Systemic Retinoic Acid Treatment Induces Sodium/Iodide Symporter Expression and Radioiodide Uptake in Mouse Breast Cancer Models

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ABSTRACT

Lactating breast tissue and some breast cancers express the sodium/iodide symporter (NIS) and concentrate iodide. We recently demonstrated that all-trans retinoic acid (tRA) induces both NIS gene expression and iodide accumulation in vitro in well-differentiated human breast cancer cells (MCF-7). In the present study, we investigated the in vivo efficacy and specificity of tRA-stimulated iodide accumulation in mouse breast cancer models. Immunodeficient mice with MCF-7 xenograft tumors were treated with systemic tRA for 5 days. Iodide accumulation in the xenograft tumors was markedly increased, ~15-fold greater than levels without treatment, and the effects were tRA dose dependent. Iodide accumulation in other organs was not significantly influenced by tRA treatment. Significant induction of NIS mRNA and protein in the xenograft tumors was observed after tRA treatment. Iodide accumulation and NIS mRNA expression were also selectively induced in breast cancer tissues in transgenic mice expressing the oncogene, polyoma virus middle T antigen. These data demonstrate selective induction of functional NIS in breast cancer by tRA. Treatment with short-term systemic retinoic acid, followed by radioiodide administration, is a potential tool in the diagnosis and treatment of some differentiated breast cancer.

INTRODUCTION

Lactating breast tissue and some breast cancers express the sodium/iodide symporter (NIS) and concentrate iodide (1–3). In the lactating mammary gland, NIS is expressed on the basolateral membrane (1, 2), and iodide is concentrated 6–15-fold in milk, relative to the plasma iodide concentration (4). Iodide uptake and NIS expression in the mammary gland is induced by oxytocin (1, 2), and the induction is enhanced by prolactin and estrogen (1). Iodide trapped in mammary glands is secreted into breast milk and used by the developing neonate for thyroid hormone synthesis. NIS is also expressed on the basolateral membrane of thyrocytes and is responsible for the ability of the thyroid gland to concentrate iodide. In the thyroid, thyrotropin (TSH) is the major regulator of iodide accumulation and stimulates iodide uptake as well as NIS gene expression (5–8). TSH, however, does not influence NIS expression in extrathyroidal tissues such as mammary gland, salivary gland, and gastric mucosa (reviewed in Ref. 9).

Radioiodide is commonly used in the treatment of differentiated thyroid cancer after thyroidectomy. Thyroid cancer, however, has reduced NIS activity compared with normal thyroid and requires high levels of TSH to maximally stimulate radioiodide uptake. This is accomplished in athyreotic patients by withdrawal of thyroid hormone supplement for several weeks or administration of recombinant TSH (10). After TSH stimulation of NIS, thyroid cancer is effectively imaged by 123I or 131I, and treatment with 131I reduces or eliminates metastatic disease in most cases.

NIS gene therapy has been used in a variety of animal models (9, 11–14). In a mouse prostate cancer model modified to express NIS, a reduction of the tumor volume (≥84%) was achieved after a bolus injection of 131I (13). Selective iodide accumulation by NIS after systemic radioiodine, therefore, was sufficient to significantly reduce tumor size.

Endogenous NIS protein expression, as determined by immunohistochemistry, was recently reported in >80% of human breast cancer specimens (1). In a subsequent study, NIS mRNA expression was reported in six of seven human primary breast cancer tissues using reverse transcriptase-PCR (RT-PCR; Ref. 15). Only one of the six tumors, however, was visualized by in vivo 99mTc scintigraphic imaging, whereas no 99mTc uptake was observed in the other five tumors. The single tumor with iodide uptake had abundant NIS expression, whereas the other five had only modest NIS expression (15). Although NIS expression in breast cancer has been considered a potential therapeutic target (16, 17), the iodide concentration in breast cancer specimens is significantly lower than that in normal thyroid tissue (18). Selective stimulation of NIS expression, therefore, is likely to be important for diagnostic and therapeutic use of radioiodide in breast cancer.

