Signaling through 3',5'-Cyclic Adenosine Monophosphate and Phosphoinositide-3 Kinase Induces Sodium/Iodide Symporter Expression in Breast Cancer

KATHERINE A. B. KNOSTMAN, JE-YOEL CHO, KWON-YUL RYU, XIAOQIN LIN, JAMES A. MCCUBREY, TIMOTHY HLA, CATHERINE H. LIU, EMMA DI CARLO, RUTH KERI, MING ZHANG, DAE Y. HWANG, WILLIAM C. KISSEBERTH, CHARLES C. CAPEN, AND SISSY M. JHIANG

Departments of Veterinary Biosciences (K.A.B.K., C.C.C.), Veterinary Clinical Sciences (W.C.K.), and Physiology and Cell Biology (S.M.J.), The Ohio State University, Columbus, Ohio 43210; Department of Biochemistry (J.Y.C.), School of Dentistry, Kyungpook National University, Daegu 700-42, Republic of Korea; Department of Biological Sciences (K.Y.R.), Stanford University, Stanford, California 94305; Department of Biological Chemistry (X.L.), University of California, Irvine, Irvine, California 92697; Department of Microbiology and Immunology (J.A.M.), East Carolina University School of Medicine, Greenville, North Carolina 27858; Center for Vascular Biology (T.H.), University of Connecticut Health Center, Farmington, Connecticut 06093; Department of Molecular Genetics, Microbiology, and Immunology (C.H.L.), University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854; Department of Oncology and Neurosciences (E.D.C.), “G. d’Annunzio” University, 66100 Chieti, Italy; Department of Pharmacology (R.K.), Case School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106; Department of Molecular and Cellular Biology (M.Z.), Baylor College of Medicine, Houston, Texas 77030; and Division of Laboratory Animal Resources (D.Y.H.), National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul 122-704, Republic of Korea

The sodium/iodide symporter (NIS) is a membrane transport glycoprotein normally expressed in the thyroid gland and lactating mammary gland. NIS is a target for radioiodide imaging and therapeutic ablation of thyroid carcinomas and has the potential for similar use in breast cancer treatment. To facilitate NIS-mediated radionuclide therapy, it is necessary to identify signaling pathways that lead to increased NIS expression and function in breast cancer. We examined NIS expression in mammary tumors of 14 genetically engineered mouse models to identify genetic manipulations associated with NIS induction. The cAMP and phosphoinositide-3 kinase (PI3K) signaling pathways are associated with NIS up-regulation. We showed that activation of PI3K alone is sufficient to increase NIS expression and radioiodide uptake in MCF-7 human breast cancer cells, whereas cAMP stimulation increases NIS promoter activity and NIS mRNA levels but is not sufficient to increase radioiodide uptake. This study is the first to demonstrate that NIS expression is induced by cAMP and/or PI3K in breast cancer both in vivo and in vitro. (J Clin Endocrinol Metab 89: 5196–5203, 2004)

Breast cancer is one of the leading causes of cancer in women in the United States, with more than 181,000 newly diagnosed cases and in excess of 44,000 cancer-related deaths annually (1). Several specific genetic mutations and environmental factors leading to an increased risk of breast cancer have been elucidated in recent years. Conventional treatment modalities for breast cancer include radical mastectomy, lumpectomy with radiotherapy, systemic hormone modulating therapy (tamoxifen and raloxifene), and chemotherapy (1, 2). Diagnosis is currently made at an earlier stage of the disease due to increased use of mammography and routine clinical examination, which together have decreased mortality by 25–30% in women over 50 yr old. However, monitoring recurrence and metastases by frequent physical examination in breast cancer patients has not been successful in changing the clinical course, and most women with metastatic carcinoma will eventually die from the disease. Therefore, detection and treatment of recurrent and metastatic breast cancer is of high clinical importance.

The sodium/iodide symporter (NIS) is a transmembrane glycoprotein most commonly studied in the context of the thyroid gland, in which it mediates active transport of iodide (I\(^-\)) from the systemic circulation into thyroid follicular cells. NIS forms the basis of radioiodide treatment for thyroid cancer by facilitating targeted radioiodide uptake and subsequent destruction of residual and/or metastatic neoplastic cells after thyroidectomy (3, 4). Additionally, NIS-expressing thyroid tumors can be imaged using nuclear scintigraphy, improving detection of residual, recurrent, or metastatic lesions.

