23

Regulation and Enhancement of Endogenous Sodium Iodide Symporter Expression: NIS Regulatory Pathways in Thyroid and Breast Cancer

Takahiko Kogai and Gregory A. Brent Molecular Endocrinology Laboratory, VA Greater Los Angeles Healthcare System, Departments of Medicine and Physiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90073, USA

Abstract

The sodium iodide symporter (NIS) gene is expressed at a high level in the thyroid gland and the lactating breast. Thyroid-stimulating hormone, or thyrotropin (TSH) is the major regulator of NIS expression in the normal thyroid and also stimulates NIS in more than 80% of differentiated thyroid cancers. TSH stimulates NIS gene expression through the proximal promoter and the NIS far upstream enhancer (NUE). The NUE requires at least two transcription factors, Pax-8 and CREB, for stimulation. Reduced expression of these transcription factors may contribute to the reduction in NIS expression that is seen in some differentiated thyroid cancer. High levels of endogenous TSH, or administration of recombinant TSH, are required to enhance the iodide uptake in thyroid cancer before radioiodide therapy. NIS gene expression in lactating breast tissue is primarily regulated by oxytocin and prolactin. A modest level of NIS expression has been reported in approximately 80% of cases of breast cancers. A number of strategies have been used to enhance NIS expression and radioiodide uptake in thyroid cancer and breast cancer. These include redifferentiation agents, such as retinoic acid and other nuclear hormone receptor ligands, as well as epigenetic modifiers. The results from application of these agents to *in vitro* models of thyroid and breast cancer will be described. Insights into NIS regulation, as well as agents that promote endogenous NIS expression, should lead to new approaches to using radioiodine in the treatment of thyroid and breast cancers.

Abbreviations

B-ZIP	Basic-leucine zipper
cAMP	Cyclic AMP, or 3′–5′-cyclic adenosine monophosphate
CRE	cAMP-responsive element
CREB	Cyclic AMP-responsive element binding protein
CREM	cAMP response element modulator
Dex	Dexamethasone
DR2	Direct repeat-2
DR-5	Direct repeat-5
hCG	Human chorionic gonadotropin
HER2/neu	Human epidermal growth factor receptor 2
NIS	Sodium iodide (Na^+–I^-) symporter
NTF-1	NIS TSH-responsive factor-1
NUE	NIS far upstream enhancer
Pax-8	Paired-domain containing transcription factor-8
PI3K	Phosphoinositide-3 kinase
PKA	Protein kinase-A
PPARγ	Peroxisome proliferator-activated receptor-γ
PyVT	Polyoma virus middle T antigen
RA	Retinoic acid
RAR	Retinoic acid receptor
RARE	Retinoic acid response element
Ref-1	Redox factor-1
RET/PTC	Rearranged in transformation/papillary thyroid carcinoma
RXR	Retinoid X receptor, 9-*cis* retinoic acid receptor
RPL18a	Ribosomal protein L18a
Tg	Thyroglobulin gene
TPO	Thyroperoxidase gene
TSA	Trichostatin A
TSH	Thyroid-stimulating hormone, or thyrotropin
TSHR	TSH receptor
TTF-1	Homeodomain containing thyroid transcription factor-1

Comprehensive Handbook of Iodine
ISBN: 978-0-12-374135-6

The primary regulator of sodium iodide symporter (NIS) expression in the thyroid is the thyroid-stimulating hormone, or thyrotropin (TSH). TSH stimulates adenylyl cyclase through the G-protein coupled TSH receptor (TSHR) resulting in accumulation of cyclic AMP (cAMP) in thyroid follicular cells. The elevation of endogenous cAMP induces NIS gene expression by stimulating factors that bind to specific regions of its promoter and the NIS upstream enhancer (NUE) (Kogai *et al.*, 2006). TSH also increases NIS protein half life and stimulates the trafficking of NIS to the plasma membrane in thyroid cells (Riedel *et al.*, 2001). Most well-differentiated thyroid cancers accumulate radioiodide in response to TSH through stimulation of the endogenous NIS gene.

The lactating mammary gland concentrates iodide to a degree similar to that seen in the thyroid, providing iodine for thyroid hormone synthesis to the developing infant. NIS is abundantly expressed in the lactating breast, and is also expressed at low levels in some breast cancer (Kogai *et al.*, 2006). Oxytocin and prolactin, but not TSH, are the primary stimulators of NIS expression in the lactating breast. Retinoids stimulate NIS expression in breast cancer cells, but suppress NIS expression in thyroid cells (Kogai *et al.*, 2006). Retinoids stimulate NIS expression at both the transcriptional and the post-transcriptional levels in breast cancer cells, but do not stimulate translocation of NIS protein to the plasma membrane (Kogai *et al.*, 2000b, 2004).

Structure of the Human and Rat NIS Gene

The difference in the factors that regulate NIS gene expression in thyroid and breast suggests that there are tissue-specific pathways involved. Many of these NIS regulatory factors have been identified, and are active in both human and rodent cell models (Kogai *et al.*, 2006). This suggests that the regulatory region(s) for NIS gene transcription are in sequences common to the human and rat NIS genes. This has been an important guide to mapping key NIS gene regulatory regions, focusing on regions with a high similarity in humans and rats.

The human NIS gene maps to 19p13.2-p12 (Smanik *et al.*, 1997) and contains 14 introns in 22116 bases, as measured from the first to the last exon. The rat NIS gene is 9260 bases, significantly smaller in size compared to the human gene, although the number of exons is the same and the full-length mRNA is similar in size. The next gene upstream of *NIS*, ribosomal protein L18a (*RPL18a*), is located 8657 bases in the human genome or 2130 bases in the rat genome upstream from the 1st exon of *NIS* (Figure 23.1). The similarity of the 5′-flanking region (from the NIS coding sequence to the RPL18a coding sequence) between humans and rats is only 11.8%. There are three regions with high homology between the human and the rat sequences in the 5′-flanking region of *NIS* (Figure 23.1),

