were chosen for 125I retention assays. After the removal of 125I, the remaining cell-associated radioactivity of each sample at various time points (5–30 min) was measured. All three of the NIS/TPO cotransduced H1299 clones had enhanced radiodiode retention capacity in addition to markedly increased 125I uptake compared with the NIS-transduced clone (31–34% radiodiode retention at 30 min after the removal of 125I in three NIS/TPO clones versus only 8% in the NIS clone; P < 0.01; Fig. 2B). The results additionally confirm our previous findings that TPO enhanced NIS-mediated radiodiode uptake and retention in NSCLC (12).

Fig. 1. Expression of NIS and TPO mRNA in the genetically modified H1299 cells by RT-PCR. M, molecular weight marker; Lanes 1 and 2, H1299 parental cells; Lanes 3 and 4, H1299 cells that were transduced with the NIS gene only; Lanes 5 and 6, H1299 cells that were transduced with both NIS and TPO genes; Lanes 1, 3, and 5, NIS expression; Lanes 2, 4, and 6, TPO expression.
Nonradioactive Iodide Potently Induces Apoptosis in the NIS/TPO-modified Lung Cancer Cells. Studies showed that iodide excess induces cytotoxicity in thyroid cells (13, 18, 19). To test whether nonradioactive iodide induces apoptosis in genetically modified lung cancer cells, H1299 NIS/TPO (clone 320X), H1299 NIS (clone 17X), and parental H1299CV clones were incubated in the presence of 30 mM KI for 48 h, and apoptosis was assessed by the APOPercentage assay method. KI induced apoptosis in 95% of H1299 NIS/TPO cells (Fig. 3A) but <1% of H1299NIS and H1299CV cells (Fig. 3, B and C) underwent apoptosis. In contrast, KCl in the same molar concentration had no significant toxic effect on H1299NIS/TPO cells (Fig. 3D), indicating that the cell death in the H1299NIS/TPO cells was not because of potassium toxicity.

NIS/TPO-modified Tumor Xenografts Markedly Increase 125I Uptake in SCID Mice. On the basis of the capacity of NIS/TPO-modified lung cancer cells to markedly increase in 125I uptake in vitro, we tested whether the H1299 xenograft tumors, which were genetically modified with both NIS and TPO genes (H1299 NIS/TPO, clone X320), NIS gene alone (H1299 NIS, clone 17X), or empty vector as controls (H1299CV) could increase 125I uptake in vivo. Three h after administration of 125I (1 μCi/mouse) by i.p. injection, tumors from each group of mice were removed, weighed, and the tumor-associated radioactivity was determined by a gamma counter. Consistent with our in vitro findings, the highest 125I uptake was observed in tumors expressing both NIS and TPO genes (P < 0.001, compared with control vector-modified tumors; Fig. 4). The NIS-modified tumors also had a significant increase in 125I uptake compared with the control vector-modified tumors (P < 0.01; Fig. 4). However, the increase in 125I uptake in the NIS-modified tumors was less than the NIS/TPO-modified tumors (P < 0.01). The results suggest that the NIS/TPO-modified tumor xenografts are capable of concentrating significantly higher levels of iodide in vivo compared with the NIS-modified tumor xenografts.
Nonradioactive Iodide Effectively Limits the Growth of NIS/TPO-modified Tumor Xenografts in SCID Mice. On the basis of our results indicating that nonradioactive iodide potently induces apoptosis in the NIS/TPO-modified lung cancer cells in vitro, we evaluated the effect of iodide on genetically modified tumor growth in a murine model. Five × 10⁶ of H1299 cells modified with both NIS and TPO genes (H1299NIS/TPO, clones X164 and X320 in duplicate experiments) or empty vectors as controls (H1299CV) were implanted via s.c. injection in the right suprascapular region in SCID mice. When the tumors were ~5 mm in diameter, KI (5 mg/mouse/day) was administered five times per week by i.p. injection. The potential adverse effects of KI were monitored by daily observation of the mice. No significant adverse effects were noted in the mice receiving KI except for minor skin irritation at the injection site. Tumor growth was monitored and documented twice a week. After treatment with KI, a marked reduction of tumor volume was evident only in mice bearing the NIS/TPO-modified tumors (Fig. 5A). KI had no effect on tumor volume in mice bearing empty vector-modified tumor xenografts, indicating the critical roles of NIS and TPO genes. Mice were euthanized at day 26 of KI administration, and tumors from each group were isolated, weighed, and photographed (Fig. 5B). Whereas no statistical differences in tumor weights were noted in the H1299CV (mean = 0.9788 ± 0.1798 g), H1299CV+KI (mean = 0.9504 ± 0.2785 g), and H1299NIS/TPO (mean = 1.2829 ± 0.2220 g) groups, KI treatment markedly reduced tumor weight in mice bearing H1299NIS/TPO tumors (mean = 0.4031 ± 0.1482g; P < 0.001). The results indicate that nonradioactive iodide effectively limits the growth of NIS/TPO-modified tumor xenografts in SCID mice.

