



Expression of Sodium Iodide Symporter in Breast Cancer Tissue after Thyroid Cancer

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Background and Objectives: Radioactive iodine (RAI) therapy may be associated with the risk of breast cancer (BC) among thyroid cancer (TC) patients, and sodium iodide symporter (NIS) is thought to mediate this association. This study examined NIS expression and hormone receptors in BC that occurred after TC (TC→BC cases). **Materials and Methods:** Tissue microarrays with paired BC and normal breast tissues were constructed from 39 of TC→BC cases with a latency period ≥ 2 years and 36 age-matched BC patients. The expression of NIS, estrogen receptor (ER α and ER β), and thyroid hormone receptor (TR α and TR β) was evaluated by immunohistochemistry. **Results:** The frequency of NIS expression was higher in TC→BC cases compared with BC controls across both cancer tissues (61.8% vs. 21.9%, $p=0.001$) and normal (55.3% vs. 27.6%, $p=0.028$) tissues. In TC→BC cases, NIS expression was associated with prior RAI therapy while the overall free thyroxine levels measured during the latent period showed no association. ER α and TR α expression was more frequently observed in TC→BC cases, and their expression was associated with NIS expression independently of RAI therapy in cancer tissues. **Conclusion:** NIS expression was upregulated in BC tissues with known TC, which might be influenced by both prior RAI therapy and the hormone receptor status in BC tissues. Further studies are needed to clarify the molecular mechanism of their association.

Key Words: Sodium iodide symporter, Breast cancer, Thyroid cancer, Radioactive iodine therapy, Estrogen receptor

Introduction

Sodium iodide symporter (NIS) is a membrane-bound glycoprotein that actively transports iodide.⁽¹⁾ NIS is mainly expressed in the thyroid tissues and provides iodine for thyroid hormone synthesis. It mediates highly selective iodide uptake of thyrocytes;

thus, radioactive iodine (RAI) has been widely used for to treat thyroid cancer (TC). Although its levels are low compared to in the thyroid, NIS has been demonstrated in various non-thyroidal tissue including breast tissues, particularly in lactating mammary glands.⁽²⁾ Regarding the physiological role of NIS in the breast, providing sufficient iodine in milk has been proposed

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to be beneficial for the development of newborn.^{3,4)} Interestingly, NIS expression in breast cancer (BC) was reported to be as high as 50%–80%, while expression is only 20%–30% in normal breast tissues.^{2,5)} The regulation of NIS within breast tissue appears largely unaffected by thyroid-stimulating hormone (TSH) or thyroid hormone, which are recognized as the principal regulators within thyroid tissues,^{6,7)} despite their established role as primary regulators in the thyroid,⁸⁾ while estradiol, prolactin, or oxytocin modulate NIS expression in breast tissues.^{2,9)} Recently, estrogen receptor (ER) α was proposed as an important regulator of NIS expression in BC,^{7,10–12)} although its role remains unclear. NIS regulation in BC cells has been under active investigation because of the potential of therapeutic application of RAI treatment for BC.^{13–15)}

In contrast, recent meta-analysis studies^{16,17)} revealed an increased risk of BC among TC survivors. Because RAI therapy is a major modality for TC treatment, concerns regarding the radiation hazards of the RAI therapy on subsequent BC development have increased. Previous studies have shown controversial results; some researchers reported an increased risk of BC following exposure to RAI therapy,^{18–20)} while others, including a study by our group, found that the overall risk of BC did not increase after RAI therapy.^{21–24)} Moreover, patients surviving TC who underwent high-dose RAI treatment experienced a reduction in BC incidence, and RAI exposure correlated with a decreased rate of BC recurrence.²¹⁾ The presence of NIS in BC^{18,21,22)} might serve as an explanation for how RAI influences BC risk, but no studies have examined the expression of NIS in BC developed after TC and its association with RAI therapy.

Regarding the hormonal effects on the increased risk of BC after TC, a role for estrogen,²⁵⁾ thyroid hormone, or thyroid hormone receptor (TR) has been proposed.^{25,26)} We also found higher expression of ER in BC after TC patients (TC→BC cases) than in those with BC alone,²⁷⁾ which is consistent with the results of two recent studies.²⁸⁾ However, the association between the expression of NIS and hormone receptors of BC has not been examined.

This study focused on examining the presence of

NIS expression in patients with BC developed after TC compared to in BC controls. We then evaluated whether NIS expression differed according to the treatments applied for TC, thyroid hormone suppression, and RAI treatment. The association between NIS expression and ER or TR status was also evaluated.

Materials and Methods

Subjects and Clinicopathological Characteristics

Human breast tissues were obtained from 55 previously reported TC→BC cases who were diagnosed with BC after TC during follow-up with an at least 2-year latency period.²⁷⁾ Of these cases, 39 cases (mean age 51.1 ± 9.6 years) were available for preparing formalin-fixed paraffin-embedded blocks. Twenty-seven had total thyroidectomy. Subsequently, 20 of these subjects received RAI therapy, administered at a median dose of 90 mCi (ranging from 60 to 190 mCi) across 1 to 3 cycles following TSH stimulation. Levothyroxine (50–250 $\mu\text{g/day}$; median 150 $\mu\text{g/day}$) was prescribed to all but one patient for a duration spanning 1.8 to 20.4 years (median 4.4 years) leading up to their BC operation. One subject developed Graves' disease within the residual thyroid lobe following their prior lobectomy for TC. This occurred concurrently with the diagnosis of BC, necessitating the administration of methimazole. Additionally, formalin-fixed paraffin-embedded blocks were procured from 36 BC controls who were matched by age (mean age 51.4 ± 11.8 years).