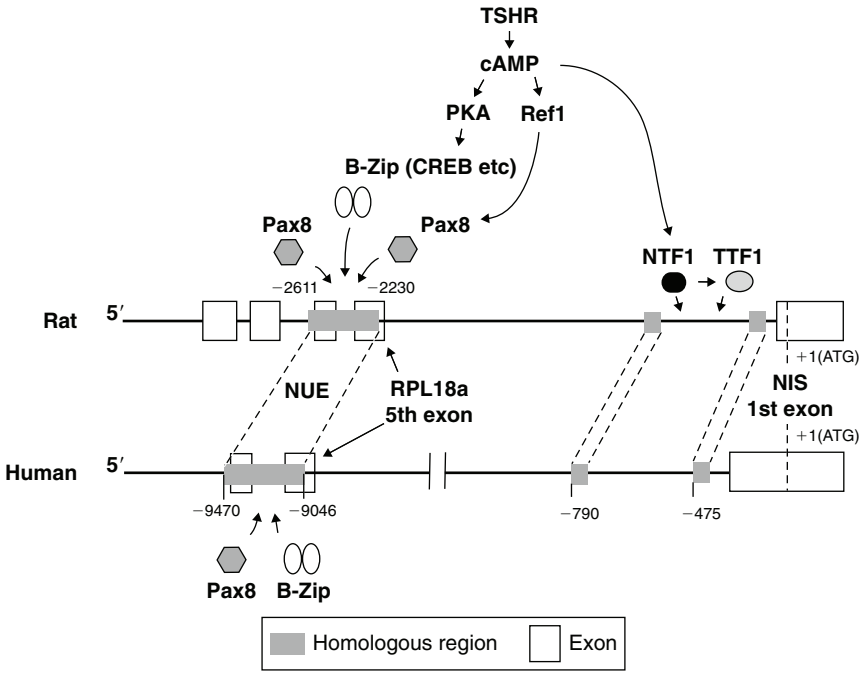

Figure 23.1 Comparison of the human and rat NIS gene structure. Three homologous regions (more than 60% homology) are shown in the 5′-flanking region. The TSH stimulatory signaling pathway to the NIS gene in thyroid cells is shown. NUE, NIS far upstream enhancer; RPL18a, ribosomal protein L18a; TSHR, TSH receptor; cAMP, cyclic AMP; CREB, cAMP responsive element binding protein; B-Zip, basic-leucine zipper transcription factor; Ref1, redox factor-1; Pax8, paired-domain containing transcription factor-8; TTF1, homeodomain containing thyroid transcription factor-1; NTF1, NIS TSH-responsive factor-1.

including the *NIS* proximal promoter and the NUE, a strong TSH-responsive enhancer (Kogai *et al.*, 2006). The human genomic sequence of *NIS* and *RPL18a* contains no consensus sequence for insulator, suggesting these genes are a single transcription regulatory unit. The NUE is located in an intron of *RPL18a*; however, the element up-regulates the *NIS* promoter, but has no effect on the *RPL18a* promoter (Taki *et al.*, 2002).

Regulation of NIS Proximal Promoter in Thyroid Cells

Both Pax-8 (a paired-domain containing transcription factor) and homeodomain containing thyroid transcription factor-1 (TTF-1) (or Nkx-2.1, a homeodomain containing thyroid transcription factor) are required for thyroid development and differentiation. These factors regulate the NIS promoter/enhancer in thyroid cells, although there is some variation among species (Kogai *et al.*, 2006). The rat NIS proximal promoter, but not human NIS promoter, contains a TSH-responsive element that binds NTF-1 (NIS TSH-responsive factor-1). TTF-1 also stimulates the rat proximal promoter (Endo *et al.*, 1997). NTF-1 is required for TTF-1-mediated thyroid-specific NIS gene expression (Ohmori *et al.*, 1998). The human NIS promoter, however, does not have the functional *cis*-elements for NTF-1/TTF-1. Since the human NIS proximal promoter does not respond to TSH stimulation (Kogai *et al.*, 2001), the thyroid-selective NUE is likely critical for the expression of NIS in human thyroid cells.

Characterization of the NIS Upstream Enhancer

The NUE responds strongly to TSH and cAMP stimulation in thyroid cells (Kogai *et al.*, 2006). In the rodent NIS gene, the NUE contains two Pax-8 elements (PA and PB, see **Figure 23.2**). A cAMP-responsive element (CRE)-like sequence is located between the two Pax-8 elements (Ohno *et al.*, 1999). In the human NUE, one of the Pax-8 elements downstream of the CRE is not present (Taki *et al.*, 2002) (**Figure 23.2**). Both the Pax-8 element(s) and the CRE-like sequence are required for the NUE activity (Taki *et al.*, 2002). TSH and cAMP agonists significantly activate the NUE through both the Pax-8 element and the CRE-like sequence in human and rodent cells (Taki *et al.*, 2002; Ohno *et al.*, 1999).

cAMP activates both protein kinase-A (PKA)-dependent pathways and PKA-independent pathways in thyroid cells. PKA phosphorylates CREB (cAMP-responsive element binding protein) and other B-ZIP (basic-leucine zipper) proteins, such as ATF1 and CREM (cAMP response element modulator). The B-ZIP proteins form dimers, bind to the CRE-like sequence of the NUE, and likely stimulate the NUE. TSH/cAMP induces both cytoplasm-to-nucleus translocation and transcription of the antioxidative redox factor-1 (Ref-1), which activates Pax-8 by its reduction. The induction of Pax-8 expression by TSH also has been reported in dog thyroid cells (Van Renterghem *et al.*, 1996).

Thyroid-specific genes, *Tg* (thyroglobulin) and *TPO* (thyroperoxidase), require both Pax-8 and TTF-1 for maximal gene expression (Kogai *et al.*, 2006). A critical role of Pax-8 for TSH regulation of NIS through the NUE has been established (Ohno *et al.*, 1999; Taki *et al.*, 2002), while the role of TTF-1 in human NIS gene expression is likely to be less important.

Transcriptional Regulation of NIS in Thyroid Cancer

Some thyroid papillary cancer cells express reduced NIS mRNA, likely due to the reduced activity of the NIS proximal promoter (Kogai *et al.*, 2001). Binding of nuclear factor(s) to the region −596 to −415 is decreased or absent in a papillary cancer cell line, compared to that in the FRTL-5 normal rat thyroid cell line (Kogai *et al.*, 2001). Over-expression of exogenous antioxidative factor Ref-1 increases the NIS proximal promoter activity (Puppin *et al.*, 2004), suggesting that oxidation of a *trans*-acting factor(s) reduces the NIS promoter activity in some papillary thyroid cancer cells.