Apoptosis Plays an Important Role in the Iodide-mediated Antitumor Effect in Vivo. To quantify apoptotic events in paraffin-embedded tumor sections, we used a Klenow-FragEL DNA fragmentation detection method to determine apoptotic index. The tumor apoptotic index was expressed as percentage of brown-stained squares divided by total squares examined. Using this method, a higher apoptotic index indicates more extensive apoptosis in tumors examined. The apoptotic index for untreated H1299NIS/TPO tumors (clone 320X) was 0.15 and 0.48 after KI treatment (P < 0.001). Morphologically, the untreated H1299NIS/TPO tumor sections under low-power (×4 objective) examination revealed large tumor masses with scattered brown-stained areas (Fig. 6A). In contrast, the KI-treated mice bearing H1299NIS/TPO tumors showed extensive cell death (Fig. 6B). The corresponding brown areas in H&E sections under high-power (×20 objective) examination showed amorphous necrotic debris in the untreated H1299NIS/TPO tumors (Fig. 6C) and clearly demonstrated groups of cells undergoing apoptosis with features of nuclear and cytoplasmic condensation within the necrotic areas in the KI-treated H1299NIS/TPO tumors (Fig. 6D). The necrotic areas of the untreated H1299NIS/TPO tumor sections showed faintly stained cell “ghosts” without nuclear details (Fig. 6E). In comparison, the apoptotic cells within the necrotic areas were strongly stained in the KI-treated H1299NIS/TPO tumors (Fig. 6F).

Iodide Induces an Increase in ROS Level and Iodide-induced Apoptosis Is Sensitive to N-Acetylcysteine Inhibition. Iodide-induced apoptosis in the NIS/TPO-modified NSCLC cells was associated with DNA damage as early as 12–24 h as determined by TUNEL assays (data not shown). ROS are well known to cause oxidative damage to DNA (20–24). Vitale et al. (13) have shown that ROS level was markedly increased during KI treatment in human thyroid cells. To evaluate whether ROS were generated in the NIS/TPO-modified NSCLC cells (clone 320X) during KI treatment, cells were incubated in a range of KI concentrations (0–30 mm) for 24 h. Ten μM DCFH-DA was added to the culture and cells were incubated for 1 h before FACS analysis. The results showed that KI induced a marked increase in ROS level in a dose-dependent manner (Fig. 7, A–D). To define whether an increase in ROS level during KI treatment leads to apoptosis, the NIS/TPO-modified NSCLC cells were incubated in a range of KI concentrations (0–30 mm) in the absence or presence of N-acetylcysteine (5 μM). Preliminary studies showed that N-acetylcysteine (5 μM) is not toxic to NSCLC cells and provides optimal antioxidant effect (data not shown). After a 48-h incubation,
iodine-131 therapy is the standard treatment for well-differentiated thyroid cancers that express the NIS gene (26). Endogenous NIS expression or induced expression after retinoic acid stimulation may mediate effective radiiodide therapy in breast cancer (9, 32, 33). Because the treatment is very effective for thyroid cancer, Mandell et al. (28) described iodine-131 uptake in normal thyroid tissue and tumors. However, iodine-131 therapy is rarely used in lung cancer due to the lack of NIS expression in lung cancer cells. Our data demonstrated that iodine-131 therapy is effective in iodine-resistant lung cancer cells.

The iodide-mediated antitumor effect in vivo was also demonstrated by the decreased tumor growth rate in treated animals. The results showed that N-acetylcysteine potently inhibits iodine-induced apoptosis (Fig. 7E, *, P < 0.01).