Clinicopathological data for BC were retrospectively collected including age at diagnosis, body mass index (BMI), smoking history, family history of BC, postmenopausal status, parity, type of surgery, and pathologic classification. Furthermore, details regarding pathologic staging were included, specifically covering tumor size, lymph node and distant metastasis, adjuvant therapy administered, the follow-up duration, recurrence status, and the time elapsed until any recurrence was detected. In TC→BC cases, data gathered for analysis encompassed the total RAI dose, the latency period extending from the initial RAI admin-

istration date to the BC diagnosis, the median levothyroxine dose, and the total duration for which levothyroxine was prescribed. All available laboratory thyroid function test results were acquired during the latent period, defined as the entire follow-up duration for TC prior to the BC diagnosis. The representative value for free T4 (FT4) was calculated by averaging all recorded measurements; similarly, TSH levels preceding RAI therapy were determined using the mean, median, and final observed values. Serum concentrations of FT4, TSH, and total triiodothyronine (T3) were quantified via an immunometric assay (Abbott, North Chicago, IL, USA). The established reference ranges for these analytes were as follows: TSH (0.4–4.1 uIU/mL), FT4 (0.70–1.80 ng/dL), and total T3 (87–184 ng/dL). The study protocol was designed in accordance with the guidelines proposed in the Declaration of Helsinki (<http://www.wma.net>) involving humans and was approved by the Institutional Review Board (H 0912-009-302 and H 1303-109-476).

Tissue Microarrays and Immunohistochemical Evaluation

Tissue microarrays pertaining to the breast were generated utilizing a representative, paired specimen set comprising both the BC tissue and the adjacent normal tissue, as previously described.²⁹⁾ Microscopic examinations were performed on hematoxylin and eosin-stained sections, and suitable 2 mm core tissues were subsequently selected from each respective case. Immunohistochemical analysis was executed on the tissue microarray sections using the BenchMark XT immunohistochemistry slide staining system (Ventana, Tucson, AZ, USA). A panel of seven antibodies were utilized for this assay, including those targeting NIS (Thermo Scientific, Waltham, MA, USA; dilution of 1:200), ER α (Thermo Scientific; no dilution), ER β (Biogenex, San Ramon, CA, USA; dilution of 1:300), TR α (Thermo Scientific; dilution of 1:600), TR β (Santa Cruz Biotechnology, Dallas, TX, USA; dilution of 1:800), Ki-67 (Dako, Carpinteria, CA, USA; dilution of 1:100), and phosphatase and tensin homolog (PTEN, Dako; dilution of 1:100). The resulting immunoreactivities for each marker were subjected to independent

evaluation in a blinded fashion by two specialized histopathologists (H.S. Min and Y.A. Kim) and a single trained endocrinologist (Y.A. Kim). This rigorous assessment methodology yielded interobserver correlation coefficients that ranged from 87.7% to 97.2%, which is a highly reliable level.³⁰⁾ Each representative field was taken at five areas at 20X magnification. The expression of NIS was initially evaluated by histochemical score (H-score) by multiplying the staining intensities (either 0 or 1; negative and positive, retrospectively) by the percentage of stained cells (0%–100%). Since NIS showed relatively uniform cytoplasmic and membranous staining or no staining, NIS expression was defined as positive when the H-score was $\geq 50\%$ and negative when it was $< 50\%$ (Fig. 1). The expression of ER or TR was determined by the Allred score.³¹⁾ The quantification of staining intensity was defined by an average scoring system: 0 (absence of staining), 1 (weak), 2 (intermediate), and 3 (strong). Conversely, the proportion score represented the estimated fraction of tumor cells exhibiting positive staining, categorized as follows: 0 (none), 1 (1/100), 2 (1/100 to 1/10), 3 (1/10 to 1/3), 4 (1/3 to 2/3) and 5 ($> 2/3$). The total score which could range from 0 to 8, was derived by summing the individual proportion score and intensity score.³²⁾ ER and TR positivity was determined by an Allred score ≥ 2 . In the context of BC, Ki-67 index measurement of $\geq 14\%$ was adopted as the threshold for determining a positive result. The evaluation of both nuclear and cytoplasmic PTEN expression in the tumor was conducted by referencing the adjacent normal epithelium and stroma, which served as an internal positive control. PTEN expression was quantified using a three-tiered scoring system: 0 (absence of immunoreaction), 1 (weak positive staining relative to normal epithelium), and 2 (positive staining demonstrating equivalent intensity to normal epithelium). A score of 0 was defined as evidence of PTEN loss.³²⁾

Statistical Analysis

All statistical analyses were conducted utilizing SPSS for Windows, version 19.0 (SPSS, Inc., Chicago, IL, USA). To compare the clinicopathological features