Pax-8, one of the main regulators of the NUE, is down-regulated in some thyroid cancer cells (Kogai *et al.*, 2006). Endogenous Pax-8 expression is markedly reduced in 70% of differentiated thyroid cancers, especially in aggressive disease (Fabbro *et al.*, 1994). Colocalization of Ref-1 and

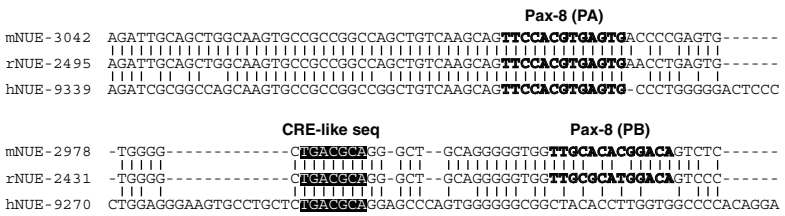

Figure 23.2 Comparison of the NIS upstream enhancer (NUE) sequences of humans and rodents. The human NUE does not contain a Pax-8 element (PB). Reproduced from Kogai *et al.*, (2006). Copyright, Society for Endocrinology 2006.

Pax-8 in the nucleus is likely required for the full activation of Pax-8. Decreased Ref-1 localization in the nuclei, however, has been observed in some thyroid cancer cells and tissues (Russo *et al.*, 2001). In addition, somatic rearrangements of the RET receptor (rearranged in transformation/papillary thyroid carcinoma, RET/PTC), frequently observed in papillary thyroid cancer, impair the activity of Pax-8 (De Vita *et al.*, 1998).

The PKA-dependent pathway is also down-regulated in some thyroid cancer cells. Reduced localization of PKA to the nucleus has been observed in PCCl3 rat thyroid cells constitutively expressing RET/PTC1 (Venkateswaran *et al.*, 2004). A study of specimens from 20 patients with thyroid cancer indicates that CREB mRNA and protein expression is significantly reduced in differentiated thyroid cancer tissue compared to normal tissue (Luciani *et al.*, 2003). The level of CREB expression, however, is not correlated with the NIS expression (Luciani *et al.*, 2003).

NIS Gene Regulation in Breast Cancer

NIS gene regulation in lactating breast tissue and breast cancer has been partially characterized. Oxytocin, a critical regulator of NIS in lactating mammary glands, activates the cAMP/PKA pathway and/or the inositol triphosphate-Ca^{2+} pathway. 8-bromoadenosine-cAMP or endogenous cAMP inducer, IBMX, and cholera toxin increase NIS expression in MCF-7 breast cancer cells, demonstrating a role for the cAMP pathway in mammary gland NIS expression (Knostman *et al.*, 2004).

Phosphoinositide-3 kinase (PI3K) is generally up-regulated in breast cancer. Transgenic mouse models of breast cancer, over-expressing HER2/neu (human epidermal growth factor receptor 2) or PyVT (Polyoma virus middle T antigen) targeted to breast tissue, express modest NIS (Tazebay *et al.*, 2000; Kogai *et al.*, 2004; Knostman *et al.*, 2004). HER2/neu and PyVT have a tyrosine kinase domain and activate PI3K. Expression of constitutively activating PI3K increases NIS expression in MCF-7 cells (Knostman *et al.*, 2004). The PI3K, therefore, could contribute to the NIS expression in some breast cancer.

Retinoic acid (RA) markedly induces NIS in some breast cancer cells, including MCF-7 (Kogai *et al.*, 2000b). RA-stimulated MCF-7 cells express three isoforms of retinoic acid receptor (RAR): α, β, and γ. An RAR β/γ agonist (AGN190168) is a more potent inducer of functional NIS expression than AGN195183 (RARα agonist) and AGN194433 (RARγ agonist), suggesting a central role of RARβ in NIS induction by retinoids (Kogai *et al.*, 2005). RAR with its ligand forms a heterodimer with retinoid X receptor (RXR), and acts as a *trans*-acting factor, stimulating a target gene through a retinoic acid response element (RARE). The consensus sequence of RARE contains two of the core motifs, 5′-PuG[G/T][T/A]CA-3′, directly repeating with a spacer of two bases or five bases (DR-2 or DR-5). The human NIS gene revealed two consensus DR-2 elements (AGGTCAggAGTTCA) in the 1st intron. These putative DR-2 elements, however, do not respond to all-*trans* RA in MCF-7 cells. Interestingly, the DR-5, but not the DR-2, responds to the all-*trans* RA stimulation in MCF-7 cells, although no consensus sequence of the DR-5 RARE has been found around the human NIS gene (Kogai *et al.*, 2008). On the other hand, RA induces the cardiac homeobox transcription factor, Nkx-2.5, in MCF-7 cells. Since Nkx-2.5 stimulates the rat proximal promoter in MCF-7 cells, RA possibly regulates the NIS gene through Nkx-2.5 (Dentice *et al.*, 2004), but not through the RARE on the NIS gene.

Enhancement of the NIS Expression in Tissue Culture Cells

Extensive experience with ^{131}I treatment of thyroid cancer has demonstrated the importance of maximizing the magnitude of specific radioiodide uptake in tumors. The regulation of NIS expression in normal and malignant thyroid cells, therefore, has been widely investigated. Various agents have been recognized to influence NIS expression in thyroid cells, and are summarized in **Table 23.1**. Lactating mammary glands express functional NIS at a high level. Stimulation of NIS expression has been investigated in normal breast and in breast cancer. Many "redifferentiation agents" have been identified that induce NIS expression in breast cancer cells (**Table 23.2**).