We previously demonstrated that all-trans retinoic acid (tRA) induces both NIS gene expression and iodide accumulation in vitro in well-differentiated human breast cancer cells (MCF-7; Ref. 19). An in vitro clonogenic assay demonstrated selective cytotoxicity with 131I treatment after tRA stimulation of MCF-7 cells (19). A recent study confirmed significant induction of NIS with retinoid receptor ligands in some breast cancer cell lines (20). tRA also enhances prostate-specific antigen promoter-directed exogenous NIS gene expression in prostate cancer cells (21). Confirmation of these in vitro effects in an in vivo system would strongly support a role for systemic retinoid treatment in the diagnosis and treatment of breast cancer (22). In the present study, we used mouse models of breast cancer to determine whether systemic tRA treatment could selectively induce NIS-mediated iodide uptake in breast cancer tissue.

MATERIALS AND METHODS

Cells and Tissues. Three lots of MCF-7 cells (lot nos. F-15100, 1566199, and 205623) and two lots of MDA-MB 231 cells (lot nos. 1224699 and 1227150) were purchased from American Type Culture Collection (Manassas, VA), and maintained as recommended. Thyroid tissue from colloid goiter patients was obtained after thyroidectomy and used as a control. Serum TSH levels of all patients were normal (1.6–3.0 microunit/ml). The use of human surgical material was approved by the institutional human subjects protection committee.

Iodide Uptake in Vitro. Iodide accumulation in cultured cells was measured with 20 mCi/mmol Na125I as described previously (19). Briefly, cells were grown in 24-well dishes, washed with HBSS, and incubated for 2 h at 37°C with 500 µl of HBSS containing ~0.1 µCi carrier-free Na125I (Amer sham Biosciences, Piscataway, NJ). After incubation, the cells were washed twice with ice-cold HBSS, scraped from each well, and radioactivity measured.

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in a gamma counter. The radioactivity was normalized to the cell number at the time of the assay. Non specific 125I- binding was determined in duplicate assays in the presence of 30 μg/ml KClO4.

**Animal Models.** Xenograft tumors derived from MCF-7 cells were grown in female severe combined immunodeficient (SCID)/beige mice from Harlan Sprague Dawley (Indianapolis, IN) by s.c. injection of ~10^7 cells suspended in 0.1 ml of PBS. To stimulate growth of the tumor, the SCID/beige mice were treated with ~12 μg/day of 17β-estradiol by s.c. implantation of a time-release pellet (Innovative Research of America, Sarasota, FL). Murine mammary tumor virus-polyoma virus middle T antigen (MMTV-PyVT)-transgenic mice (23) were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were treated according to the NIH Guidelines on Animal Use and Care. The animal studies were approved by the institutional committee for the protection of animal subjects.

**Analysis of Iodide Uptake in Vivo.** When the size of the tumor reached ~8 mm in diameter, mice were treated with tRA for 0–7 days (time-release pellets; Innovative Research of America). In some experiment, ~1.8 μg/day β-thyroxine was used for 12 days to block the iodide uptake in thyroid gland. The animals were then injected i.p. with 1 μCi of carrier-free Na125I (Amer sham Biosciences) in 0.1 ml of PBS and euthanized 4 h after the injection. Tissues were harvested, weighed, and radioactivity was counted by gamma counter. Data were normalized to the weight of each tissue, and a tissue/blood or tumor/blood ratio was calculated. For imaging study, mice were euthanized 2 h after the i.v. injection of 5 μCi Na125I and exposed to an X-ray film for 8 h.