In addition to the thyroid gland, the salivary glands, gastric mucosa, lacrimal system, placenta, and lactating mammary gland express NIS and thus have the capacity to actively accumulate iodide (5). Our unpublished data as well
as that of others (5, 6) have demonstrated NIS protein in the majority of human breast cancers using immunohistochemistry. The fact that NIS is up-regulated in lactating mammary epithelial cells and that increased NIS expression is detected in many human breast tumors raises the potential for developing NIS-mediated radionuclide therapy as a safe and effective treatment for breast cancer.

The use of genetically engineered mice (GEM) has greatly increased understanding of mammary tumorigenesis, offering a convenient system for studying the interaction of specific genetic manipulations with external factors. Tumors from Her-2/neu and v-Ha-ras transgenic mice have been reported to develop NIS-mediated radionuclide therapy as a safe and effective treatment for breast cancer.

The objectives of the current study included evaluating NIS expression in mammary tumors of 14 GEM models to identify genetic manipulations that lead to increased NIS expression in vivo and investigating the role of associated signaling pathways in NIS induction in vitro using MCF-7 human mammary carcinoma cells. Based on the four transgenes associated with NIS induction, we identified that signaling pathways mediated by cAMP and phosphoinositide-3 kinase (PI3K) are important in NIS up-regulation. We further demonstrated that activation of PI3K alone is sufficient to increase NIS expression and radioiodide uptake in MCF-7 human breast cancer cells, whereas cAMP stimulation increases NIS promoter activity and NIS mRNA levels but is not sufficient to increase radioiodide uptake.

**Materials and Methods**

**Transgenic mice**

We requested unstained tissue sections from 14 GEM models of breast cancer. Table 1 summarizes the models investigated for NIS expression using immunohistochemistry and references for each model. Investigators who made tissue samples available are indicated in Acknowledgments at the conclusion of this manuscript. The four transgenic mouse models with high NIS expression are discussed in further details as follows.

**Tissue sections** from Ubi-human chorionic gonadotropin (hCGβ) transgenic mice were obtained from Dr. Ilpo Huhtaniemi (University of Turku, Turku, Finland). The transgene, which was introduced into an FVB/N background, consisted of a 579-bp cDNA segment of the hCGβ gene driven by a 1.2-kb ubiquitin C promoter (8). Transgenic female Ubi-hCGβ mice were obese, underwent precocious puberty, and had abnormal estrous cycles leading to infertility. Females had abnormally high serum estradiol levels until 2 months of age, at which time it returned to normal. Serum progesterone, testosterone, and prolactin levels gradually increased from the age of 4–6 months until 12 months. Pituitary glands in female Ubi-hCGβ transgenic mice developed lactotrope hyperplasia and adenomas by the age of 10–12 months. Mammary glands had marked lobuloalveolar hyperplasia by age 6 months, similar in phenotype to pregnancy, which progressed to mammary tumors by age 12 months in 91% of transgenic females. The histologic appearance of mammary adenocarcinomas varied from well-differentiated papillary carcinomas with abundant intraluminal secretions to poorly differentiated solid variants. Tumors had high mitotic indices and were highly invasive, with metastases to liver, lung, spleen, uterus, and adrenal glands occurring in nearly half of all cases. Ovariecomy at 6 wk of age completely prevented glandular hyperplasia and neoplasia.

Tissue sections from mouse mammary tumor virus (MMTV)-cyclooxygenase (Cox)-2 mice were obtained from Dr. Timothy Hla (University of Connecticut Health Center, Farmington, CT). The transgene, consisting of the human Cox-2 gene open reading frame driven by the promoter of the MMTV, was injected into a CD1 background strain (9). High Cox-2 expression was detected in mammary gland tissue, particularly during lactation, and transgenic females exhibited premature mammary development. Involution was delayed from the normal 1–2 d until 7 d post weaning by virtue of decreased apoptosis of mammary epithelial cells. Multiparous females developed frequent hyperplastic alveolar nodules, and approximately 85% developed mammary adenocarcinomas. Histologically, tumors consisted of moderately to well-differentiated ductal and lobuloalveolar adenocarcinomas with frequent squamous metaplasia, scirrhous reaction, and neovascularization. Metastases to lymph nodes occurred in many MMTV-Cox-2 females.

