Activation of the PI3 Kinase Pathway By Retinoic Acid Mediates Sodium/Iodide Symporter Induction and Iodide Transport in MCF-7 Breast Cancer Cells

Emi Ohashi, Takahiko Kogai, Hiroyuki Kagechika, and Gregory A. Brent

Molecular Endocrinology Laboratory, VA Greater Los Angeles Healthcare System, Departments of Medicine and Physiology, David Geffen School of Medicine at University of California at Los Angeles, Los Angeles, California and 1School of Biomedical Science, Tokyo Medical and Dental University, Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan

Abstract

Iodide uptake in the thyroid and breast is mediated by the sodium/iodide symporter (NIS). NIS activation is used for radioiodide imaging and therapeutic ablative therapy of thyroid carcinoma. NIS is expressed in >70% of breast cancers but at a level insufficient for radioiodine treatment. All-trans retinoic acid (tRA) induces NIS gene expression and functional iodide uptake in human breast cancer cell lines and mouse breast cancer models. tRA usually regulates gene expression by direct interaction of RA receptor (RAR) with a target gene, but it can also act through nongenomic pathways. We report a direct influence of tRA treatment on the phosphoinositide 3-kinase (PI3K) signal transduction pathway that mediates tRA-induced NIS expression in MCF-7 breast cancer cells. MCF-7 cells express all three RAR isoforms, α, β, and γ, and RXRα. We previously identified RARβ2 and RXRα as important for NIS induction by tRA. Treatment with LY294002, the PI3K inhibitor, or p85α knockdown with siRNA abolished tRA-induced NIS expression. Immunoprecipitation experiments and glutathione S-transferase pull-down assay showed a direct interaction between RARβ2, RXRα, and p85α. RA also induced rapid activation of Akt in MCF-7 cells. Treatment with an Akt inhibitor or Akt knockdown with siRNA reduced NIS expression. These findings indicate that RA induction of NIS in MCF-7 cells is mediated by rapid activation of the PI3K pathway and involves direct interaction with RAR and retinoid X receptor. Defining these mechanisms should lead to methods to further enhance NIS expression, as well as retinoid targets that influence growth and differentiation of breast cancer. [Cancer Res 2009;69(8):3443–50]

Introduction

The sodium/iodide symporter (NIS) is a transmembrane glycoprotein that transports iodide into the thyroid to maintain adequate thyroid hormone production (1). NIS is expressed on the basolateral membrane of alveolar cells in lactating mammary gland, concentrating iodide in milk, but is not expressed in the nonlactating breast (2–4). It has been reported that >70% of breast cancer tissue expresses NIS, although the level of basal iodide transport is very low (3, 4). A variety of approaches have been used to enhance functional NIS expression in breast cancer, with the goal of using radioiodide to image and treat some differentiated breast cancer (5). Our previous studies have shown that all-trans retinoic acid (tRA) significantly induces NIS gene expression and iodide uptake in human breast cancer cell line and breast cancer mouse models, but the precise mechanism has not been established (6, 7).

Retinoids, active metabolites of vitamin A, comprise both naturally occurring and synthetic compounds that have been used in animal models and humans as differentiation agents for various types of cancers, including breast cancer and promyelocytic leukemia (8, 9). The classic retinoid pathway involves the ligand-activated nuclear receptors, RA receptors (RAR) and retinoid X receptors (RXR; ref. 9). Both RAR and RXR have three isoforms, α, β, and γ. RAR-RXR heterodimers bind to RA or retinoid X-response element and activate transcription (9, 10). Many hormones classically acting through nuclear receptors, however, have been shown to also act through nongenomic pathways (11, 12). It has been reported that nuclear receptors can initiate second messenger production and interact with other cellular systems (11–13). The mechanism of rapid nuclear receptor signaling, however, is not established and a variety of models have been described (12, 13). We have recently studied the human NIS gene and directly characterized 44 kb of upstream and downstream sequence but could not identify a functional RA response element (14). We used signal transduction inhibitors to show that RA induction of NIS is likely to be through a nongenomic pathway (14). Signal transduction pathways have been implicated by several studies of NIS expression in breast cancer (15, 16).