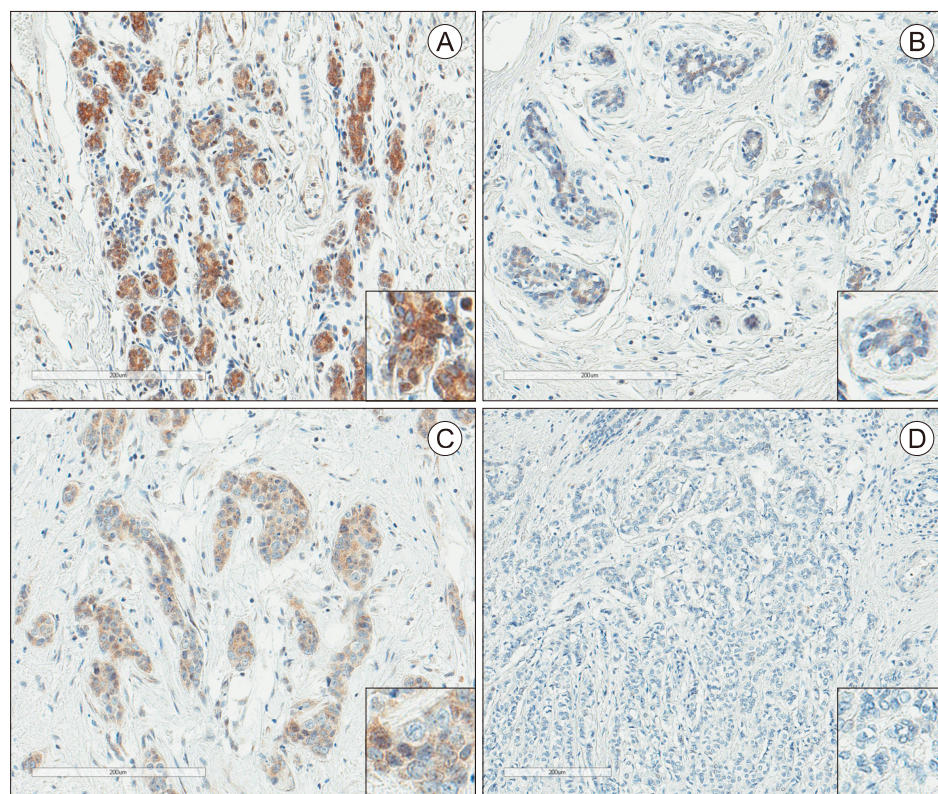


Fig. 1. Sodium iodide symporter (NIS) expression in breast cancer and normal breast tissues (200× magnification). NIS-positive (A) and NIS-negative (B) in normal breast. NIS-positive (C) and NIS-negative (D) in breast cancer. Immunohistochemical staining was performed to evaluate NIS expression.

of TC→BC cases against the BC controls, chi square or Fisher's exact test was applied when analyzing dichotomous variables. For the evaluation of continuous variables, analysis of variance, independent Student's t test, or Mann-Whitney U test was employed as appropriate. To evaluate the effect of RAI or thyroxine suppression therapy on NIS, ER, and TR expression in TC→BC cases, continuous variables including RAI dose, stimulated TSH level, and dose and duration of thyroxine were dichotomized with their median cutoff values. Kaplan-Meier estimation was used to analyze the prognostic significance of NIS expression for the recurrence of BC. All conducted statistical tests employed a two-sided criterion, and a p value <0.05 was designated as the threshold for statistical significance.

Results

Clinical Characteristics of the Subjects

The subjects' clinicopathological profiles are detailed in Table 1, reflecting data documented at the time of BC diagnosis. The TC→BC cases were statistically

comparable to the BC controls with respect to age at the time of BC diagnosis, BMI, obstetric history, and menopausal stage. In TC→BC cases, serum FT4 was higher than that in BC controls ($p=0.011$). Although the pathologic stage and tumor size were comparable between the two groups, TC→BC cases showed more favorable pathologic features than the BC controls; a higher proportion of ductal carcinoma *in situ* (DCIS, 23.1% vs. 0%, $p=0.008$), lower positive rate of Ki-67 (2.9% vs. 33.3%, $p=0.001$), and higher ER α positivity (89.2% vs. 48.6%, $p<0.001$). Thus, they received more hormonal therapy (83.8% vs. 61.1%, $p=0.030$) and, conversely, underwent chemotherapy less frequently (40.5% vs. 80.6%, $p<0.001$). When we subgrouped the TC→BC cases into an RAI-received (RAI group) or RAI-not received group (No-RAI group), there was no difference among the clinicopathological characteristics except age and serum creatinine level between groups (Table 1).

Expression of NIS and Hormone Receptors

The expressional profiles of the TC→BC cases for NIS, ER α , ER β , TR α , and TR β were compared with