**Iodide-induced Apoptosis Is Associated with Overexpression of CDKN1A and Down-Regulation of Survivin.** To begin to identify potential pathways involved in iodine-induced apoptosis in the NIS/TPO-modified lung cancer cells, we performed gene array analysis in the NIS/TPO-modified NSCLC cells after a 24-h KI treatment. Among the genes studied, overexpression of CDKN1A and down-regulation of survivin were associated with iodine-induced apoptosis in the NIS/TPO-modified NSCLC cells. The expression levels of other apoptosis-associated genes, such as p53, Bcl-2, caspases, and ataxia telangiectasia mutated (ATM) pathway genes were not modified. In addition, death ligands and receptors, such as TNF ligand, TNF receptor, TNF-associated factor, death domain, and death effector domain families were not altered. Other genes associated with apoptosis signaling, DNA damage and repair, other members of the growth arrest/senescence family, or other members of the inhibitors of apoptosis family did not show significant changes in expression levels (data not shown).

**Discussion**

NIS expression mediates the therapeutic efficacy of radioiodide in thyroid diseases (25, 26). Endogenous NIS expression has not been found in normal lung tissue or lung cancer (27). Transfection of the NIS gene into a variety of malignancies, including melanoma, colon carcinoma, ovarian adenocarcinoma, and lung and prostate cancers, confers radioiodide uptake (28–30). Introduction of the TPO gene alone into human anaplastic thyroid carcinoma cells provided no significant increase in iodide uptake, although the TPO expression in the genetically modified cell line was 1800-fold above the background levels (31).

**Fig. 6.** Apoptosis plays an important role in the iodide-mediated antitumor effect in vivo. The apoptotic/necrotic cells stain brown, whereas healthy living cells remain unstained with the Klenow-FragEL method. A, untreated H1299-NIS/TPO tumors showed scattered brown-stained areas (Klenow-FragEL, original magnification ×40); B, H1299-NIS/TPO tumors with KI treatment showed extensive cell death (Klenow-FragEL, original magnification ×40); C, untreated H1299-NIS/TPO tumors showed amorphous necrotic debris (H&E, original magnification ×200); D, H1299-NIS/TPO tumors with KI treatment clearly demonstrated groups of cells undergoing apoptosis with features of nuclear and cytoplasmic condensation within the necrotic areas (H&E, original magnification ×200); E, necrotic debris was stained faintly in untreated H1299 tumors, whereas healthy living cells remain unstained. The apoptotic/necrotic cells stain brown-stained scattered areas (Klenow-FragEL, original magnification ×40); F, apoptotic cells were strongly stained in H1299-NIS/TPO tumor sections with KI treatment (Klenow-FragEL, original magnification ×200).

apoptosis was quantified by measuring the amount of released NMP in the culture supernatants from apoptotic cells by NMP assays. The results showed that N-acetylcysteine potently inhibits iodine-induced apoptosis (Fig. 7E, *, P < 0.01).

**Fig. 7.** Iodide induces an increase in ROS level and iodide-induced apoptosis is sensitive to N-acetylcysteine inhibition. The NIS/TPO-modified NSCLC cells were treated with variable concentration of KI (A, diluent only; B, 10 m M; C, 20 m M; D, 30 m M) for 24 h. Cells were then incubated with DCFH-DA for 1 h and analyzed by FACScan. The green peaks (–) show the background level of mean fluorescence in cells receiving diluent only. The purple peaks (–) demonstrate a progressive shift of mean fluorescence intensity to the high end in cells when KI supplement is increased. The purple peak overlaps the green peak when KI concentration is reduced to 5 m M (data not shown). E, the NIS/TPO-modified NSCLC cells were treated with variable concentration of KI (0–30 m M) in the absence or presence of N-acetylcysteine (5 m M) for 48 h. The culture medium from each sample was collected for NMP ELISA. *, P < 0.01; bars, ±SD.
initially proposed NIS-based radioisotope concentrator gene therapy for extrathyroidal malignancies. We reported previously that the peak 125I uptake occurred rapidly, and cells maintained a high steady-state intracellular concentration provided that radioiodide was present in the medium (12). However, if radioiodide was removed from the medium, the cells displayed a rapid loss of iodide, suggesting that there was a pronounced egress of radioiodide. This led us to conclude that the cytotoxic efficacy of NIS gene transfer may be limited by radioiodide efflux in lung cancer cells and that enhancing the intracellular retention of radioiodide may confer a therapeutic advantage.