TSH Receptor Stimulation in Thyroid Cells

TSH maintains the expression of NIS in the thyroid gland. TSH stimulates NIS mRNA and protein expression in rodent thyroid cell lines, FRTL-5 cells (Kogai *et al.*, 1997), PCCl3 cells (Trapasso *et al.*, 1999), and human primary thyroid cells (Kogai *et al.*, 2000a; Saito *et al.*, 1997). FRTL-5 cells require TSH to grow in monolayer for more than 10 days. Removal of TSH significantly decreases iodide uptake and NIS expression on plasma membrane in about 3 days (Riedel *et al.*, 2001). The addition of TSH to the quiescent FRTL-5 cells markedly induces the NIS mRNA expression within 6 h, reaching the maximum in 24 h (Kogai *et al.*, 1997). The maximum induction of iodide uptake, 20–30 fold above baseline, requires 60–72 h of treatment with TSH (**Figure 23.3a**) (Kogai *et al.*, 1997). Translocation of NIS to the plasma membrane is also stimulated by TSH in FRTL-5 rat thyroid cells (Riedel *et al.*, 2001). Stimulators of the cyclic AMP pathway, forskolin and $(Bu)_2$cAMP, mimic the effects of TSH on NIS expression, indicating that the effects of TSH are mediated through the cAMP pathway (Kogai *et al.*, 1997). Direct stimulation of TSHR by autoantibody in Graves'

Table 23.1 Agents that stimulate NIS expression in thyroid

Agent	*Pharmacology*	*Experimental system*	*Iodide uptake*	*NIS mRNA*	*NIS protein*
Bovine TSH	TSHR agonist	FRTL5 rat cell line	X	X	X
		PCCl3 rat cell line	X	X	
		WRT rat cell line			X
		Primary human thyroid cell culture (normal)	X	X	X
		KAT50 human cell line	X	X	
		Long-term culture of human thyroid (normal)	X	X	X
		Rat normal thyroid, *in vivo*			X
hCG	TSHR agonist	FRTL5 rat cell line	X	X	X
Forskolin	Adenylyl cyclase activator	FRTL5 rat cell line	X	X	X
		PCCl3 rat cell line		X	
		Primary human thyroid cell culture (normal)	X	X	X
Adenosine	A_1 receptor agonist	FRTL5 rat cell line	X	X	X
All *trans* RA	RAR agonist	FTC133 and 238, follicular cancer cell lines		X	
Troglitazone	PPARγ ligand	FTC133, follicular cancer cell line		X	
		TPC1, papillary cancer cell line		X	
Depsipeptide	HDAC inhibitor	FTC133 and 236, follicular cancer cell lines	X	X	
		SW1736 and KAT4, anaplastic cancer cell lines	X	X	
		BHP18–21v, papillary cancer cell line	X	X	X
		BHP18–21v xenograft *in vivo*	X		
		ARO, anaplastic cancer cell line	X	X	X
Trichostatin A	HDAC inhibitor	TPC1, papillary cancer cell lines		X	
		FTC133, follicular cancer cell line		X	
		XTC1, Hurthle cell cancer cell line		X	
		BHP18–21v, papillary cancer cell line		X	X
		ARO, anaplastic cancer cell line		X	X
Valproic acid	HDAC inhibitor	NPA, papillary cancer cell line	X	X	X
		ARO, anaplastic cancer cell line		X	X
5-azacytidine	Demethylation agent	NPA, KAT5, KAT10, papillary cancer cell lines	X	X	
Clinical use					
hrTSH		Metastatic/recurrent cancer (50%–90%)	X		
13-*cis* RA	Pro-drug of tRA	Metastatic/recurrent cancer (0–42%)	X		

Note: Agents that significantly induce NIS are shown with an X. TSH, thyroid-stimulating hormone; TSHR, TSH receptor; hCG, human chorionic gonadotropin; RA, retinoic acid; RAR, retinoic acid receptor; PPAR, peroxisome proliferator-activated receptor; HDAC, histone deacetylase; hrTSH, human recombinant TSH. Reproduced from Kogai *et al.,* (2006). Copyright, Society for Endocrinology (2006).

Table 23.2 NIS expression stimulator in breast tissues

Agent	*Experimental system*	*Iodide uptake*[a]	*NIS mRNA*	*NIS protein*	*Note*
Oxytocin	Rat normal breast, *in vivo*	X		X	E_2 required
	Cancer primary culture (3-D)		X		
Prolactin	Rat normal breast, *in vivo*	X		X	E_2 required
	Mouse breast explant	X		X	
	Cancer primary culture (3-D)		X		
	MCF-7, ER+ cancer cell line	~10[b]	X	X	
Estradiol	Rat normal breast, *in vivo*	X		X	
8-Bromo-cAMP, cholera toxin	MCF-7, ER+ cancer cell line	~3.3[b]	X		
hCG	MCF-7, ER+ cancer cell line	~3.0[c]			
Prostaglandin-E_2	MCF-7, ER+ cancer cell line	~2.3[c]			
Insulin/IGF-I/IGF-II	MCF-7, ER+ cancer cell line	11–14[b]	X		
all *trans* RA	MCF-7, ER+ cancer cell line	~9.2[d]	X	X	
	MCF-7 xenograft *in vivo*	X	X	X	
	MMTV-PyVT *in vivo*	X	X		
Dex + RA	MCF-7, ER+ cancer cell line	12–18[e]	X		Synergistic effect with RA
AGN190168 (RARβ/γ ligand)	MCF-7, ER+ cancer cell line	~9.3[f]	X		Long duration
AGN194433 (RARγ agonist)	MCF-7, ER+ cancer cell line	~4.0[f]			
AGN197496, 195183 (RARα ligands)	MCF-7, ER+ cancer cell line	~3.0[g], ~3.3[f]	X		

(*Continued*)

Table 23.2 (Continued)

Agent	*Experimental system*	*Iodide uptake*[a]	*NIS mRNA*	*NIS protein*	*Note*
AGN195203, 194204, 196060 (RXR ligands)	MCF-7, ER+ cancer cell line	~2.5[g], ~6.0[f]	X		
9-*cis* RA	MCF-7, ER+ cancer cell line	~14[g], ~9.0[d]	X		
	T47D, ER+ cancer cell line		X		
	BT474, ER+ cancer cell line		X		
Troglitazone (PPARγ ligand) + RA	MCF-7, ER+ cancer cell line	~9.6[h]	X		Synergistic effect with RA

Note: X in column indicates significant induction by agent. Reproduced from Kogai *et al.,* (2006). Copyright, Society for Endocrinology (2006). Abbreviations: 3-D, three-dimensional; ER, estrogen receptor; E2, 17β-estradiol; cAMP, 3′–5′-cyclic adenosine monophosphate; hCG, human chorionic gonadotropin; IGF, insulin-like growth factor; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid-X receptor; PPAR, peroxisome proliferator-activated receptor.