Table 1. Clinicopathological characteristics of subjects

Characteristics	BC controls (n=36)	TC→BC cases (n=39)			p [*]	p [†]
		Total (n=39)	No RAI (n=19)	RAI (n=20)		
Age at diagnosis, years	51.4±11.8	51.1±9.6	47.8±7.6	54.2±10.4	0.890	0.036
Body mass index, kg/m ²	23.2±2.6	23.8±2.8	23.7±3.2	23.9±2.5	0.284	0.827
Ever smokers, n (%)	3 (8.3)	1 (2.6)	0 (0)	1 (5)	0.282 [‡]	0.343 [‡]
Family history of BC, n (%)	5 (13.9)	1 (2.6)	1 (5.6)	0 (0)	0.078 [‡]	0.292 [‡]
Postmenopausal status, n (%)	15 (41.7)	17 (44.7)	6 (33.3)	11 (55.0)	0.818	0.186 [‡]
Parity, n (%)	34 (94.4)	33 (89.2)	17 (89.5)	16 (88.9)	0.672	0.955
Serum creatinine, mg/dL	0.77±0.11	0.79±0.23	0.82±0.11	0.72±0.10	0.648	0.024[§]
TSH, mIU/L	2.03 [0.55, 2.6]	0.20 [0.10, 0.40]	0.20 [0.10, 0.90]	0.30 [0.10, 0.38]	0.058 [§]	0.708 [§]
Free T4, ng/dL	1.18±0.29	1.76±0.52	1.80±0.48	1.72±0.56	0.011	0.659
Total T3, ng/dL	112.8±25.9	124.1±22.1	115.4±19.5	130.4±22.1	0.304	0.059
Type of surgery, n (%)						
Breast conserving/mastectomy	14/22 (38.9/61.1)	22/14 (59.5/37.8)	9/7 (50/41.2)	13/7 (65/35)	0.103	0.474
Tumor size, cm	2.5 [1.5, 3.7]	2.0 [1.0, 3.0]	1.5 [1.0, 3.5]	2.1 [1.1, 2.9]	0.066 [§]	0.737 [§]
IDC/DCIS/others, n (%)	35/0/1	27/9/3	12/6/1	15/3/2	0.008	0.440
	(97.2/0/2.8)	(69.2/23.1/7.7)	(63.2/31.6/5.3)	(75/15/10)		
Lymph node metastasis, n (%)	16 (44.5)	10 (27.0)	4 (23.5)	6 (30.0)	0.220	0.725
Distant metastasis, n (%)	1 (2.8)	0 (0)	0 (0)	0 (0)	0.304 [‡]	NA
Pathologic stage, n (%)	12/12/8/4	16/11/10/0	8/5/4/0	8/6/6/0	0.185	0.883
	(33.3/33.3/22.2/11.1)	(43.2/29.7/27.0/0)	(47.1/29.4/23.5/0)	(43.2/30.0/30.0/0)		
ERα positivity, n (%)	17/35 (48.6)	33/37 (89.2)	16/18 (88.9)	17/19 (89.5)	<0.001[‡]	0.955 [‡]
Ki-67 positivity, n (%)	12 (33.3)	1 (2.9)	1 (7.1)	0 (0)	0.001[‡]	0.232 [‡]
Ki-67 index	11.7±14.0	4.5±4.1	4.0±5.1	4.9±3.4	0.005	0.563
PTEN loss, n (%)	8 (22.9)	6 (17.1)	3 (17.6)	3 (16.7)	0.157	0.940 [‡]
Adjuvant or neoadjuvant therapy						
Adjuvant hormone therapy, n (%)	22 (61.1)	31 (83.8)	13 (76.5)	18 (90.0)	0.030	0.383
Chemotherapy, n (%)	29 (80.6)	15 (40.5)	7 (41.2)	8 (40.0)	<0.001	1
Radiation therapy, n (%)	19 (52.8)	25 (67.6)	12 (70.6)	13 (65.0)	0.197	1

BC: breast cancer, DCIS: ductal carcinoma *in situ*, ER: estrogen receptor, IDC: invasive ductal carcinoma, NA: not applicable, PTEN: phosphatase and tensin homolog, RAI: radioactive iodine, T3: triiodothyronine, T4: thyroxine, TC: thyroid cancer, TSH: thyroid stimulating hormone

*p from the comparison of total subjects of BC after TC and BC controls. [†]p from the comparison of subgroups of BC after TC (No RAI vs. RAI). [‡]Calculated from Fisher's exact test. [§]Calculated from Mann-Whitney U test.

those of the BC controls in both cancer and paired normal tissues (Table 2). NIS expression was significantly elevated in the TC→BC cases relative to the levels observed in BC controls in both normal breast (55.3% vs. 27.6%, $p=0.028$) and cancer tissues (61.8% vs. 21.9%, $p=0.001$, Table 2). The proportion of ER α positive tumors was significantly elevated in the TC→BC cohort than in BC control group (89.2% vs. 48.6%, $p<0.001$). However, in normal tissue, the expression of ER α was comparably high with no differences between groups (88.2% vs. 80.0%, $p=0.396$). The incidence of TR α positivity was likewise greater in the

TC→BC cohort compared to the BC control group in normal tissues (47.2% vs. 22.6%, $p=0.044$) as well as in cancer tissues (19.4% vs. 2.9%, $p=0.028$). ER β and TR β expression was statistically similar between the TC→BC and BC control cohorts in both normal and tumor tissues (Table 2).

Effects of RAI and Thyroxine Suppression Treatments for TC on NIS Expression in TC→BC Cases

Because NIS expression in TC→BC cases was significantly higher in both normal and cancer tissues, we

Table 2. Comparison of the expression of NIS, ER and TR in normal breast and BC tissues between TC→BC cases and BC controls

Type of tissue	BC controls			TC→BC cases			p^*	$p^\dagger$
	Normal	Cancer	p^a	Normal	Cancer	p^b		
NIS, n (%)	8/29 (27.6)	7/32 (21.9)	0.769	21/38 (55.3)	21/34 (61.8)	0.637	0.028	0.001
ER α , n (%)	24/30 (80.0)	17/35 (48.6)	0.011	30/34 (88.2)	33/37 (89.2)	1.000	0.396	<0.001
ER β , n (%)	1/31 (3.2)	4/35 (11.4)	0.212 [†]	2/34 (5.9)	2/33 (6.1)	0.669 [†]	0.613 [†]	0.439 [†]
TR α , n (%)	7/31 (22.6)	1/35 (2.9)	0.015[†]	17/36 (47.2)	7/36 (19.4)	0.023	0.044	0.028[†]
TR β , n (%)	10/30 (33.3)	3/35 (8.6)	0.014[†]	12/31 (38.7)	2/36 (5.6)	0.001[†]	0.791	0.622 [†]