**RT-PCR Analysis.** Total RNA was isolated by RNeasy mini kit with on-column DNase I digestion (Qiagen, Valencia, CA) from tissues in euthanized mice or surgical specimen. Purified total RNA (3 μg) was reverse transcribed by using 50 units of Superscript II (Invitrogen, Carlsbad, CA) in 20 μl of reaction with oligo(dT)20 primer, and 0.3 μl of the product were used for subsequent PCR. Primers for human NIS (hNIS), mouse NIS (mNIS), human apical iodide transporter (AIT), and mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed as shown in Table 1. Primers for human retinoid receptors and human β-actin were designed as described previously (24). Primers for PyVT were designed as recommended by the Jackson Laboratory.3 PCR for hNIS was performed in 25 μl of reaction with the Expand High Fidelity PCR System (Roche) was exponentially increased with the template amount in the range of 10^-16 to 10^-13 g/reaction at 34 cycles. The initial amount of hNIS cDNA was normalized to β-actin for each reaction and expressed as NIS/β-actin ratio.

**Western Blot Analysis.** Postnuclear membrane fractions were prepared from breast cancer tissues after the animals were euthanized, and Western blot analysis was performed as previously described (19) with a polyclonal rabbit anti-hNIS antibody (26) provided by Dr. Tsukasa Saito (Yamanashi University, Yamanashi, Japan). Equal amounts of protein were applied to each lane, and a duplicate gel was stained with Coomassie blue to normalize protein loading.

**Immunocytochemistry and Immunohistochemistry.** For immunocytochemistry, cells grown on glass slides were fixed with methanol:acetone, 50:50, and stained as previously described (27) with polyclonal rabbit anti-hNIS antibody (1) provided by Drs. Orsolya Doha and Nancy Carrasco (Albert Einstein College of Medicine, Bronx, NY) at a concentration of 1:1000. For immunohistochemistry, 4–6-μm thick sections of formalin-fixed and paraffin-embedded xenograft tissues from euthanized mice were stained as previously described (27) with polyclonal rabbit anti-hNIS antibody (26) provided by Dr. T. Saito (Yamanashi University, Yamanashi, Japan) at a concentration of 1:1000. The staining procedure was performed in the automated Dako staining system (Dako, Carpinteria, CA) with Dako Envision System (Dako) to avoid nonspecific staining.

**Statistical Analysis.** Unless otherwise noted, statistical significance was determined by conducting a paired Student’s t test. Mann-Whitney U test was performed for data that was not normally distributed.

**RESULTS**

**tRA Induces In Vitro Iodide Uptake and NIS Expression in VariousLots of MCF-7 Cells.** Clonal variation in hormone response of MCF-7 cells has been previously reported (28), therefore, we tested three lots of MCF-7 cells (lot nos. F-15100, 1566199, and 205623 from American Type Culture Collection) to determine tRA induction of iodide uptake. As shown in Fig. 1A, 48 h of tRA (10^-6 M) treatment induced iodide uptake up to ~10 fold with some variation in magnitude among the lots. In contrast, two lots of MDA-MB 231 (lot nos. 1227150 and 1224699), an estrogen receptor-negative breast cancer cell line, showed no response to tRA treatment (Fig. 1A). Our immunocytochemical staining with anti-hNIS antibody indicated faint staining in MCF-7 cells without tRA treatment (Fig. 1B), whereas the staining was dramatically increased in tRA-treated MCF-7 cells (Fig. 1C). No staining was observed in MDA-MB 231 cells (Fig. 1D). These data are consistent with the functional iodide uptake.

**tRA Induces Iodide Uptake in MCF-7 Xenograft Tumors.** To evaluate selective tRA induction of iodide accumulation in breast cancer tissue in vivo, we developed xenograft tumor models from the three lots of MCF-7 cells (lot no. F-15100 is shown in Fig. 2A). When

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3 Internet address: http://jaxmice.jax.org.
5 T. Kogai and G. A Brent, unpublished data.