Mammary tissue sections from MMTV-polymyosin virus middle T antigen (PyMT) transgenic mice were obtained from Dr. Donna Kusewitt

**TABLE 1.** Immunohistochemical detection of NIS expression in mouse models of breast cancer

<table>
<thead>
<tr>
<th>Model</th>
<th>Ref.</th>
<th>n</th>
<th>No. positive (%)</th>
<th>% Cells positive</th>
<th>Intensity</th>
<th>Subcellular localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubi-hCGβ</td>
<td>8 5</td>
<td>5 (100)</td>
<td>100</td>
<td>4+</td>
<td>PM</td>
<td></td>
</tr>
<tr>
<td>MMTV-neu</td>
<td>12, 13 9</td>
<td>8 (100)</td>
<td>100</td>
<td>4+</td>
<td>PM, C</td>
<td></td>
</tr>
<tr>
<td>MMTV-PyMT</td>
<td>10 3</td>
<td>3 (100)</td>
<td>100</td>
<td>4+</td>
<td>PM</td>
<td></td>
</tr>
<tr>
<td>MMTV-Cox-2</td>
<td>9 2</td>
<td>2 (100)</td>
<td>100</td>
<td>3+</td>
<td>PM, C</td>
<td></td>
</tr>
<tr>
<td>α-LH/CTP</td>
<td>38 4</td>
<td>4 (100)</td>
<td>100</td>
<td>1–2+</td>
<td>PM, C</td>
<td></td>
</tr>
<tr>
<td>WAP-p53R172H + DMBA + pituitary isograft</td>
<td>39 4</td>
<td>4 (100)</td>
<td>100</td>
<td>1+</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>C3(1)-Tag</td>
<td>40 5</td>
<td>2 (40)</td>
<td>50</td>
<td>1+</td>
<td>PM, C</td>
<td></td>
</tr>
<tr>
<td>WAP-des-IGF-1</td>
<td>41 3</td>
<td>3 (100)</td>
<td>100</td>
<td>1+</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>WAP-des-IGF-1 + WAP-des-IGF-1</td>
<td>42 4</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>WAP-HPV16E6β-casein</td>
<td>43 1</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>WAP-Tag + WAP-maspin</td>
<td>44 2</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>MMTV-c-myc × MMTV-v-Ha-ras</td>
<td>45 3</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>MMTV-polymyosin virus middle T antigen</td>
<td>41 5</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

PM, Plasma membrane; C, cytoplasm; N/A, not applicable.

a) Glycoprotein α-subunit promoter driving LH/C-terminal peptide fusion gene.

b) Whey acidic protein promoter driving p53 R172H mutant gene with DMBA carcinogen and implantation of pituitary isograft.

c) Prostate steroid binding protein C3 (1) promoter driving SV40 large T antigen gene.

d) p53 null mammary explants implanted in wild-type mice treated with DMBA and pituitary isograft.

e) Whey acidic protein promoter driving IGF gene.

f) Bitransgenic mice expressing mutant p53 and IGF-I.

* Mouse mammary tumor virus promoter driving human papillomavirus 16E6 subunit/β-casein fusion gene.

h) Bitransgenic mice expressing SV40 large T antigen and maspin tumor suppressor gene.

i) Bitransgenic mice expressing c-myc and v-Ha-ras oncogenes.

j) Wild-type mice treated with DMBA and pituitary isografts.
(Ohio State University, Columbus, OH). The transgene consisted of the PγMT gene open reading frame driven by the promoter of the MMTV. Mammary tumorsogenesis was evident by the age of 5 wk, although very early lesions of atypical ductular hyperplasia sometimes preceded neo-
plasia. Histologically, tumors were solid to cystic, multicentric, often poorly differentiated, invasive, and widely metastatic (10, 11).