BC: breast cancer, ER: estrogen receptor, NIS: sodium iodine symporter, TC: thyroid cancer, TSH: thyroid stimulating hormone

p , comparison between cancer and adjacent normal tissues, p^a and p^b , comparison between BC controls and BC after TC in normal (p^*) and cancer tissues ($p^\dagger$). [†]Calculated from Fisher's exact test.

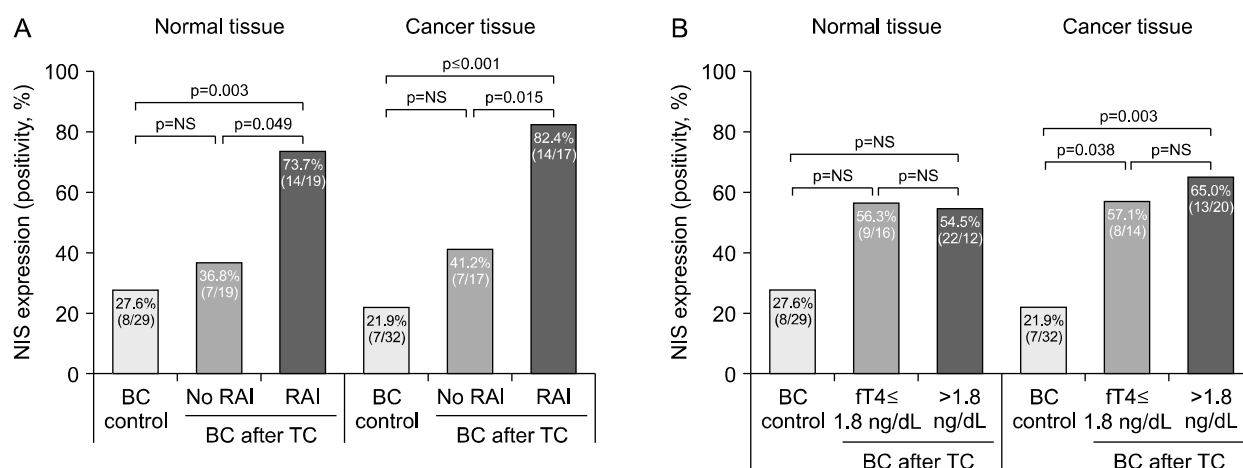


Fig. 2. (A) NIS positivity in breast tissues, both in normal and tumor areas, depending on the presence of previous TC and its treatment – RAI. p values were calculated for each group using the chi square test. (B) NIS positivity in breast tissues, both in normal and tumor areas, depending on the presence of previous TC and its treatment such as thyroxine suppression. The group showing thyroxine suppression was divided into two subgroups based on the median value of serum free thyroxine, 1.8 ng/dL, which is also the upper limit of the normal range. Serum free thyroxine was calculated as the mean of all measured values during follow-up until the time of BC diagnosis. p values were calculated for each group using chi square test. BC: breast cancer, ft4: serum free thyroxine, NIS: sodium iodide symporter, NS: not significant, RAI: radioactive iodine, TC: thyroid cancer

next investigated whether NIS expression was associated with the RAI therapy or thyroxine suppression treatments, which are the main treatment methods for TC.

RAI therapy showed a strong association with NIS expression in TC→BC cases. Higher NIS positivity in both normal (73.7% vs. 36.8%, $p=0.049$) and cancer tissues (82.4% vs. 41.2%, $p=0.015$) in the RAI group compared to in the No-RAI group (Fig. 2A). NIS positivity in the No-RAI group also showed a higher tendency than BC controls, but the difference was not significant. The NIS positivity status did not show statistical association with RAI-related metrics, encompassing the total administered RAI dose and the time elapsed between the first RAI treatment and the BC diagnosis, nor with stimulated TSH levels (Supplementary Table 1).

To estimate the effects of thyroxine suppression therapy, FT4 levels and other related factors (Supplementary Table 1) were analyzed. TC→BC cohort was stratified into two subgroups using the median serum FT4 concentration (1.8 ng/dL, which is also the upper limit of normal range). NIS positivity did not differ between the two subgroups according to the FT4 cut-off (Fig. 2B). Other thyroxine therapy-related factors, such as dose, duration, and dose $\times$ duration with the sum of prescription data, also showed no significant

difference from NIS positivity (Supplementary Table 1).

Association of ER α or TR α Expression with NIS Expression

Because the TC→BC cohort exhibited an upregulation in the expression level of both ER α and TR α , we next evaluated the association between NIS positivity and the expressional status of ER α or TR α (Table 3). To exclude any possible confounding effects of TC treatments, ER α and TR α positivity was assessed to determine their association with TC treatments, which revealed no significant difference according to TC treatment (Supplementary Tables 1, 2).