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**Table 1** The reverse transcriptase-PCR primer sequences for NIS, PDS, AIT, and GAPDH genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank accession no.</th>
<th>Forward/reverse primer sequence (5′→3′)</th>
</tr>
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<tbody>
<tr>
<td>hNIS, nt 324 to 1838</td>
<td>NM_000453</td>
<td>TCTTCTACCGCCTGGGCCT/ACAGACGATCCTCATTGGTGGGC</td>
</tr>
<tr>
<td>hNIS, nt 324 to 1012</td>
<td>NM_000453</td>
<td>TCTTCTACCGCCTGGGCCT/AGGCAGATCTTCGAAGATGT</td>
</tr>
<tr>
<td>hNIS, nt 773 to 1012</td>
<td>NM_000453</td>
<td>ATGTATGGCGTGAACCAGGC/AGGCAGATCTTCGAAGATGT</td>
</tr>
<tr>
<td>hPDS, nt 1463 to 1901</td>
<td>NM_000441</td>
<td>TGTCATTCAATCGGCTGGA/ATTTCCTGTGGTATCGGAA</td>
</tr>
<tr>
<td>hAIT, nt 767 to 1836</td>
<td>AY081220</td>
<td>TCTCAGGTCGTCACAACCC/AGGCTCTCCAAACAGGATGCTATGC</td>
</tr>
<tr>
<td>mNIS, nt 590 to 1515</td>
<td>NM_053246</td>
<td>TAATGCTCGTCGGCTTCTGGGTG/AGAACTGGGGATCCCTCTGGAAGTG</td>
</tr>
<tr>
<td>Mouse GAPDH</td>
<td>NM_000804</td>
<td>AGCAACTCCACTTCTCA/CCGTGTCCTGAGCCGTGAT</td>
</tr>
</tbody>
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the tumors grew to ≥8 mm in diameter, the animals were treated with 160 mg/kg/day tRA by using s.c. time-release pellets for a period of up to 7 days, and iodide accumulation was evaluated 4 h after i.p. injection of 1 μCi Na125I. Iodide uptake in xenograft tumors before the tRA treatment (tumor/blood ratio = 0.37 ± 0.23, Fig. 2B) was not significantly different from that in the liver (tissue/blood ratio = 0.34 ± 0.18) without endogenous NIS gene expression (3, 7, 9), suggesting no active iodide uptake in the tumor without treatment. Significantly increased iodide uptake in the tumors was detected after 3 days of tRA treatment (tumor/blood ratio = 1.13 ± 0.47, 3.08 ± 1.27-fold greater than without treatment), and maximum induction was seen after 5 days of treatment (tumor/blood ratio = 5.31 ± 2.01, 14.8 ± 5.7-fold greater than without treatment; Fig. 2B). The uptake induced by tRA was significantly inhibited (~82%) by injection of NaClO4 (2 mg/mouse), a specific inhibitor of NIS-mediated iodide transport (29). The uptake in the tumor at day 5 was increased in a dose-dependent manner (Fig. 2C). Although clonal variation of MCF-7 cells has been reported (28), the magnitude of induction was not significantly different in tumors from the three lots of MCF-7 cells (data not shown). As shown in Table 2, iodide uptake in various organs, other than the xenograft tumors, was not significantly influenced by 5 days of tRA treatment.

Autoradiographic imaging of the tRA treated mice clearly displayed the accumulation of radioiodide in the tumors 2 h after the i.v. injection of 125I, whereas the tumors in the mice without tRA treatment were not visualized (Fig. 3). Normal mammary glands were not
Table 2. Iodide uptake in tumors and tissue of MCF-7 xenograft model mice

<table>
<thead>
<tr>
<th></th>
<th>Iodide uptake (ratio relative to blood)</th>
<th>Average ratio</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>tRA -</td>
<td>tRA +</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.48 ± 0.13</td>
<td>5.22 ± 1.48*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.41 ± 0.11</td>
<td>0.37 ± 0.24</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.47 ± 0.31</td>
<td>0.31 ± 0.24</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.29 ± 0.15</td>
<td>0.29 ± 0.19</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.39 ± 0.21</td>
<td>0.42 ± 0.31</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.08 ± 0.80</td>
<td>3.08 ± 0.98</td>
</tr>
</tbody>
</table>