Phosphoinositide 3-kinase (PI3K)/Akt signaling pathway influences cell growth, cell survival, and cell movement, and signaling defects have been identified in a wide spectrum of human cancers (17, 18). PI3Ks are a family of enzymes and can be subdivided into three classes—class I, II, and III (19). Class IA PI3Ks are heterodimers of regulatory and catalytic subunits (18, 19). A regulatory subunit of class IA PI3Ks, p85α, is a phosphoprotein substrate of many cytoplasmic and receptor tyrosine kinases (17, 19). Members of the nuclear receptor superfamily, such as the estrogen, thyroid hormone, and RARs, have been shown to interact with p85α, and activate the PI3K/Akt signaling pathway (20–23).

We have investigated the mechanism of tRA induction of NIS. We show that rapid activation of PI3K/Akt by RA induces NIS expression in MCF-7 cells. We also show that this rapid activation of PI3K/Akt pathway is initiated by a direct interaction between p85α and RARβ2, in association with RXRα.

Materials and Methods

Cells and culture conditions. MCF-7 cells and COS-7 cells were obtained from the American Type Culture Collection and maintained...
according to the recommended conditions. In the performance of immunoprecipitation, Akt phosphorylation, and cell fractionation studies, cells were serum-starved in phenol red–free DMEM for at least 24 h, and then treated with 1 μmol/L tRA for the indicated time.

**Reagents and antibodies.** tRA and 9cRA were purchased from Sigma. LT294002 and Akt inhibitor VIII were purchased from EMD Biosciences. Antibodies for phosphorylated 5473 Akt, total Akt, β-actin, and PARP were purchased from Cell Signaling Technology. RXRα antibody was purchased from Santa Cruz Biotechnology. p85α antibody was purchased from Millipore. FLAG antibody was purchased from Sigma. NIS antibody was a gift from Dr. Nancy Carrasco (Albert Einstein College of Medicine, Bronx, NY).

**Whole blot analysis.** Whole cell lysates were prepared as described previously (21) with minor modifications. Briefly, cells were washed with ice-cold PBS and lysed with cell lysate buffer (Tris-HCl [20 mmol/L (pH 7.4)], EDTA [10 mmol/L], NaCl [100 mmol/L], Nonidet P-40 [1%], sodium deoxycholate [5%], Na2VO4 [1 mmol/L], sodium PPI [2.5 mmol/L], β-Glycerophosphate [1 mmol/L], and the protease inhibitor cocktail [Sigma]). Cells were sonicated for 20 s, centrifuged for 10 min at 14,000 g at 4°C. Supernatant was collected and 2× sample buffer was added, then incubated at 95°C for 5 min. Membrane fraction was prepared as previously described (6). Protein concentrations of the samples were measured using the Bio-Rad protein assay (Bio-Rad). Western blot was performed as described previously (24), except that the protein was visualized by ECL. Western blotting detection reagents (GE Healthcare Bio-Sciences). For the same experiment, membranes were stripped with a stripping buffer (Pierce) and immunoblotted with another antibody.

**Immunoprecipitations.** Cells were washed with ice-cold PBS and lysed with cell lysis buffer. Immunoprecipitating antibody was added to equal amounts of cell lysates (3–5 mg) in 1 mL of lysis buffer for 12 h at 4°C rotating. Then, 40 μL of 1:1 Protein-A agarose was added and rotated for another 3 h at 4°C. The immunoprecipitates were washed with an ice-cold lysis buffer for 5 times, then resuspended in a sample buffer and incubated at 95°C for 5 min. The bound proteins were analyzed by Western blot analysis. The FLAG-IP, anti-FLAG M2 affinity gel (Sigma) was used and immunoprecipitation was performed according to the manufacturer’s protocol.