NIS expression was more common in cancer tissues with positive expression for ER α (58.1% vs. 14.3% in ER α -negative BC, $p=0.001$) or TR α (83.3% vs. 40.4% in TR α -negative BC, $p=0.046$) (Table 3). However, those association was not observed in the subgroup, RAI group and No-RAI group, because of the limited number of subjects. However, a similar trend was observed in the No-RAI group, suggesting an independent association between NIS expression and the effects of RAI treatment. The frequency of NIS positivity was high, regardless of ER α or TR α status, in the RAI group (Table 3), supporting the strong effect of RAI on NIS expression, which may reduce the influence of ER α or TR α .

Table 3. NIS positivity in normal breast or BC tissues according to the ER α or TR α status

Type of tissue	Normal, n (%)			Cancer, n (%)		
	ER α (-)	ER α (+)	p	ER α (-)	ER α (+)	p
Total	4/7 (57.1)	22/52 (42.3)	0.462	3/21 (14.3)	25/43 (58.1)	0.001
BC control	1/3 (33.3)	7/22 (31.8)	0.959	2/18 (11.1)	5/13 (38.5)	0.077
BC after TC						
No RAI	0/1 (0)	5/16 (31.3)	0.519	0/2 (0)	7/14 (50.0)	0.197
RAI	3/3 (100)	10/14 (71.4)	0.304	1/1 (100)	13/16 (82.4)	0.643
	TR α (-)	TR α (+)	p	TR α (-)	TR α (+)	p
Total	16/38 (42.1)	11/24 (45.8)	0.798	23/57 (40.4)	5/6 (83.3)	0.046
BC control	6/19 (31.6)	2/7 (28.6)	0.885	7/31 (22.6)	–	NA
BC after TC						
No RAI	3/8 (37.5)	3/10 (30.0)	0.744	5/13 (38.5)	2/3 (66.7)	0.390
RAI	7/11 (63.6)	6/7 (85.7)	0.322	11/13 (84.6)	3/3 (100.0)	0.482

BC: breast cancer, ER: estrogen receptor, NA: not applicable, NIS: sodium iodine symporter, RAI: radioactive iodine, TC: thyroid cancer, TR: thyroid hormone receptor

Chi square test or Fisher's exact test were used to compare the NIS expression according to the ER α / TR α positivity.

Table 4. Comparison of clinicopathologic characteristics according to the NIS expression in BC tissues

NIS expression	Total		BC controls		TC→BC cases	
	Positive (n=28)	Negative (n=38)	Positive (n=7)	Negative (n=25)	Positive (n=21)	Negative (n=13)
Age at diagnosis, years	48.3±7.8	51.3±10.9	41.3±6.5	51.9±9.9*	50.6±6.8	50.2±12.9
Body mass index, kg/m ²	22.8±2.5	23.6±2.7	23.2±2.8	23.2±2.8	22.9±2.7	24.2±2.4
Postmenopausal status, n (%)	9 (33.3)	16 (42.1)	0 (0)	12 (48.0)*†	9 (42.9)	4 (30.8)†
TSH, mIU/L	2.5±7.8	1.2±1.3	NA	2.0 [0.5, 1.3]	0.2 [0.1, 0.3]	0.3 [0.1, 0.7]†
Free T4, ng/dL	1.7±0.6	1.6±0.5	NA	1.1 [1.0, 1.3]	1.8 [1.4, 2.2]	1.9 [1.5, 2.0]†
Total T3, ng/dL	122.9±23.6	119.8±18.6	NA	114.0 [87.5, 137.5]	125.0 [106.8, 133.0]	124.0 [107.0, 126.0]†
Serum creatinine, mg/dL	0.8±0.1	0.8±0.2	0.7±0.1	0.8±0.3†	0.7±0.1	0.8±0.1†
IDC/DCIS/others, n (%)	19/6/3	34/3/1	7/0/0	24/0/1	12/6/3	10/3/0
Tumor size, cm	(67.9/21.4/10.7)	(89.5/7.9/2.6)†	(100/0/0)	(70.6/0/4.0)†	(57.1/28.6/14.3)	(76.9/23.1/0)†
Lymph node metastasis, n (%)	2.0 [1.0, 3.0]	2.5 [1.5, 3.0]†	2.0 [1.2, 2.5]	2.5 [1.7, 4.0]†	1.8 [1.0, 3.0]	1.8 [0.8, 2.5]†
Distant metastasis, n (%)	7 (25.9)	14 (37.8)	3 (42.9)	10 (40.0)	4 (20.0)	4 (33.3)†
0 (0)	0 (0)	1 (2.7)†	0 (0)	1 (4.0)†	-	-
Ki-67 positive, n (%)	7 (26.9)	5 (14.3)	4 (57.1)	5 (20.8)†	3 (15.8)	0 (0)†
PTEN loss, n (%)	4 (15.4)	9 (25.0)†	1 (14.3)	7 (29.2)†	3 (15.8)	2 (16.7)†
ERα positive, n (%)	25 (89.3)	18 (50.0)*†	5 (71.4)	8 (33.3)	20 (95.2)	10 (83.3)†
TRα positive, n (%)	5 (17.9)	1 (2.9)*†	0 (0)	0 (0)	5 (23.8)	1 (9.1)†
Follow up period, years	4.9 [4.2, 7.4]	4.5 [4.0, 5.1]†	4.4 [4.0, 4.5]	4.4 [4.0, 4.8]†	6.5 [4.3, 8.3]	6.2 [3.6, 9.3]†
Recurrence, n (%)	1 (3.6)	8 (21.1)*†	1 (14.3)	5 (20.0)†	0 (0)	3 (23.1)*†
Time to recurrence, years	1.18	1.9 [0.5, 2.5]†	1.18	1.3 [0.7, 2.5]†	-	2.5 [0.2, 2.5]

BC: breast cancer, DCIS: ductal carcinoma *in situ*, ER: estrogen receptor, IDC: invasive ductal carcinoma, NA: not applicable, NIS: sodium iodine symporter, PTEN: phosphatase and tensin homolog, TC: thyroid cancer, TR: thyroid hormone receptor, TSH: thyroid stimulating hormone

*<0.05 by Student's t test or chi-square test. Mann Whitney U test or Fisher's exact test were used for non-parametric comparison, and the values were labeled with † and ‡, respectively.