[a]Fold induction compared to without treatment is shown for data from MCF-7 cells.

[b]The induction at 12 h. Cells were maintained in DMEM with 0.2% FBS.

[c]The induction at 24 h (PGE2) or 48 h (hCG). Cells were maintained in DMEM: F12, 50:50 with 10% FBS.

[d]The duration is 36–72 h. Cells were maintained in MEM with 10% FBS or serum replacement.

[e]The duration is 2–4 days with 10^{-7} M tRA or 2–5 days with 10^{-6} M AG190168. Cells were maintained in MEM with 10% FBS or serum replacement.

[f]The induction at 48 h. Cells were maintained in MEM with 10% FBS or serum replacement.

[g]The induction at 24 h.

[h]The induction at 24 h with 10^{-7} M 9-*cis* RA.

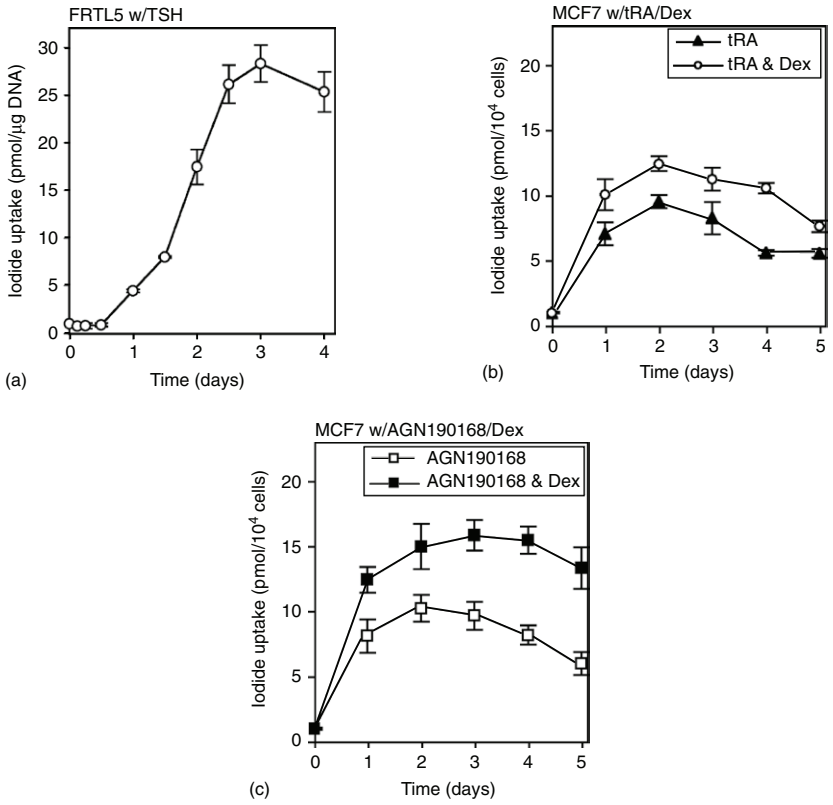

Figure 23.3 Induction of iodide uptake in FRTL-5 rat thyroid cells and MCF-7 breast cancer cells. Iodide uptake was measured with ^{125}I and 10 μM of sodium iodide, and normalized with DNA content (a); or cell number (b and c). (a) TSH (1 mU/ml) significantly induces iodide uptake in FRTL-5 cells. Although the growth medium for FRTL-5 cells contains TSH, cells were grown without TSH for 8 days before the TSH treatment. Reproduced from Kogai *et al.,* (1997). Copyright 1997, The Endocrine Society. (b and c) Effects of long-term treatment with a retinoid, a pan-RAR agonist all-*trans* RA (tRA) or an RAR β/γ ligand tazarotene (AGN190168), and dexamethasone (Dex) on iodide uptake in MCF-7 cells. RAR stimulator markedly induces iodide uptake in MCF-7 cells. The duration and magnitude of the NIS induction by RA is markedly increased by the addition of Dex, especially in combination with an RAR β/γ ligand AGN190168. Reproduced from Kogai *et al.,* (2005). Copyright 2005, The Endocrine Society.

disease, constitutive activating mutations of TSHR in a hyperfunctioning thyroid adenoma, or the weak agonist human chorionic gonadotropin (hCG) (Hershman, 1999) activates the cAMP pathway and results in marked NIS expression.

More than 70% of differentiated thyroid cancer concentrates radioiodine after TSH stimulation (Robbins *et al.*, 1991; Jarzab *et al.*, 2003). Some differentiated thyroid cancer (approximately 10–20%), as well as anaplastic thyroid cancer, however, do not concentrate radioiodide, even after TSH stimulation (Robbins *et al.*, 1991). Since almost all differentiated thyroid cancer expresses TSHR (Brabant *et al.*, 1991), the absence of NIS induction in response to TSH is most likely due to defects in postreceptor signaling pathways. Recent studies have demonstrated the potential for NIS induction in poorly differentiated thyroid cancer by "redifferentiation" agents, such as nuclear receptor ligands, RA and peroxisome proliferator-activated receptor-γ (PPARγ) ligands, and inhibitors of epigenetic modifications.

Effects of Redifferentiation Agents on Thyroid NIS

RA induces NIS mRNA expression in two follicular thyroid cancer cell lines, FTC-133 and FTC-238, but not in rat thyroid cells (Schmutzler *et al.*, 1997). Based on the findings, clinical trials have been conducted to evaluate the efficacy of RA for improving radioiodide uptake in recurrent/metastatic thyroid cancer. Twenty to 42% of aggressive differentiated thyroid cancer responds to RA treatment by an increase in radioiodide uptake (Kogai *et al.*, 2006). The studies, however, have not been randomized prospective studies of matched groups that would be necessary to confirm an effect of RA treatment.