* P = 0.007, when compared with tumors from the untreated group.

visualized by 125I, even after the tRA treatment (Fig. 3, A and C). Thyroxine (~1.8 μg/day) significantly diminished thyroid gland iodide uptake (>80%), as previously described (14), but did not significantly change the uptake in the xenograft tumors (data not shown). These data indicate selective induction of iodide accumulation in MCF-7 xenograft tumors by systemic tRA treatment. Longer treatment of tRA (~5 days) was required for the induction of iodide uptake in the xenograft tumor model compared with in vitro (48 h; Fig. 1A and Ref. 19), likely because of a longer time period to achieve an effective tRA dose in the in vivo tumor model.

No significant retinoid toxicity such as hair loss, skin scaling, liver dysfunction (serum alanine transaminase value were normal), anemia, or hyperlipidemia was observed after 5 days of treatment (data not shown). Longer treatment (>10 days), however, was associated with signs of retinoid toxicity, including weight loss, hair loss, and skin scaling. These findings are consistent with previous reports of retinoid toxicity (30).

**tRA Induces NIS mRNA Expression in MCF-7 Xenograft Tumor.** Our previous *in vitro* study demonstrated that tRA stimulates NIS gene expression in MCF-7 cells (19). To determine the effect of tRA on NIS mRNA levels in the xenograft model, quantitative PCR was performed with cDNA of tRA-treated or untreated MCF-7 xenograft tumors using primers spanning exons 1–15 of NIS cDNA. Amplification plots of each PCR were obtained from the ethidium bromide-stained gels (Fig. 4A), and the initial amount of the template was estimated using regression equations ($y = a \times b^n$, $y$ is the intensity and $n$ is the number of cycle) fit to the data in the linear portion of the semilogarithmic graphs (Fig. 4B) as described previously (25). tRA treatment for 2 days markedly increased NIS mRNA expression to ~40-fold in the xenograft tumors (Fig. 4B). To eliminate the possibility of cross-reaction of our primers (spanning exons 1–15) with unknown sequence, we additionally used other primer pairs (spanning exons 1–8 and exons 6–8) and confirmed the reproducibility of the results of RT-PCR in the MCF-7 xenograft tumors (data not shown). These results were also consistent with Northern blot analysis with hNIS cRNA probe (data not shown). Time course studies showed that the maximum level of NIS mRNA expression in the xenograft tumor was at day 2 of the tRA treatment, followed by reduction in levels at days 3 and 4 (Fig. 5).

**NIS Protein Induction by tRA in MCF-7 Xenograft Tumor.** Our time course study of tRA treatment indicated maximum induction of iodide accumulation at day 5, whereas NIS mRNA was maximal at day 2 and reduced in the next 2 days. To confirm the tRA induction of NIS protein along with the maximal induction of iodide uptake, we performed Western blot analysis of postnuclear membrane fraction from the xenograft tumors with anti-NIS antibody (26). A weak band of $M_r \sim 68,000$ was observed before the tRA treatment (Fig. 6A), consistent with our previous *in vitro* data (19). NIS protein in the membrane fraction was markedly increased (8.18 ± 2.39-fold before the induction) after 5 days treatment of tRA (Fig. 6, A and B).