Clinicopathological Characteristics of NIS-positive BC

Clinicopathological characteristics were compared according to the NIS positivity of BC (Table 4). NIS-positive cases showed higher ER α (89.3% vs. 50.0%, $p=0.001$) or TR α (17.9% vs. 2.9%, $p=0.046$) positivity among total subjects, and although significance was not observed, similar trends were observed in both TC $\rightarrow$ BC cases and BC controls. No other clinicopathological characteristics differed between NIS-positive and negative groups, except for age and menopausal status; NIS positive cases were all premenopausal women and younger than the NIS-negative cases (41.3 years, contrasting with 51.9 years, $p=0.026$) in BC controls.

Interestingly, the overall recurrence rate was lower in NIS-positive cases (3.6% vs. 21.1%, $p=0.042$). Only one recurrence occurred among NIS-positive BC controls, and there was no recurrence among 21 NIS-positive TC $\rightarrow$ BC cases. However, Kaplan-Meier analysis revealed that recurrence-free survival did not significantly diverge between the two cohorts (Log-rank test, $p=0.280$).

Discussion

The current investigation revealed elevated NIS expression specifically in BCs that developed subsequent to TC (TC $\rightarrow$ BC cases), and this finding exhibited a strong association with the prior RAI therapy administered for TC. Given the negative association between NIS expression and BC recurrence, RAI treatment might have protective effects on BC progression. Furthermore, the upregulation of both ER α and TR α expression levels was apparent within the TC $\rightarrow$ BC cohort in association with NIS expression, which might be implicated in the development of BC after TC.

NIS is physiologically expressed in lactating mammary glands and in approximately 20%–30% of normal breast and 50%–80% of BC tissue.^{2,5)} Our results also showed similar rates of 20%–30% in BC controls with higher positivity (50%–80%) in TC $\rightarrow$ BC cases, particularly 70%–80% in those who received RAI therapy

preceding TC treatment. Although a minor portion of NIS within the breast tissue might be functional, this fraction retains the potential capacity to accumulate radioiodine and consequently leading to localized radiation exposure. Considering higher NIS positivity of RAI exposed breast tissues, NIS in the breast might further amplify the risk of BC. However, our previous report showing no increase in BC after RAI therapy or decreased risk of BC after high-dose RAI²¹⁾ suggest the protective effects of RAI or NIS on the development of BC. Moreover, our study showed the better prognosis of BC patients with positive NIS expression. NIS might be involved in the ablation effects in breast tissues, which is consistent with our previous results revealing the favorable effects of RAI therapy on BC prognosis.²¹⁾ These findings support that RAI therapy can be applied for the indicated TC patients without concerns of increased subsequent BC. Moreover, RAI therapy may be a therapeutic modality for BC. However, we could not demonstrate the effects of NIS on the development or progression of BC, and thus further investigations are required.

NIS expression is clinically significant in BC, with ER α ^{7,10–12)} suggested as its regulator. In several BC cell lines, the NIS positive phenotype exhibited concordance with the patient's intrinsic ER α status, and the existence of a TATA box, situated in close proximity to the estrogen response elements within the NIS promoter, provided additional evidence confirming ER α -mediated transcriptional activation of the NIS gene in *in vitro*.¹⁰⁾ Recent studies also detected increased NIS expression in ER α -positive human breast tissues compared to in negative tissues.^{7,12)} We demonstrated positive correlations between NIS and ER α in BC tissues, although no significance for TC $\rightarrow$ BC cases. The positive correlation may not be observed in these cases because of the high NIS positivity in RAI-treated cases.

In our study, RAI treatment showed a stronger correlation than ER α status with NIS expression in BC. The underlying molecular mechanism responsible for RAI-related NIS upregulation in BC developed after TC remains largely ambiguous; nonetheless, prior research has documented sustained activation of the

PI3K/Akt pathway in mammary glands exposed to external radiation *in vivo*.³³⁻³⁵⁾ In addition, ER α is suggested to be competent in orchestrating the activation of the PI3K/Akt pathway within BC cells,^{36,37)} a mechanism that could potentially lead to augmented NIS expression in cell lines like MCF-7.^{38,39)} Therefore, RAI therapy or the presence of ER α may activate the PI3K/Akt pathway, resulting increased NIS transcription in BC.