**Glutathione S-transferase pull-down assay.** Glutathione S-transferase (GST) fusion protein was expressed in BL21 bacteria cells and purified using standard techniques. For the pull-down assays, bovine recombinant GST-p85α fusion protein or GST bound to glutathione-agarose beads was suspended in 500 μL of AM-1 Buffer [20 mmol/L Tris-HCl (pH 8.0), 20% Glycerol, 100 mmol/L KCl, 1 mmol/L DTT, and 0.2 mmol/L EDTA] and incubated with 1 μg human recombinant RXR (Active Motif) for 1 h at 4°C. The samples were washed 5 times with ice-cold PBS, then resuspended in a sample buffer and incubated at 95°C for 5 min. The bound proteins were analyzed by Western blotting.

**Reverse transcription-PCR and quantitative real-time PCR analysis.** Total RNA isolation, reverse transcription-PCR (RT-PCR), and real-time PCR analysis were carried out as previously described (5, 7). Sequences of PCR primer pairs for RARα, p85α, and Akt5 are shown in Supplementary Data. Primers for NIS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have been described previously (5).

**Plasmid constructs.** pcDNA3.0-RARα was subcloned from pcMX-RARα by HindIII-BamHI site of pcDNA3.0 (Invitrogen) and pSGXFLAG-14 vector (Sigma), respectively. For RARα, inserts were created by PCR amplification of cDNA obtained from the breast cancer cell line, Hs578T. cDNA was obtained as described above. Primers used for cloning are as follows: 5′-CAAGCTTACGCCCTGTTGAGGAGTGA-3′ (forward primer), 5′-ACCTGAGAAGCTTCTGGAATGGTGTAGTGA-3′ (reverse primer). TOPO-Cloning kit was used and performed according to the manufacturer’s manual. RARα construct was then subcloned into HindIII-Xhol site of pcDNA3.0. Because there was another ATG site at nucleotide 411, 37 bp 5′ of the RARα transcription start site at nucleotide 448, the ATG site at 411 was modified to TTG by site-directed mutagenesis using PCR. Primer used for mutagenesis was 5′-GAAAAAGGCCCCATACACCCCTACAGGCCCCT-3′. pcMX-RARα was a gift of Dr. Masato Kasuga (Kobe University, Kobe, Japan). pSG5-RXRα was a gift from Dr. Ronald Evans (Salk Institute, La Jolla, CA).

**Figure 1.** Influence of retinoids on NIS mRNA expression and iodide uptake in MCF-7 cells. A, response to treatment with natural and synthetic RAR/RXR ligands. MCF7 cells were incubated with each ligand at a concentration of 1 μmol/L for 24 h and iodide uptake was measured. Perchlorate (ClO4−, 30 μmol/L) is a specific inhibitor of NIS function, and was used to determine NIS-independent 125I− transport. *, statistical significance at a P value of <0.05; NS, not significant. B, pan-RAR and pan-RXR antagonists block the iodide uptake induced by tRA and 9cRA. MCF7 cells were incubated with a combination of RA (0.1 μmol/L) and an antagonist (5 μmol/L) for 24 h and iodide uptake was measured. C, effect of RAR/RXR ligands on NIS mRNA expression. MCF7 cells were incubated with each ligand at a concentration of 1 μmol/L for 12 h, and RT-qPCR of NIS and GAPDH mRNA was performed. The average ratio of NIS/GAPDH in untreated cells was set as 1.
Small interfering RNA preparation. Small interfering RNAs (siRNA) targeting RAR\(\beta\)2 gene were designed and were chemically synthesized by Dharmaco RNA Technologies. The sequences for siRNAs are as follows: GTATGGATGTCTGGCAGT and GGATTCTACACTGCGAGT. Nontargeting control siRNA (SMARTpool control) and predesigned siRNAs against RXRa, p85a, Akt1, and Akt2 (siGENOME siRNA) were also purchased from Dharmaco.

Transfection. Plasmids were transfected into MCF-7 cells and COS-7 cells with Effectene and Polyfect (Qiagen), respectively. Transfection was performed into cells at a final concentration of 100 nmol/L by using transfection reagent, DharmaFECT 1 (Dharmacon). Transfection was performed according to the manufacturer’s protocol.