Troglitazone, a PPARγ ligand, also significantly increases the NIS mRNA in some differentiated thyroid cancer cell lines, FTC-133 and TPC-1 (Park *et al.*, 2005). Troglitazone inhibits cell proliferation and induces apoptosis in some papillary thyroid cancer cell lines *in vitro* and *in vivo* (Ohta *et al.*, 2001). Although troglitazone is no longer available for clinical use, a combination of PPARγ agonist and radioiodide therapy might provide a synergistic inhibitory effect on some thyroid cancers.

Histone deacetylase inhibitors, depsipeptide (FR901228), trichostatin A (TSA), and valproic acid, increase NIS expression in thyroid cancer cell lines (Kitazono *et al.*, 2001; Kogai *et al.*, 2006). Depsipeptide significantly induces NIS mRNA and iodide uptake in follicular thyroid cancer cell lines (FTC 133 and FTC 236) and two anaplastic cancer cell lines (SW-1736 and KAT-4) at a low concentration (1 ng/ml) *in vitro* (Kitazono *et al.*, 2001). Pharmacokinetics of depsipeptide in patients have indicated that levels of more than 500 ng/ml are achieved without significant toxicity, promising to obtain the NIS-inducible concentration in patients (Kitazono *et al.*, 2001).

The human NIS gene has three CpG-rich regions around the translation start site: the core promoter region (about 100 bp from the transcription start site), the 5′-untranslated region, and the coding region of the 1st exon (Venkataraman *et al.*, 1999). The demethylation agent, 5-azacytidine, restores NIS mRNA expression and iodide uptake in three papillary cancer cell lines, NPA, KAT-5, and KAT-10, but not in two follicular cancer cell lines, MRO and WRO (Venkataraman *et al.*, 1999). A correlation has been observed between the successful restoration of NIS expression by 5-azacytidine and demethylation of the 5′-untranslated region (Venkataraman *et al.*, 1999).

NIS Expression in Lactating Mammary Glands

NIS is predominantly expressed on the basolateral membrane of alveolar cells in mammary glands (Spitzweg *et al.*, 1998) and markedly induced during lactation (Tazebay *et al.*, 2000; Cho *et al.*, 2000). Treatment of the mice with the combination of oxytocin, prolactin and estradiol markedly induces NIS in mammary glands, while each hormone alone is not sufficient for NIS induction (Tazebay *et al.*, 2000). Basal levels of these three hormones are significantly increased in late pregnancy, and the lactogenic hormones, prolactin and oxytocin, are still elevated during the first few months of the postpartum period. The surge of oxytocin during lactation is required for maximum induction of NIS in mammary glands.

Enhancement of NIS Expression in Breast Cancer Cells

More than 80% of breast cancer tissues express NIS, although the fraction of tumors that functionally concentrate iodine is likely to be much lower (Wapnir *et al.*, 2003). Agents that stimulate NIS expression in breast cancer sufficient to concentrate radioiodide, therefore, have been considered as a potential therapy for some differentiated breast cancer (Boelaert and Franklyn, 2003). Recent *in vitro* studies have demonstrated significant induction of NIS in breast cancer cells by lactogenic hormones, insulin, and some nuclear receptor ligands, such as retinoids, PPARγ ligands, and glucocorticoids (Kogai *et al.*, 2006) (**Table 23.2**).

Prolactin and oxytocin treatment induces NIS mRNA in some human breast cancer tissues cultured primarily on collagen gel, while the combination of these hormones does not produce an additive effect (Cho *et al.*, 2000). A significant induction of iodide uptake and NIS mRNA by prolactin has been reported in MCF-7 breast cancer cells

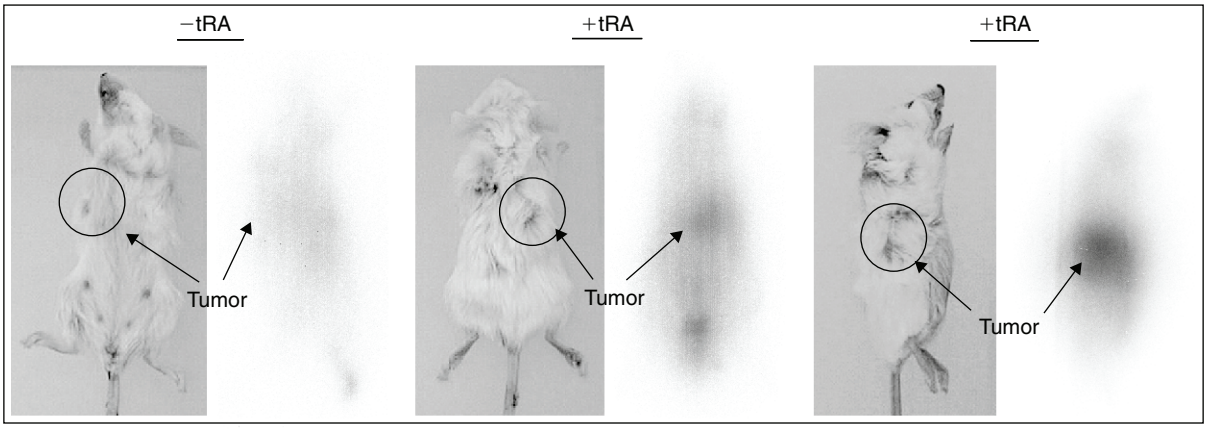

Figure 23.4 Imaging of the MCF-7 xenograft tumors with ^{125}I. In animals treated with systemic all-*trans* RA (tRA, 160 mg/kg/day) for 5 days, the tumor was visualized 2 h after the administration of ^{125}I. Reproduced from Kogai *et al.,* (2004) with permission from The American Association for Cancer Research.

(Arturi *et al.*, 2005). The duration of the prolactin induction, however, was relatively short with maximum iodide uptake at 12 h, but reduced at 24 h.