We developed a strategy to introduce the NIS gene into tumor cells to promote radioiodide uptake and the TPO gene to enhance retention of radioiodide by promoting its organification (12). Our rationale for choosing the NIS and TPO genes is that intracellular concentration of iodide in the normal thyroid is determined by a balance between NIS-mediated iodide influx and TPO-promoted iodine organification (25, 26, 34–36). An increase in radiodinated intracellular proteins that was sensitive to methimazole inhibition in the NIS/TPO cotransfected NSCLC cells correlated with an improved radioiodide uptake and retention, suggesting that an increase in intracellular 125I concentration was attributable to TPO-mediated organification (12). Because the substrates for TPO in NSCLC may not be the same as those in the thyroid cells, TPO presumably uses other tyrosine-rich protein substrates to achieve efficient iodination of intracellular proteins in the NIS/TPO-transfected lung cancer cells. Furthermore, the therapeutic efficacy of radioiodide incorporation may not be affected in other cancers such as breast cancer, because the efflux of radioiodide is relatively slow (32). Results in the current study from single clones that stably express NIS or both NIS and TPO genes by retroviral transduction additionally confirm our previous findings (12).

We demonstrated previously radiation-induced apoptosis in lung cancer cells using the NIS/TPO system in vitro (12). Although this novel strategy may have therapeutic potential for locally advanced solid tumor, radiation-induced toxicity to normal tissues could limit its clinical application. Consistent with previous studies in thyroid cells (13, 18, 19), our results indicate that nonradioactive iodide potently induces apoptosis in the NIS/TPO-modified lung cancer cells in vitro and in vivo. Hao et al. (37) have shown that administration of nonradioactive iodide after radioactive iodide therapy in patients with Graves’ disease is safe and effective. On the basis of these findings, we determined whether the growth of the NIS/TPO-modified human tumors could be controlled by the administration of nonradioactive iodide in vivo. Results in the current study show that NIS/TPO-modified lung cancer cells in vitro and tumor xenografts in vivo significantly concentrate therapeutic levels of nonradioactive iodide resulting in enhanced apoptosis.

We repeated apoptosis studies using different methods including flow cytometry (data not shown), TUNEL assays (Fig. 6) and NMP assays (Fig. 7), and confirmed the above results. Furthermore, we performed apoptosis studies in different NIS/TPO-modified NSCLC cell lines and different clones from the same cell line with variable levels of 125I uptake. Nonradioactive iodide-induced apoptosis was directly related to the level of 125I uptake without significant cell line preference in NSCLC (data not shown). Whereas clones in the low or moderate 125I uptake categories (an increase in 125I uptake <10 times or 10–50 times) did not show significant apoptosis, all of the clones in the high 125I uptake category (an increase in 125I uptake >100 times) underwent marked apoptosis after a 48-h incubation in KI (Fig. 3). Clones in the moderately high category (an increase in 125I uptake 50–100 times) showed statistically significant apoptosis in vitro compared with the controls (data not shown). The efficacy of nonradioactive iodide-induced tumor volume reduction in vivo in the moderately high category has not been determined and requires additional investigation. In addition, kinetic studies of nonradioactive iodide-induced apoptosis in the NIS/TPO-modified lung cancer cells (clones 164X and 320X) showed a slight but statistically significant increase in apoptosis that was evident as early as 12 h after KI supplement and reached a maximal level at 48–72 h (95–100% cell death; data not shown).

Iodide administration is associated with a variety of potential complications. The most prominent side effect of iodide is salivary gland inflammation (38, 39). We obtained salivary gland specimens from mice with or without KI treatment. No acute or chronic inflammation was noted in the histological sections from the KI-treated group compared with the controls (data not shown). Another reported effect of iodide is stimulation of autonomous thyroid hormone production (Jod-baslow syndrome; Ref. 40). Most susceptible patients could be identified by screening for underline nodular thyroid disease. Finally, a “down-regulation” of iodide-uptake is seen in the thyroid after iodide exposure (Wolff-Chaikoff effect). This was shown recently to be related to down-regulation of NIS expression (41). NIS and TPO expression in genetically modified tumors is autonomous and under the control of their own promoters, independent of plasma iodide levels.

Thyroid toxicity of iodide excess has been demonstrated previously in vitro and in vivo (13, 19, 42). The potential mechanisms of iodide-induced apoptosis in thyroid cells were proposed in connection to the overproduction of ROS by oxidation of excess intracellular iodide (13). Oxidative stress occurs in cells when the generation of ROS overwhelms the natural antioxidant defenses of the cell. There is a growing consensus that oxidative stress and the antioxidant state of a cell plays a pivotal role in regulating apoptosis (20–24). Consistent with previous observation by others in thyroid cells (13), iodide markedly induced ROS in a dose-dependent manner in the NIS/TPO modified NSCLC cells (Fig. 7, A–D). Antioxidants such as N-acetyl-


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