In thyroid follicular cells, TSH up-regulates NIS expression at the transcriptional (5, 7, 8) and posttranslational levels (31), and the protein half-life is 3–5 days. NIS is predominantly distributed on the plasma membrane under TSH stimulation, whereas removal of TSH results in redistribution of NIS to the intracellular space (31). Recent immunohistochemical studies have shown that the predominant distribution of NIS in breast cancer tissue is in the intracellular space (1,
32). To evaluate the effect of tRA on the NIS protein distribution in breast cancer tissue, we performed immunohistochemistry with an anti-hNIS antibody (26) in the MCF-7 xenograft tumors. Positive staining of NIS was observed in the xenograft tissue (Fig. 6C), and 5 days of treatment with tRA substantially increased the staining (Fig. 6D), consistent with the in vitro data (Fig. 1C). The NIS-positive tumor cells had predominantly intracellular staining, consistent with previous data (1, 32), with some immunoreactivity of NIS at the plasma membrane (Fig. 6E, indicated by arrow). Although tRA induced NIS protein at the membrane, no significant effect of tRA on intracellular NIS distribution was demonstrated.

Expression of Other Iodide Transporters in MCF-7 Xenograft Tumors. The thyroid gland expresses several iodide transporters in addition to NIS, including pendrin, apical iodide transporter, and a predicted iodide-permeable chloride channel (9). Those transporters are expressed on the apical membrane of thyrocytes and are responsible for transport of iodide from thyrocytes into the follicular lumen (33, 34). Recent in vitro studies, however, revealed that excess expression of those transporters and/or excess concentration of iodide outside of the cells give these transporters the ability to promote iodide influx in monolayer cells (33, 34). To assess if the expression of these iodide transporters is induced by tRA in the MCF-7 xenograft tumor, we determined the level of PDS, whose product is pendrin, and apical iodide transporter mRNA by RT-PCR. The MCF-7 xenograft expressed both PDS and apical iodide transporter mRNA; however, the expression levels were significantly lower than that in human colloid goiter tissue, and tRA did not significantly change the expression levels in the xenograft tumors (data not shown).

Retinoic Acid Receptor (RAR) Expression in MCF-7 Xenograft Tumors. Our studies with retinoid agonists indicate that the stimulation of the NIS gene up-regulation is primarily mediated by RAR (19). The tRA-RAR complex likely recruits retinoid X receptor (RXR) and other cofactors and binds to a cis-element in the regulatory regions of the retinoic acid-responsive gene (35, 36).

NIS mRNA level and iodide uptake was reduced with long-term tRA treatment. To explore the mechanisms responsible for the decline of NIS mRNA, we determined mRNA levels of RAR and RXR.
INDUCTION OF IODIDE TRANSPORTER IN BREAST CANCER MODELS

Fig. 7. Expression profile of retinoid receptors in the MCF-7 xenograft tumor treated with all-trans retinoic acid (tRA). Reverse transcriptase–PCR was performed with total RNA from the tumors of mice treated with or without 160 mg/kg/day tRA for 2 days. Taq polymerase was used for the PCR. A, RT-PCR for retinoic acid receptors (RARs). Quantitative analysis of the fluorescence intensity indicated that those reactions were in exponential phase up to 40 cycles (data not shown). B, RT-PCR for retinoic X receptors (RXRs) and β-actin. Reactions were stopped at 37 cycles for RXRs and 25 cycles for β-actin. C, quantitative analysis of the RT-PCR for RARs. The fluorescence intensity of the corresponding RAR mRNA bands was quantitated and normalized by the intensity of β-actin. Values are mean ± SD (n = 3), *P = 0.02 and **P = 0.04, when compared with the untreated group (for RARα and β).

subtypes in MCF-7 xenograft tumors with or without 2 days of tRA treatment. Abundant expression of RARα, γ, and RXRα was observed before tRA treatment (Fig. 7, A and B), consistent with a previous study (37), as well as modest expression of RARβ and RXRβ. The expression of RARα was significantly decreased by the tRA treatment (to levels ~18% of those before the treatment; Fig. 7C). In contrast, RXRβ mRNA was significantly increased (~3.1-fold) by the treatment (Fig. 7C). The levels of RXRα and β were not significantly changed by the treatment (Fig. 7B). Because our preliminary study with retinoid analogues showed that RARα was likely one of the critical isoforms for NIS induction in MCF-7 cells in vitro,3 the reduction of NIS mRNA expression may be attributable to the selective reduction of RARα.