Iodide uptake. The iodide uptake assay was performed as described previously (5). The uptake was normalized to the cellular protein concentration measured with Bio-Rad protein assay.

Statistical analysis. For statistical analysis, one-factor ANOVA followed by the Scheffe’s F test was used. Values were expressed as means ± SD, and \(P\) value of <0.01 was considered significant. All the experiments were performed with at least three replicates.

Results

Response to natural and synthetic retinoids. We used isoform-specific retinoid receptor ligands to characterize the isoform specificity of tRA induction of NIS expression. We have reported previously that an RAR \(\beta/\gamma\) agonist was the most potent retinoid stimulator of NIS expression (5). We extended these studies and performed iodide uptake and NIS mRNA expression with the RAR \(\alpha/\beta\) agonist, Am80, a pan-RAR agonist, Re80, and pan-RXR agonists, PA024 (Fig. 1A and C). Although a natural pan-RAR agonist, tRA, and a natural pan-RAR/RXR agonist, 9cRA, induced iodide uptake and NIS mRNA expression to the greatest level of expression, Am80 induced NIS to a similar level as Re80. This result, along with those from our previous study (5), indicates that RAR\(\beta\) plays the major role in NIS induction by tRA. In addition, we performed iodide uptake after treatment with an RAR antagonist, LE540, and an RXR antagonist, HX531. Interestingly, both LE531 and HX531 blocked tRA-stimulated iodide uptake (Fig. 1B). The RXR antagonist, HX531, is known to selectively inhibit the activity of RAR-RXR heterodimers (25). Inhibition of NIS induction by HX531 indicates that heterodimerization of RAR and RXR is required for the tRA-induction of iodide uptake.

Nuclear receptor RAR\(\beta2\) and RXRa are required for the NIS induction by tRA in MCF-7 cells. The pharmacologic studies showed that RAR\(\beta\) and RXR are important for tRA-mediated NIS induction. We further evaluated the role of RAR\(\beta\) and RXR by determining the influence of loss- and gain-of-function experiments on NIS induction. MCF-7 breast cancer cells express RXRa, \(\beta,\gamma,\) and RXRa (26, 27). RAR\(\beta\) generates multiple variants by using two promoters and alternative splicing (26). Variants of RAR\(\beta\) have been identified in human breast cancer cells: \(\beta2, \beta2S, \beta4,\) and \(\beta5\) (Fig. 2A; refs. 26, 28, 29). RAR\(\beta\) encodes the full-length isoform, whereas RAR\(\beta2S, \beta4,\) and \(\beta5\) are truncated variants of RAR\(\beta2,\) and the corresponding sequences are indicated by gray boxes.

![Figure 2](https://example.com/image2.jpg)

**Figure 2.** RAR\(\beta2\) and RXRa are required for NIS induction by tRA. A, schematic representation of RAR\(\beta\) variants expressed in MCF-7 cells. B, stable transfection \((TX)\) of RAR\(\beta2\) and \(\beta4\) expression vectors. RT-qPCR for NIS and GAPDH mRNA was performed with transfected cells treated with or without tRA (1 \(\mu\)mol/L) for 12 h (left). The average ratio of NIS/GAPDH in untreated cells was set as 1. *, statistical significance from empty vector transfected cells \((P < 0.01)\). RT-PCR confirming the expression of RAR\(\beta\) variants in stable transfected cells (right). Bottom right, the position of PCR primers used in B. RAR\(\beta2\) primers detect RAR\(\beta2\) only (left arrow below RAR\(\beta2\)), whereas RAR\(\gamma\) primers detect both \(\beta2\) and \(\beta4\) (right arrow below both RAR\(\beta2\) and \(\beta4\)). C, MCF-7 cells transfected with siRNAs against RAR\(\beta2\) and/or RXRa were treated with 1 \(\mu\)mol/L tRA for 12 h and RT-qPCR for NIS and GAPDH was performed (left). The average ratio of NIS/GAPDH in untreated cells was set as 1. Control siRNA, nontargeting siRNAs; *, statistical significance compared with control \((P < 0.01)\). Efficiency of the knockdown is shown by RT-PCR of RAR\(\beta2,\) RXRa, and \(\beta\)-actin mRNA (right).
(26, 28, 29). Two kinds of protein are translated from these variants. One is the product of RARβ2 and β2S, which include the receptor domains A to F, and the other is the product from β4 and β4S, which lack the A, B, and C domains (Fig. 2A). MCF-7 cells express β2, β2S, β4, and β5 mRNA as determined by RT-PCR (data not shown). We stably transfected MCF7 cells with vectors expressing one of the two RARβ protein product variants, either RARβ2 or β4, and performed RT-qPCR of NIS mRNA and iodide uptake (28, 29). Overexpression of RARβ2 significantly increased tRA-induction of NIS mRNA and iodide uptake (data not shown), whereas RARβ4 expression reduced both (Fig. 2B). Selective knockdown of RARβ2, as well as RXRα, significantly decreased RA-induced NIS mRNA expression in MCF-7 cells (Fig. 2C). These data indicate that tRA-induced NIS expression uses RARβ2 and RXRα, likely as heterodimers, whereas the truncated RARβ4 functions as an inhibitor.