Tissue sections from MMTV-neu transgenic mice were acquired from Dr. Emma Di Carlo (G. d’Annunzio University, Chieti, Italy), and tissue sections from MMTV-c-neu transgenic mice were obtained from Dr. William Kisselberth (The Ohio State University, Columbus, OH). MMTV-
neu mice from G. d’Annunzio University were created by insertion of the transgene, consisting of the MMTV promoter driving expression of the activated rat neu oncogene, into a BALB/c background strain (12). Widespread atypical hyperplasia of small lobular ducts and lobules was already evident in mammary tissue at the third week of age, progressing to lobular carcinoma in situ by the 10th to 11th week and finally to multiple invasive carcinomas at approximately 20 wk of age, which involved all 10 mammary glands by the age of 33 wk. MMTV-c-neu mice from The Ohio State University were created by insertion of the transgene, consisting of the MMTV promoter driving expression of the un-
activated rat c-neu protooncogene, into an FVB/N background strain (13). These mice developed focal mammary carcinomas between the ages of 4 and 11 months of age with development of lung metastases in approximately 40–75% of mice (variation was present among different transgenic mouse lines).

Immunohistochemistry

Paraffin-embedded formalin- or parafomaldehyde-fixed murine mammary tissue was sectioned to 5 µm thickness and affixed to glass slides. Deparaffinization and rehydration were performed as follows: slides were submerged in 100% xylene for three repetitions of 5 min each, followed by submersion into 100% ethyl alcohol for two repetitions of 2 min each, 95% ethyl alcohol for 2 min, and 70% ethyl alcohol for two repetitions of 2 min each. Next, slides were gently washed in double-distilled water (ddH2O) for 2 min. Endogenous peroxidase activity was blocked by soaking slides in 3% hydrogen peroxide in methanol for 15 min. After gently washing slides in ddH2O, antigen retrieval was performed by dipping slides in steaming ddH2O and placing them into steaming citric acid buffer (ci-
trate, sodium citrate, and ddH2O) for 30 min. After antigen retrieval, slides were rinsed in ddH2O three times with gentle agitation. Next, slides were placed in a humidified chamber and blocked at 4°C overnight using 2% BSA in TBS. The primary antibody, which cross-labels mouse NIS (PA716, a kind gift from Dr. Bernard Rousset, Institut National de la Sante et de la Recherche Medicale, Lyon, France; diluted 1:1000 in 1% BSA) was immediately applied to slides, which were then incubated in a humidified chamber at room temperature for 1 h. Negative control slides were incubated with 1% BSA alone. After 1 h, slides were washed with TBS to remove residual primary antibody. Horseradish peroxidase-conjugated goat antimouse secondary antibody (Bio-Rad Laboratories, Hercules, CA) was diluted 1:250 in 1% BSA and applied to slides for 20 min incubation in a hum-
idified chamber at room temperature. Slides were rinsed in TBS to remove residual secondary antibody. Antigen-antibody complexes were detected by incubation of tissue sections with chromagen DAB (Dako, Carpinteria, CA) for 5 min in a dark chamber at room temperature. Slides were then gently washed in ddH2O three times with gentle agitation, counterstained with Gill’s hematoxylin (Fisher Scientific, Pittsburgh, PA) for 30 sec, dipped in running lukewarm tap water, placed in clarifier for 1 min, rinsed a second time in running lukewarm tap water, and dipped in a bluing agent (1% NH4OH). Dehydration was performed by dipping slides in a progression of 70% ethanol, 95% ethanol, 100% ethanol, and 100% xylene. Slides were then air dried and coverslipped.

Cell culture

MMCF-7 cells were maintained in medium consisting of equal parts DMEM and Ham’s F12, 10% fetal bovine serum (FBS), and 1% penicillin/ streptomycin (Invitrogen, Carlsbad, CA) and kept in a 37°C incubator with 5% CO2. When applicable, cells were treated with 2, 5, or 10 IU/ml hCG (Dr. A. F. Parlow, National Hormone and Peptide Program, Tor-

DNA constructs

NIS promoter-luciferase reporter gene constructs, 144-bp hNISp and 2.9-kb hNISp, were engineered using the PGLB vector as described by Ryu et al. (14). Recombinant retroviruses encoding v-Ha-ras, PI3K p110 active, and empty vector pBpuo were obtained from Dr. Michael Weber (University of Virginia, Charlottesville, VA). Dr. Julian Downward (Im-
perial College Research Fund, London, UK), and Dr. Martin McMahon (University of California, San Francisco). MCF-7 stable clones expressing pLXSN-v-Ha-ras, pLXSN/PI3K p110 CAAX (constitutively active), and empty vector pBpuo were generated by retroviral infection of MCF-7 cells in the laboratory of Dr. James McCubrey (East Carolina University, Greenville, NC) as described by Davis et al. (15).