NIS Induction by tRA in a Transgenic Mouse Model of Breast Cancer. To determine whether the tRA induction of iodide accumulation in breast cancer tissue is limited to the MCF-7 model, we used a transgenic mouse model of breast cancer, MMTV-PyVT in which the expression of the PyVT is controlled by the MMTV, mammary gland-specific promoter. The mice spontaneously develop multiple breast adenocarcinomas by 10 weeks of age as described previously (23). Overexpression of the transgene, PyVT, was confirmed by RT-PCR in the tumors (Fig. 8A). RT-PCR demonstrated NIS mRNA expression in those tumors (Fig. 8B, Lanes 1–3), and tRA significantly increased the NIS mRNA levels (Fig. 8B, Lanes 4–6, ~3.5-fold). No NIS mRNA signal was detected in the spleen with up to 36 cycles of PCR (data not shown), in agreement with previous studies showing reduced or absent NIS expression in spleen (3, 38). Breast tumors in that model accumulated radioidide (~1.84-fold over the level of the blood), and tRA treatment for 5 days significantly enhanced the uptake (~1.8-fold, Fig. 8C). Iodide uptake in other organs such as liver, spleen, muscle, and stomach was not significantly influenced by the tRA treatment (data not shown). These results indicate tRA induction of NIS expression in the transgenic breast cancer model mice as well as the MCF-7 xenograft model, although at a lower magnitude.

DISCUSSION

We have demonstrated that systemic tRA treatment specifically increases iodide uptake in three different lots of MCF-7 breast cancer cells grown as xenografts in mice and breast cancer tissues in MMTV-PyVT-transgenic mice. tRA increased NIS mRNA and protein expression but did not influence PDS or apical iodide transporter gene expression, and the induced uptake was blocked by NaClO4 [an inhibitor for NIS function (29)]. On the basis of these observations, we conclude that the induced iodide uptake was mediated by NIS.

Our time course study of tRA treatment in the MCF-7 xenograft model indicated NIS mRNA reached a maximum level at day 2, whereas iodide uptake was maximal at day 5, with abundant expression of NIS protein. This is consistent with the long half-life of NIS (>3 days) in rat thyroid cells (31). NIS is glycosylated in lactating mammary glands (1, 2) as well as in thyroid cells (26, 39). Our Western blot analysis showed NIS as a Mr ~68,000 protein in the xenografts, larger than the deglycosylated form (26, 39), suggesting some glycosylation. NIS is also phosphorylated in thyroid cells, and the importance of its phosphorylation was proposed for targeting to the plasma membrane (31). The time lag between mRNA and iodide uptake, therefore, may be attributable to the processing of NIS such as glycosylation and phosphorylation and/or targeting to the membrane. A recent clinical study showed that iodide uptake in the thyroid was higher 3 days after recombinant TSH compared with patients studied after 1 day (40).

A significant reduction of iodide uptake was observed after the maximum induction by tRA in our xenograft model. MCF-7 cells stably transfected with NIS cDNA retain the ability to concentrate iodide for several months (11), indicating that constant NIS gene expression is sufficient for constitutive iodide accumulation. The reduction of iodide uptake in our xenograft model, therefore, is likely...
because of the reduction of NIS mRNA expression observed after the maximal expression. Our RT-PCR indicated significant down-regulation of RARα, which is likely involved in the stimulation of NIS. The inhibition of RARα mRNA by tRA is consistent with reports in different cell lines and tissues (41–43). In addition, tRA induces degradation of RAR α and γ in MCF-7 cells in vitro by ubiquitination (44).