**Activation of PI3K is required for NIS induction by tRA.** The specific inhibitor of PI3K, LY294002, blocked the induction of NIS mRNA expression by tRA in a time-dependent manner (Fig. 3A). LY294002 treatment also inhibited NIS protein expression on the membrane in a dose-dependent fashion (Fig. 3B). This is consistent with our recent study showing that LY294002 blocked the RA-induction of iodide uptake at an IC50 of 7.87 ± 1.1 μmol/L (14).

Furthermore, tRA-induction of NIS mRNA did not require de novo protein synthesis because cotreatment of tRA and cycloheximide did not influence NIS mRNA expression (data not shown). These pharmacologic data, as well as our previous studies in which a functional RA response element was not identified in the NIS gene locus or adjacent sequences (14), suggest that tRA treatment induces NIS expression in MCF-7 cells by modulating the PI3K signal transduction pathway.

We further investigated the involvement of PI3K in NIS induction by tRA, by using siRNA targeting p85α, one of the regulatory subunit of class IA PI3Ks. Knockdown of p85α significantly decreased the NIS mRNA induced by tRA compared with the control cells (Fig. 3C).

**RARβ2 and RXRα forms a complex with p85α.** To determine the mechanism of PI3K activation by tRA, we investigated whether RARβ2 and RXRα directly interact with p85α, by performing coimmunoprecipitation and pull-down assays. Immunoprecipitation experiments using COS-7 cells transfected with RARβ2, p85α, and RXRα showed a direct interaction between RARβ2 and p85α in a tRA ligand-independent manner (Fig. 4A). Conversely, a similar stable interaction between RARβ2 and p85α was observed with immunoprecipitation performed with anti-p85α antibody (Fig. 4B). These finding show direct ligand-independent binding of RARβ2.
and p85α. We then examined the interaction between RXRα and p85α in a cell-free system by performing a pull-down assay using GST-p85α fusion protein. GST-p85α fusion protein bound to the human recombinant RXRα but GST alone did not. Interestingly, both pan-RAR and -RXR antagonist inhibited the binding of RXRα to p85α (Fig. 4D). This is consistent with findings from the functional studies using pharmacologic antagonists that blocked tRA-induced NIS expression (Fig. 1B). Furthermore, using the coimmunoprecipitation assay with endogenous p85α and RXRα from cell lysates of MCF-7 cells, we found that tRA increases the association of p85α and RXRα (Fig. 4C).