Luciferase assay

Approximately 1 × 106 MCF-7 cells were seeded in each well of a 6-well plate. After 24 h, cells were transfected with human NIS pro-
moters (2.9-kb hNISp, 144-bp hNISp, or PGLC control vectors) using Lipofectamine (Invitrogen). The culture medium was then changed to 5% charcoal-stripped medium, and cells were treated with IBMX (10 µM) and CT (10 ng/ml) for 24 h. Cells were harvested, and luciferase assays were performed using a luciferase assay kit (Promega, Madison, WI) and luminometer (Lumat; PerkinElmer, Boston, MA). As a positive control, the PGLC-C control vector with the Simian virus 40 (SV40) promoter showed high basal activities but no further activation by IBMX+CT treatment. Normalization of transfection efficiency was determined by cotransfecting cells with the plasmid pCH110 (SV40-LacZ). Within each treatment group, luciferase activity was normalized to β-galactosidase activity.

Quantitative real-time PCR

Quantitative real-time PCR assay was performed as described by our laboratory (16). Briefly, 5 × 105 MCF-7 cells were seeded in normal culture medium in 35-mm plates. Medium was changed to charcoal-
stripped MCF-7 medium 1 d before treatment with 8-Br-cAMP or IBMX and CT. RNA was isolated 24 h after treatment using TRIzol reagent (Invitrogen). One microgram of RNA was used for the reverse trans-
scription reaction. Two microliters of cDNA template were used for quantitative real-time PCR, which used the human NIS dual-probe quantification method. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH) normalization, 236 bp human GAPDH was amplified by PCR using two primers: GAPDH-F1 (TTC ACC ACC ATG GAG AAG GC) and GAPDH-R1 (GGC AGC TGT GGT CAT GA). The amplified DNA fragment was cloned into the TA cloning vector, maxiprepared (Qagen, Valencia, CA) and used as the standard for SYBR Green I quantitative real-time PCR. The data were presented as a fold increase over control after NIS mRNA levels were normalized with GAPDH mRNA levels.

Radioiodide uptake assay

Radioiodide uptake assay was performed essentially as in La Perle et al. (17). Twelve-well plates were seeded with 5 × 106 MCF-7 cells per well. Assays were performed in triplicate. MCF-7 cells were treated with hCG or PGE2 for 24 or 48 h when applicable, after which the cells were...
were incubated with 2 μCi/well of 125I in NaI for 30 min at 37°C. Cells were rapidly washed twice with 1 ml cold Hanks’ balanced salt solution and incubated in 1 ml 95% ethanol at room temperature for 20 min to release intracellular 125I. The supernatant was counted in a γ-radiation counter. Counts per minute were normalized to cell number (1 × 10⁵). MCF-7 cells treated with tRA were used as a positive control (18).

Statistical analysis

Statistical analysis consisted of Student’s t test and was performed using GraphPad software (GraphPad Inc., San Diego, CA). Each experimental group was compared with its respective control group.

Results

Of the 14 GEM models evaluated (Table 1), mammary tumors from transgenic mice expressing the oncogenes PyMT and neu (both activated and unactivated forms) were strongly positive for NIS using immunohistochemical staining, as were transgenic mice expressing the hCGβ subunit and the enzyme Cox-2 in the mammary gland (Fig. 1, A–K). NIS staining was primarily plasma membrane localized in all four models, although light cytoplasmic staining was present in Cox-2 and neu tumors. Lesions of lobular hyperplasia were available from activated neu and hCGβ mice. All of these lesions had strong plasma membrane staining for NIS (Fig. 1, G and J). Additionally, two samples from histologically normal juvenile c-neu mouse mammary glands were found to be strongly positive for NIS (Fig. 1F), whereas nonlactating mammary glands of nontransgenic mice did not express NIS (data not shown).