The induction of NIS in breast cancer tissues in our models required a relatively large dose of tRA. The daily dose for the maximum NIS induction in breast cancer tissues was ~17-fold of the maximum tolerable dose in human for solid tumor patients on a mg/m² basis (according to Ref. 45), although the treatment for 5 days did not cause detectable toxic effects in our mice. A pharmacokinetic study with pregnant mice given a single oral dose of 10 mg/kg, a quarter of the dose required for significant NIS induction, showed that plasma levels of tRA reached a peak ~4.5 μg/ml in 1 h, followed by quick elimination in 8 h (46). Daily bolus doses of tRA do not keep plasma tRA levels constant because of the instability of tRA in plasma. Indeed, our preliminary studies indicated wide variability of NIS induction by daily i.p. injection of tRA (data not shown). On the other hand, relatively high stability of tRA in culture medium with serum has been reported. The addition of serum stabilizes the tRA and >80% remains at 72 h (47). Our previous in vitro study has shown that 10⁻⁶ M (0.3 μg/ml) tRA in the medium, >10 times lower than the peak plasma concentration in the mice with 10 mg/kg oral dose, are enough to induce the maximum NIS expression in MCF-7 cells (19). A continuous stimulation, which was achieved by time-release pellets in our study, is likely important for reproducible in vivo NIS induction in breast tumor. In addition, the high metabolic rate of tRA in MCF-7 cells, previously reported (48), may be involved in the requirement of high doses. Retinoid analogues with greater in vivo stability, less toxicity, and preferably without down-regulation of RARα, may be more efficacious for NIS stimulation.

A significant concern is whether the fraction radiiodiode concentrated in the tumors and the retention time is adequate for a therapeutic effect. Spitzweg et al. (13) reported that intratumoral injection of adenovirus vector expressing NIS under the control of cytomegalovirus promoter, followed by administration of ¹³¹I, significantly reduced the tumor volume, >80%, in a prostate cancer xenograft model. The adenovirus vector they used allows transient expression of NIS so that the iodide uptake in vitro after the infection of the vector with NIS is reduced >50% in 2 days after the maximum induction (13), similar to our data in MCF-7 xenografts stimulated by tRA. In our study, the maximum fraction of radiiodiode uptake we achieved with systemic tRA (~18% of the total radiiodiode injected) is in the range of fraction (20.2 ± 11.4%) that achieved in the NIS-transfected prostate cancer xenografts, which was sufficient to reduce tumor volume (13).

A biological half-life of ¹³¹I in the NIS-transfected prostate cancer xenografts was ~5.6 h (13), whereas our preliminary data of biological half-life of ¹²⁵I in the MCF-7 xenografts was ~4 h,⁵ consistent with a previous data in a rat NIS-transfected MCF-7 xenograft model (11). The fraction of radiiodiode taken up by thyroid cancers is often only 0.5–1% of the ingested dose, but it is still therapeutically effective. Use of radiiodiode for ablation of normal thyroid tissue, as well as thyroid cancer, demonstrates a prolonged effect of tissue damage over months, well after radioactivity is undetectable.

Radioiodiode therapy for thyroid cancer is generally performed after total thyroidectomy. Elevated serum TSH, either from thyroid hormone withdrawal or recombinant TSH administration, is required to enhance iodide uptake in target cancer tissue. TSH preferentially induces iodide uptake in normal thyroid tissue. In contrast, we have demonstrated that tRA markedly induced iodide uptake in breast cancer tissues but not in normal mammary gland or other organs. Thyroxine supplements blocked iodide uptake in thyroid gland but not in breast cancer. Systemic treatment with tRA, therefore, has a potential for targeted breast cancer therapy with radioiodiode. Low iodine diet, in combination with the thyroxine, may additionally enhance the tumor uptake of radioiodiode in addition to the tRA treatment. Previous studies showed that tRA increases radiosensitivity in breast and squamous cell carcinomas (49, 50), suggesting an additional advantage of tRA treatment before radioiodiode therapy.

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INDUCTION OF IODIDE TRANSPORTER IN BREAST CANCER MODELS


Systemic Retinoic Acid Treatment Induces Sodium/Iodide Symporter Expression and Radioiodide Uptake in Mouse Breast Cancer Models

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