RAR agonists strongly induce functional NIS in MCF-7 cells. All-*trans* RA and the synthetic RAR ligands, TTNPB and tazarotene (AGN190168), markedly induce iodide uptake in MCF7 cells, up to 15-fold above baseline, which is sustained for more than 5 days (**Figure 23.3b, c**) (Kogai *et al.*, 2005). The induction is faster than that seen in FRTL-5 cells in response to TSH; NIS mRNA and iodide uptake reach the maximum around 12 h and 36–48 h (**Figure 23.3b, c**), respectively (Kogai *et al.*, 2000b). Tumor-selective induction of functional NIS and its mRNA expression has been confirmed in mouse breast cancer models, MCF-7 xenograft tumors (**Figure 23.4**), and transgenic mice over-expressing the PyVT oncogene in breast tissue (Kogai *et al.*, 2004). Isomers of all-*trans* RA, 9-*cis* RA, and 13-*cis* RA also significantly induce NIS expression in MCF-7 cells, as that seen with all-*trans* RA. Since the effect of RXR agonists on NIS expression is markedly reduced compared to that of RAR agonists, the effects of RA isomers is likely through RAR, with *trans* RA converted from these isomers by endogenous isomerases (Kogai *et al.*, 2005).

Glucocorticoid receptor ligands significantly increase RA-induced iodide uptake and NIS mRNA, and prolong the induction of iodide uptake (**Figure 23.3b, c**) (Kogai *et al.*, 2005; Dohan *et al.*, 2006; Unterholzner *et al.*, 2006). The addition of dexamethasone (Dex) reduces the median effective concentration (EC_{50}) of RA for the induction of iodide uptake to ~7% (Kogai *et al.*, 2005). The *in vivo* systemic dose of all-*trans* RA for maximum induction of NIS is quite high. The combination treatment with Dex, therefore, has potential to reduce the dose of RA, resulting in less toxicity *in vivo*. Interestingly, the duration of iodide uptake and NIS mRNA with an isoform-specific retinoid receptor agonist is significantly longer than that with all-*trans* RA, especially in combination with Dex (Kogai *et al.*, 2005). A recent preliminary *in vivo* study, however, indicated the NIS induction by Dex in both normal and tumor tissues in a rodent breast cancer xenograft model, limiting its possible clinical application (Willhauck *et al.*, 2006). Further study is required for more specific induction of NIS in breast cancer.

NIS Expression in Placenta-Derived Cells

Placenta expresses NIS to transport iodide from the maternal circulation to the fetus. Endogenous NIS expression has been reported in two placental-derived choriocarcinoma cell lines, JAr (Mitchell *et al.*, 2001) and BrWo (Manley *et al.*, 2005). hCG increases the NIS mRNA expression and iodide uptake in JAr cells, partially through cAMP pathway (Arturi *et al.*, 2002).

Summary Points

- The primary cell signaling pathways that stimulate NIS expression differ in thyroid and lactating breast, although there is some overlap.
- The goal in thyroid cancer treatment is to optimize iodine uptake. A number of different strategies and agents have been used to stimulate NIS expression and iodine uptake in refractory thyroid cancer, although TSH remains the most potent stimulus.
- Basal NIS expression in breast cancer is relatively low, but RA and other compounds stimulate NIS expression and iodine uptake in some breast cancer cells.
- Improved understanding of NIS regulatory pathways in thyroid and breast cancer should lead to additional therapeutic options.

Acknowledgements

This study was supported by NIH RO1 CA089364 and VA merit review funds.

References

Arturi, F., Ferretti, E., Presta, I., Mattei, T., Scipioni, A., Scarpelli, D., Bruno, R., Lacroix, L., Tosi, E., Gulino, A., Russo, D. and Filetti, S. (2005). J. Clin. Endocrinol. Metab. 90, 2321–2326.

Arturi, F., Lacroix, L., Presta, I., Scarpelli, D., Caillou, B., Schlumberger, M., Russo, D., Bidart, J.M. and Filetti, S. (2002). Endocrinology 143, 2216–2220.

Boelaert, K. and Franklyn, J.A. (2003). Lancet 361, 796–797.

Brabant, G., Maenhaut, C., Kohrle, J., Scheumann, G., Dralle, H., Hoang-Vu, C., Hesch, R.D., Von Zur Muhlen, A., Vassart, G. and Dumont, J.E. (1991). Mol. Cell. Endocrinol. 82, R7–R12.

Cho, J.Y., Leveille, R., Kao, R., Rousset, B., Parlow, A.F., Burak, W.E., Jr., Mazzaferri, E.L. and Jhiang, S.M. (2000). J. Clin. Endocrinol. Metab. 85, 2936–2943.

Dentice, M., Luongo, C., Elefante, A., Romino, R., Ambrosio, R., Vitale, M., Rossi, G., Fenzi, G. and Salvatore, D. (2004). Mol. Cell. Biol. 24, 7863–7877.

De Vita, G., Zannini, M., Cirafici, A.M., Melillo, R.M., Di Lauro, R., Fusco, A. and Santoro, M. (1998). Cell Growth Differ. 9, 97–103.

Dohan, O., De La Vieja, A. and Carrasco, N. (2006). Mol. Endocrinol. 20, 1121–1137.

Endo, T., Kaneshige, M., Nakazato, M., Ohmori, M., Harii, N. and Onaya, T. (1997). Mol. Endocrinol. 11, 1747–1755.

Fabbro, D., Di Loreto, C., Beltrami, C.A., Belfiore, A., Di Lauro, R. and Damante, G. (1994). Cancer Res. 54, 4744–4749.

Hershman, J.M. (1999). Thyroid 9, 653–657.

Jarzab, B., Handkiewicz-Junak, D., Roskosz, J., Puch, Z., Wygoda, Z., Kukulska, A., Jurecka-Lubieniecka, B., Hasse-Lazar, K., Turska, M. and Zajusz, A. (2003). Eur. J. Nucl. Med. Mol. Imaging 30, 1077–1086.

Kitazono, M., Robey, R., Zhan, Z., Sarlis, N.J., Skarulis, M.C., Aikou, T., Bates, S. and Fojo, T. (2001). J. Clin. Endocrinol. Metab. 86, 3430–3435.

Knostman, K.A., Cho, J.Y., Ryu, K.Y., Lin, X., Mccubrey, J.A., Hla, T., Liu, C.H., Di Carlo, E., Keri, R., Zhang, M., Hwang, D.Y., Kisseberth, W.C., Capen, C.C. and Jhiang, S.M. (2004). J. Clin. Endocrinol. Metab. 89, 5196–5203.