Upon examination of the available scientific literature concerning signal transduction pathways activated by the four transgenes (8–13, 19–22), we identified two signaling pathways of interest for further in vitro study. We hypothesize that Ras and PI3K signal transduction pathways with potential roles in NIS induction. Her-2/neu and PyMT oncogenes use Ras and PI3K signal transduction pathways. Prostaglandins induced by the Cox-2 enzyme activate adenyl cyclase and increase cAMP levels but also interact with PI3K. hCG induces adenyl cyclase activity, increasing intracellular cAMP but also stimulates estradiol (E2) release from the ovaries in vivo.

![Image of histology and immunohistochemical staining for NIS expression in mouse models of breast cancer](image-url)

**Fig. 1.** A–K, Histology and immunohistochemical staining for NIS expression in mouse models of breast cancer (magnification, ×400). A, Carcinoma from MMTV-PyMT mouse mammary gland [hematoxylin and eosin (H&E)]. B, Strongly positive immunohistochemical staining for NIS in a PyMT mouse mammary tumor. C, Adenocarcinoma with marked lymphocytic infiltration from a MMTV-Cox-2 mouse mammary gland (H&E). D, Strongly positive immunohistochemical staining for NIS in a Cox-2 mouse mammary adenocarcinoma. E, Carcinoma from a MMTV-neu mouse mammary gland (H&E). F, Strongly positive immunohistochemical staining for NIS in a lesion of lobular hyperplasia from a MMTV-neu mouse mammary gland. G, Strongly positive immunohistochemical staining for NIS in a lesion of ductular hyperplasia from a MMTV-neu mouse mammary gland. H, Strongly positive immunohistochemical staining for NIS in a Ubi-hCGβ mouse mammary carcinoma. I, Ductular carcinoma from a Ubi-hCGβ mouse mammary gland (H&E). J, Strongly positive immunohistochemical staining for NIS in a lesion of ductular hyperplasia from a Ubi-hCGβ mouse mammary gland. K, Strongly positive immunohistochemical staining for NIS in a Ubi-hCGβ mouse mammary carcinoma. L, lower panel) Signaling pathways up-regulated in the four above transgenic mouse models with potential roles in NIS induction. Her-2/neu and PyMT oncogenes use Ras and PI3K signal transduction pathways. Prostaglandins induced by the Cox-2 enzyme activate adenyl cyclase and increase cAMP levels but also interact with PI3K. hCG induces adenyl cyclase activity, increasing intracellular cAMP but also stimulates estradiol (E2) release from the ovaries in vivo.
that cAMP elevation in hCGβ and Cox-2 mice and PI3K activation in PyMT and Her-2/neu mice are responsible for NIS induction in the corresponding mammary glands and mammary tumors (Fig. 1L, bottom panel).

Using the LH/CGR receptor-positive MCF-7 human mammary carcinoma cell line, we showed that hCG treatment induces radioidide uptake, an indication of NIS function (Fig. 2). We also showed that PGE2 treatment induces radioidide uptake (Fig. 3). Cox-2 is critical in catalyzing the conversion of arachidonic acid to prostaglandins, such as PGE2. Because cAMP signaling plays an important role in hCG signaling and is induced by PGE2 treatment of MCF-7 cells (23), we further investigated the importance of cAMP in NIS induction in vitro. We showed that increasing intracellular cAMP levels using the phosphodiesterase inhibitor IBMX in combination with the adenylyl cyclase activator ChT, or alternatively by administration of 8-Br-cAMP, markedly stimulated NIS mRNA expression in MCF-7 cells (Figs. 4 and 5). Interestingly, administration of high doses of IBMX and ChT resulted in less induction of NIS expression than did the lower doses. To determine whether cAMP has a direct effect on the NIS promoter, MCF-7 cells were transiently transfected with NIS promoter-reporter gene constructs consisting of either a 144-bp minimal promoter or a 2.9-kb 5’ flanking region driving expression of the luciferase reporter gene. In cells treated with IBMX and ChT, activities of both the minimal promoter and the 2.9-kb NIS promoter were increased more than 10-fold (Fig. 6), indicating that a cAMP response element is present in these promoter regions.

The importance of signal transduction through PI3K was investigated using MCF-7/PI3K stable clones. Stable clones expressing the constitutively active PI3K p110 catalytic subunit had greater than 4-fold increase in radioidide uptake, compared with empty vector-expressing cells (Fig. 7). Stable clones expressing v-Ha-ras, one upstream mediator of PI3K activity, had slightly increased iodide uptake, compared with negative control cells. These findings reveal that PI3K overexpression is sufficient to induce NIS function in MCF-7 breast cancer cells.