Akt, downstream of PI3K pathway, is rapidly activated after treatment with tRA in MCF-7 cells. Several nuclear receptors have been shown to rapidly activate Akt after P3K activation. We determined whether tRA activates Akt in MCF-7 cells. The cells were serum starved for 24 to 48 hours before tRA treatment. Treatment with 1 μmol/L tRA rapidly increased Ser-473 phosphorylation of Akt at 5 to 10 minutes and then decreased to the basal level at 20 minutes (Fig. 5A). Treatment with an Akt inhibitor VIII, an isozyme-specific inhibitor that inhibits Akt1 and Akt2 selectively, reduced NIS mRNA induced by tRA (Fig. 5B). Combining Akt inhibition with PI3 kinase inhibition did not further reduce NIS mRNA (Fig. 5B). High concentrations of Akt inhibitor VIII (IC50, 4.5 μmol/L) inhibits tRA-induced iodide uptake in MCF-7 cells (Fig. 5C). Combination treatment of Akt1 and Akt2 siRNAs in MCF-7 cells also decreased tRA-induced NIS mRNA expression ~25%, but it was not a significant reduction (Fig. 5D). We also performed a selective knockdown of Akt1 or Akt2 separately, but there was a trend in these studies toward increased NIS mRNA induced by tRA (data not shown).

**Discussion**

A high fraction, ~70%, of breast cancers express NIS, but functional iodide concentration is rare (3, 4). Selective induction of NIS provides the potential for developing NIS-mediated radionuclide therapy as a safe and effective treatment for breast cancer. Baseline iodide transport in breast cancer, however, is insufficient and stimulation of expression is necessary. Identification of the mechanism(s) of RA induction of NIS expression is essential to determine approaches to augment functional uptake. The involvement of signal transduction pathways, including P3K, in NIS induction in breast cancer has been suggested by several previous studies (15, 16). The current study shows that liganded RAR directly
induces the rapid activation of PI3K/Akt pathway, leading to the activation of a yet unknown transcription factor, which then induces NIS mRNA expression (Fig. 6).

The activation of PI3K/Akt has been reported by other nuclear receptors, including estrogen, thyroid hormone, and glucocorticoid receptors (20–23). Recently, it has been reported that RA can activate PI3K/Akt pathway through interaction between RARα and p85α (22, 32). In this study, we have identified a novel ligand-dependent interaction between RARβ2, RXRα, and p85α. Ligand binding in MCF7 cells facilitates the association of RXRα, subsequently activating PI3K and Akt. Although we were able to observe ligand-dependent interaction of RXRα and p85α by immunoprecipitation assay, we were not able to observe the same result in GST-p85α pull-down assay. This may be due to the differences between the in vitro and in vivo conditions. The interaction between RARβ2 and p85α, however, was ligand-independent. This finding is consistent with previous reports showing the interaction of thyroid hormone receptor and RARα with p85α (20, 22). Inhibitor for PI3K and Akt, as well as siRNAs for p85α and Akt1/Akt2, decreased tRA-induced NIS mRNA expression. We have also found that Akt was rapidly phosphorylated within 5 to 10 minutes after tRA stimulation. These results indicate that the rapid activation of the PI3K/Akt pathway by tRA led to an increase in NIS mRNA expression and iodide uptake. Several studies have shown that tRA has a negative effects on PI3K activity in MCF-7 cells (33, 34). One group showed that long-term treatment with tRA induced growth inhibition of MCF-7 cells, mediated by down-regulation of IRS-1/PI3K/Akt pathway (33). The different influences of tRA on PI3K may be due to short-term rather than long-term treatment.