Kogai, T., Curcio, F., Hyman, S., Cornford, E.M., Brent, G.A. and Hershman, J.M. (2000a). J. Endocrinol. 167, 125–135.

Kogai, T., Endo, T., Saito, T., Miyazaki, A., Kawaguchi, A. and Onaya, T. (1997). Endocrinology 138, 2227–2232.

Kogai, T., Hershman, J.M., Motomura, K., Endo, T., Onaya, T. and Brent, G.A. (2001). Endocrinology 142, 3369–3379.

Kogai, T., Kanamoto, Y., Che, L.H., Taki, K., Moatamed, F., Schultz, J.J. and Brent, G.A. (2004). Cancer Res. 64, 415–422.

Kogai, T., Kanamoto, Y., Li, A.I., Che, L.H., Ohashi, E., Taki, K., Chandraratna, R.A., Saito, T. and Brent, G.A. (2005). Endocrinology 146, 3059–3069.

Kogai, T., Ohashi, E., Jacobs, M.S., Sajid-Crockett, S., Fisher, M.L., Kanamoto, Y. and Brent, G.A.(2008). J. Clin. Endocrinol. Metab. 93, 1884–1892.

Kogai, T., Schultz, J.J., Johnson, L.S., Huang, M. and Brent, G.A. (2000b). Proc. Natl. Acad. Sci. USA 97, 8519–8524.

Kogai, T., Taki, K. and Brent, G.A. (2006). Endocr. Relat. Cancer 13, 797–826.

Luciani, P., Buci, L., Conforti, B., Tonacchera, M., Agretti, P., Elisei, R., Vivaldi, A., Cioppi, F., Biliotti, G., Manca, G., Vitti, P., Serio, M. and Peri, A. (2003). Eur. J. Endocrinol. 148, 579–586.

Manley, S.W., Li, H. and Mortimer, R.H. (2005). Placenta 26, 380–386.

Mitchell, A.M., Manley, S.W., Morris, J.C., Powell, K.A., Bergert, E.R. and Mortimer, R.H. (2001). Placenta 22, 256–258.

Ohmori, M., Endo, T., Harii, N. and Onaya, T. (1998). Mol. Endocrinol. 12, 727–736.

Ohno, M., Zannini, M., Levy, O., Carrasco, N. and Di Lauro, R. (1999). Mol. Cell. Biol. 19, 2051–2060.

Ohta, K., Endo, T., Haraguchi, K., Hershman, J.M. and Onaya, T. (2001). J. Clin. Endocrinol. Metab. 86, 2170–2177.

Park, J.W., Zarnegar, R., Kanauchi, H., Wong, M.G., Hyun, W.C., Ginzinger, D.G., Lobo, M., Cotter, P., Duh, Q.Y. and Clark, O.H. (2005). Thyroid 15, 222–231.

Puppin, C., Arturi, F., Ferretti, E., Russo, D., Sacco, R., Tell, G., Damante, G. and Filetti, S. (2004). Endocrinology 145, 1290–1293.

Riedel, C., Levy, O. and Carrasco, N. (2001). J. Biol. Chem. 276, 21458–21463.

Robbins, J., Merino, M.J., Boice, J.D., Jr., Ron, E., Ain, K.B., Alexander, H.R., Norton, J.A. and Reynolds, J. (1991). Ann. Intern. Med. 115, 133–147.

Russo, D., Arturi, F., Bulotta, S., Pellizzari, L., Filetti, S., Manzini, G., Damante, G. and Tell, G. (2001). J. Endocrinol. Invest. 24, RC10–RC12.

Saito, T., Endo, T., Kawaguchi, A., Ikeda, M., Nakazato, M., Kogai, T. and Onaya, T. (1997). J. Clin. Endocrinol. Metab. 82, 3331–3336.

Schmutzler, C., Winzer, R., Meissner-Weigl, J. and Kohrle, J. (1997). Biochem. Biophys. Res. Commun. 240, 832–838.

Smanik, P.A., Ryu, K.Y., Theil, K.S., Mazzaferri, E.L. and Jhiang, S.M. (1997). Endocrinology 138, 3555–3558.

Spitzweg, C., Joba, W., Eisenmenger, W. and Heufelder, A.E. (1998). J. Clin. Endocrinol. Metab. 83, 1746–1751.

Taki, K., Kogai, T., Kanamoto, Y., Hershman, J.M. and Brent, G.A. (2002). Mol. Endocrinol. 16, 2266–2282.

Tazebay, U.H., Wapnir, I.L., Levy, O., Dohan, O., Zuckier, L. S., Zhao, Q.H., Deng, H.F., Amenta, P.S., Fineberg, S., Pestell, R.G. and Carrasco, N. (2000). Nat. Med. 6, 871–878.

Trapasso, F., Iuliano, R., Chiefari, E., Arturi, F., Stella, A., Filetti, S., Fusco, A. and Russo, D. (1999). Eur. J. Endocrinol. 140, 447–451.

Unterholzner, S., Willhauck, M.J., Cengic, N., Schutz, M., Goke, B., Morris, J.C. and Spitzweg, C. (2006). J. Clin. Endocrinol. Metab. 91, 69–78.

Van Renterghem, P., Vassart, G. and Christophe, D. (1996). Biochim. Biophys. Acta 1307, 97–103.

Venkateswaran, A., Marsee, D.K., Green, S.H. and Jhiang, S.M. (2004). J. Clin. Endocrinol. Metab. 89, 6168–6172.

Venkataraman, G.M., Yatin, M., Marcinek, R. and Ain, K.B. (1999). J. Clin. Endocrinol. Metab. 84, 2449–2457.

Wapnir, I.L., Van De Rijn, M., Nowels, K., Amenta, P.S., Walton, K., Montgomery, K., Greco, R.S., Dohan, O. and Carrasco, N. (2003). J. Clin. Endocrinol. Metab. 88, 1880–1888.

Willhauck, M.J., Sharif Samani, B.R., Senekowitsch-Schmidtke, R., Goke, B., Morris, J.C. and Spitzweg, C. (2006). *Annual Meeting of the American Thyroid Association.* Phoenix, AZ.