Discussion

This study is the first to demonstrate the importance of cAMP and PI3K signaling in NIS regulation in the context of the mammary gland. We showed that activation of PI3K alone is sufficient to increase NIS expression and radioidide uptake in MCF-7 human breast cancer cells, whereas cAMP stimulation increases NIS promoter activity and NIS mRNA levels but is not sufficient to increase radioidide uptake (see Ref. 18 and our unpublished data).

We found that MMTV-Cox-2 and Ubi-hCGβ transgenic mice had high levels of NIS expression in their mammary tumors and that treatment of MCF-7 breast cancer cells with PGE2 or hCG stimulated radioidide uptake. The signaling cascades downstream of PGE2 and hCG are similar, involving G protein-coupled receptors, cAMP up-regulation, and protein kinase A activation. In Cox-2 transgenic mice, up-regulation of the PGE2 receptors, EP2 and EP4, has been detected in mammary tumors, confirming that PGE2 signaling is activated in this system (24). Planchon et al. (23) showed...
that treatment of MCF-7 cells with PGE2 induced cAMP levels by more than 10-fold over untreated controls. Treatment with hCG also increased NIS expression in JAr human choriocarcinoma cells via interaction with the LH/CG receptor (25) and in rat FRTL-5 thyroid cells via interaction with the TSH receptor (26).

We demonstrated that treatment of MCF-7 cells with compounds that increase intracellular cAMP levels (IBMX, ChT, and 8-Br-cAMP) led to a significant increase in NIS mRNA levels and NIS promoter activity (Figs. 4–6). Interestingly, treating MCF-7 cells with forskolin, an adenylyl cyclase activator, was not sufficient to increase radioiodide uptake (see Ref. 18 and our unpublished data). It is possible that, whereas increasing cAMP levels leads to increased NIS transcription, it does not result in sufficient protein levels or cell surface trafficking to have a significant effect on radioiodide uptake in MCF-7 cells. Thus, the effect of cAMP on NIS expression in MCF-7 cells is different from that in thyroid follicular cells. TSH up-regulates NIS expression and radioiodide uptake through interaction with TSH receptors in the plasma membrane, Gα-protein mediation, and an increase in cAMP (4, 14, 27), and NIS up-regulation can be fully reproduced through administration of cAMP agonists, such as forskolin. Thus, whereas the effects of hCG and PGE2 on NIS regulation in the mammary gland context are similar to those of TSH in thyroid follicular cells, the pathways are not identical with respect to the effect of cAMP on radioiodide uptake.

Lactogenic hormones are critical in NIS regulation in the mammary gland (5, 16, 28), yet the signal transduction pathways stimulated by these hormones leading to increased NIS expression have not been characterized. In general, prolactin functions via activation of downstream signal transduction through MAPK and/or PI3K (29, 30), and oxytocin uses protein kinase C/phospholipase C and Ras/Raf/MAPK (31). Transient transfection of mutant rasV12S35, which preferentially interacts with the downstream effector Raf-1, abolished NIS expression in TSH-stimulated Wistar rat thyroid (WRT) cells (32, 33). The rasV12C40 mutant, which interacts primarily with PI3K, maintained NIS expression. Our finding of increased radioiodide uptake in MCF-7/PI3K stable clones demonstrates that overexpression of PI3K alone is sufficient to induce NIS function in MCF-7 cells. It is interesting to note that Lin et al. (11) discovered frequent induction of neu expression in PyMT mammary tumors, which was confirmed in our study (data not shown). Thus, NIS induction in the mammary tumors of PyMT mice is potentially a PI3K-mediated phenomenon secondary to neu expression.

PI3K up-regulation plays an important role in human
breast cancer, particularly in tumors expressing Her-2/neu or Src oncogenes (34), in which PI3K overactivation is associated with antiapoptotic effect. The role of cAMP in breast carcinogenesis is less known. Cox-2 is known to play a role in human breast cancer through promotion of proliferation, angiogenesis, inhibition of apoptosis, and immunosuppression (35). On the other hand, hCG promotes mammmary epithelial cell differentiation, and hCG expression by tumor cells is a positive prognostic indicator in human breast cancer (36). However, overexpression of hCG in transgenic mice results in excessive estrogen production by the ovaries, a factor thought to be important in human breast cancer (8). All four transgenic mouse models of breast cancer have primary tumors of variable differentiation with systemic metastases. Up-regulation of signaling pathways in Her-2/neu-, PyMT-, and Cox-2-induced tumors is potentially more representative of the human disease than is up-regulation by marked hCG overexpression.