**Figure 5.** Rapid activation of Akt is involved in tRA induction of NIS. 
A, time-dependent effects of treatment with 1 μmol/L tRA on Ser-473 phosphorylation of Akt. MCF-7 cells were serum starved and treated with 1 μmol/L tRA for the indicated time period. Whole cell lysates (20 μg) were used for Western blot analysis. Band density was measured by ImageJ software. B, Akt inhibitor VIII or PI3K inhibitor LY294002 reduces RA induction of NIS. MCF-7 cells were incubated with 1 μmol/L tRA and the addition of Akt and/or PI3K inhibitor (10 μmol/L) for 12 h, and then RT-qPCR for NIS and GAPDH was performed. The average ratio of NIS/GAPDH in untreated cells was set as 1. *, statistical significance compared with RA-treated cells (P < 0.01). C, influence of the Akt inhibitor VIII at increasing concentration on iodide uptake in MCF7 cells (IC50 4.5 μmol/L). D, MCF-7 cells transfected with siRNAs targeted for Akt1 and Akt2 were treated with/without 1 μmol/L tRA for 12 h and RT-qPCR for NIS and GAPDH was performed (right). The average ratio of NIS/GAPDH in untreated cells was set as 1. Control siRNA, nontargeting siRNAs. Efficiency of the knockdown is shown by RT-PCR of Akt 1/Akt2 and β-actin (right).
Inhibition of tRA-induction of NIS required high concentrations of the Akt inhibitor. The IC_{50} for Akt inhibitor VIII using the in vitro kinase assay has been reported to be 58 and 210 nmol/L for Akt1 and Akt2, respectively (35). Even the high concentration of Akt inhibitor VIII, 10 μmol/L, only inhibited NIS mRNA expression by 50%. This indicates that Akt is likely not the only pathway required for the NIS induction by tRA. Indeed, our previous study using various kinds of signal transduction inhibitors showed that Janus-activated kinase, Rac, and p38 inhibitors also partially inhibited the induction of NIS by tRA (14). Akt inhibition also reduced iodide uptake, suggesting actions of Akt activation on NIS protein processing or membrane insertion. The combination knockdown of Akt1 and Akt2 decreased the NIS expression, but knockdown of each Akt1 or Akt2 did not decrease but rather increased the tRA-induced NIS mRNA expression. Blocking either Akt1 or Akt2 pathway may activate the other pathway and increase the overall Akt activation, resulting in an increase in NIS expression.

Growing evidence shows that rapid nongenomic actions of nuclear receptors are initiated outside the nucleus; however, considerable controversy about these actions remain (11, 13, 36). Transfection of a chimeric RARα-c-Src myristylation domain construct, which was targeted to plasma membrane, in mouse embryonic fibroblast-RAR(α/β)hL-C-null cells and COS-7 cells resulted in strong activation of PI3K/Akt signaling, indicating that interaction of RARα and p85α can occur at the plasma membrane (22). The interaction among RAR/β, RARα, and p85α might also take place in the cytoplasm or near the plasma membrane, although p85α is localized in both cytoplasm and nucleus. Phosphorylation of RARα in promyelocytic leukemia cells reduces transcriptional activation, and RAR/RXR phosphorylation may influence signal transduction (37). Further studies are required to determine the underlying mechanism.

Due to their role in the regulation of cell growth and differentiation in preclinical models, retinoids are being extensively evaluated in clinical trials of cancer prevention and therapy, including breast cancer (8). Retinoid receptors are expressed in normal and malignant epithelial breast cells and are critical for normal development. It has been suggested that biological effects of tRA are partially mediated through multiple signal transduction pathways. These nongenomic actions of RARβ may play an important role in indirectly regulating gene expression, not only for NIS induction but also for other tRA-regulated influences on cancer growth and differentiation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 8/20/08; revised 1/14/09; accepted 2/17/09; published OnlineFirst 4/7/09.

Grant support: NIH RO1 CA089364 (G.A. Brent).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We thank Tiffany Levy for excellent technical assistance.

References

Figure 6. Model for the mechanisms of retinoid induction of NIS expression in MCF-7 cells. Factors that stimulate expression are shown with a ‘+’ and those that inhibit are shown by a ‘−’. PI3K activation of additional pathways is likely required for NIS gene expression.

www.aacrjournals.org 3449 Cancer Res 2009; 69: (8). April 15, 2009


Activation of the PI3 Kinase Pathway By Retinoic Acid Mediates Sodium/Iodide Symporter Induction and Iodide Transport in MCF-7 Breast Cancer Cells

Emi Ohashi, Takahiko Kogai, Hiroyuki Kagechika, et al.


Updated version
Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-08-3234

Cited articles
This article cites 37 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/69/8/3443.full.html#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/69/8/3443.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.