In this study, we found cAMP and PI3K to be of critical importance in up-regulating NIS in MCF-7 breast cancer cells and transgenic mouse models of breast cancer. Because MCF-7 is the only immortalized cell line with inducible NIS function, it is not known whether the in vitro findings are applicable to nonneoplastic immortalized mammary epithelial cells (such as MCF-12A). However, based on the presence of NIS protein in the mammary epithelium of hCG and Her-2/neu transgenic mice before malignant transformation, it is likely that these pathways are operative in normal mammary epithelial cells as well as in breast cancer.

In summary, this study provides several significant advances in the understanding of NIS regulation in breast cancer. First, NIS expression in mammary tumors of Her-2/neu, PyMT, hCGβ, and Cox-2 transgenic mice indicates that signal transduction through cAMP and/or PI3K likely contributes to NIS up-regulation in breast cancer in vivo. Furthermore, NIS expression in normal and early hyperplastic lesions of juvenile Her-2/neu mice and in hyperplastic lesions of hCGβ-overexpressing mice indicates that NIS induction is a specific result of transgene expression because NIS is induced before malignant transformation. Second, we demonstrated that activation of cAMP and PI3K induces NIS up-regulation in vitro in MCF-7 cells. Previously, all-trans and 9-cis-retinoic acid were the only compounds known to increase NIS expression in MCF-7 cells, in which they function through nuclear receptors (37). Finally, NIS expression in these four mouse models provides a vehicle for imaging and therapy using animal models not previously known to have NIS-positive mammary tumors. Further investigation of the molecular mechanisms underlying NIS induction by these two signaling pathways will facilitate NIS-mediated radionuclide therapy for breast cancer becoming a reality.

Acknowledgments

We thank Dr. Ilpo Huhtaniemi (Imperial College London) and Dr. Matti Poutanen (the University of Turku, Turku, Finland) for providing mammmary tumor tissue from Ubi-hCGβ transgenic mice; Dr. Jeffrey Rosen, Dr. Darryl Hadsell, and Dr. Dan Medina (Baylor College of Medicine, Houston, TX) for providing mammary tumor tissues from WAP-p53 mice; Dr. Susan Ritting (University of Pennsylvania, Piscataway, NJ) for providing mammary tumor tissues from MMTV-c-myb × MMTV-v-Ha-ras; Dr. Jeffrey Green (the National Cancer Institute, National Institutes of Health (NIH), Bethesda, MD)) for providing mammary tumor tissues from C3(1)-Tag mice; and Dr. Donna Kusevitt (The Ohio State University, Columbus, OH) for providing mammary tumor tissues from MMTV-PyMT mice. Additionally, we would like to acknowledge Dr. Artemi A. P. Parlow (the National Hormone and Peptide Program, Torrance, CA) for providing recombinant hCG for in vitro experiments.

Received May 13, 2004. Accepted July 14, 2004.

Address all correspondence and requests for reprints to: Sissy M. Jhiang, 304 Hamilton Hall, 1645 Neil Avenue, Columbus, Ohio 43210. E-mail: jhiang.1@osu.edu.

This work was supported in part by the following grants: NIH NIBIB R01 EB001876–01 and DAMD17–02–1–0119 (to S.M.J.), NIH T32 RR07073 (to K.A.B.K.), and Schering-Plough Research Institute (to C.C.C.).

References


27. Taki K, Kogai T, Kanamoto Y, Hershman JM, Brent GA 2002 Pax-8 binding and cyclic adenosine 3′,5′-monophosphate response element-like sequence binding proteins for full activity and is differentially regulated in normal and thyroid cancer cells. Mol Endocrinol 16:2266–2282


32. Cass LA, Meinkoth JL 2000 Ras signaling through PI3K confers hormone-independent proliferation that is compatible with differentiation. Oncogene 19:924–932