Another possible mechanism of NIS regulation by RAI therapy is activation of the p53 signaling pathway. Elevated levels of p53-family proteins (p53, p73) led to an increase in endogenous NIS expression within liver cancer cells.⁴⁰⁾ *In vitro* studies have shown that radiation exposure promoted BC cell proliferation by triggering the accumulation of mutant p53.⁴¹⁾ Consequently, BC after TC may demonstrate a preferential manifestation of copious p53 mutant protein with inversely higher NIS expression after RAI exposure. Prior studies have indicated that radiation exposure induces microRNAs, which subsequently upregulate NIS expression in TC cells/tissues via interaction with either the 3'-untranslated region of the NIS mRNA or upstream regulatory elements, such as the paired box-8 protein.⁴²⁻⁴⁴⁾ The necessity for further investigation is underscored by the need to clarify this underlying mechanism.

In addition to ER α , TR α was also increased in TC $\rightarrow$ BC cohort, and the positivity of NIS showed a higher prevalence in TR α -positive BC. However, the association between RAI and TR α was not determined and the association between thyroxine suppression therapy and TR α expression remain unclear, although some recent studies indicated an elevated risk of BC in female subjects diagnosed with hyperthyroidism, proposing thyroid hormone as a risk factor.⁴⁵⁻⁴⁷⁾

This study has some limitations. Although NIS immunoreactivity was observed both within the cytoplasmic compartment as well as plasma membrane, the precise subcellular distribution and functionality could not be definitively established by our investigation. Some data were missing from this retrospective analysis, although we attempted to gather as much available data as possible. Additionally, a relatively small

number of subjects and short duration of follow-up were used. Notwithstanding these points, our work represents the initial investigation to successfully identify copious NIS expression within BC developed in TC survivors and evaluating their association with RAI or thyroxine therapy for TC.

In conclusion, the expression of NIS was positively associated with previous RAI therapy for TC, revealing a protective role for NIS in BC. Additionally, ER α or TR α profile similarly exhibited an increasing trend in BCs developing after TCs, thereby suggesting an influence of hormone receptor status of BC tissues on NIS expression as well as RAI therapy. Further studies are warranted to elucidate the molecular mechanism of their association.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Supplementary Table 1. Comparison of factors related with TC treatment according to NIS, ER α , or TR α positivity of BC

TC treatment related factors	n	NIS positive	n	NIS negative
RAI dose, GBq	13	60 [60.0–183.0]	3	60 [60.0–298.0]
Latency period from 1st RAI to BC diagnosis, years	14	3.9 [2.3–5.5]	3	5.7 [2.4–5.9]
TSH, mean, mIU/L	9	142.7 [72.2–205.0]	2	120.1 [73.7–147.9]
TSH, median, mIU/L	9	176.0 [68.0–201.7]	2	126.3 [68.5–160.9]
TSH, last, mIU/L	9	176 [59.1–201.7]	2	135.4 [86.8–164.6]
LT4 dose, μ g/day	17	150 [112.5–200.0]	11	125 [100.0–200.0]
LT4 duration, years	21	4.3 [3.0–7.4]	13	4.4 [3.4–5.8]
LT4 dose $\times$ duration, μ g/day $\times$ years	17	10.5 [4.3–12.6]	11	6.7 [4.0–7.7]
	n	ER α positive	n	ER α negative
RAI dose, GBq	17	90 [60.0–183.0]	1	300
Latency period from 1st RAI to BC diagnosis, years	17	4.1 [2.7–6.5]	2	2.4, 3.7
TSH, mean, mIU/L	11	142.7 [73.7–195.0]	1	239.7
TSH, median, mIU/L	12	136.5 [64.5–192.8]	1	187
TSH, last, mIU/L	12	136.5 [52.2–192.8]	1	182
LT4 dose, μ g/day	28	150 [106.3–200.0]	3	200 [100.0–200.0]
LT4 duration, years	33	4.5 [3.4–7.0]	4	4.0 [2.8–6.8]
LT4 dose $\times$ duration, μ g/day $\times$ years	28	7.0 [4.6–13.0]	3	5.0 [4.4–5.0]
	n	TR α positive	n	TR α negative
RAI dose, GBq	4	125 [37.5–272.5]	13	90 [60.0–163.0]
Latency period from 1st RAI to BC diagnosis, years	4	2.4 [0.7–5.9]	14	4.2 [2.9–7.7]
TSH, mean, mIU/L	2	161.5 [83.3–16.5]	10	154.6 [70.5–200.0]
TSH, median, mIU/L	2	142.0 [96.9–142.0]	11	63.2 [176.0–195.0]
TSH, last, mIU/L	2	139.5 [96.9–139.5]	11	45.4 [176.0–195.0]
LT4 dose, μ g/day	6	200 [137.5–212.5]	24	150 [100.0–200.0]
LT4 duration, years	7	3.5 [2.5, 4.6]	29	4.7 [3.6, 7.4]
LT4 dose $\times$ duration, μ g/day $\times$ years	6	6.8 [4.7, 12.3]	24	7.0 [4.4, 13.0]

BC: breast cancer, ER: estrogen receptor, LT4: levothyroxine, NIS: sodium iodine symporter, RAI: radioactive iodine, TC: thyroid cancer, TR: thyroid hormone receptor, TSH: thyroid stimulating hormone

Mann Whitney U test were used for the analysis. Treatment related factors for TC showed no association with molecular expressions of NIS, ER α or TR α .

Supplementary Table 2. Comparison of ER α or TR α expression according to the history of RAI or thyroxine treatment for TC

	ER α positivity				TR α positivity			
	Normal tissues		Cancer tissues		Normal tissues		Cancer tissues	
	n (%)	p	n (%)	p	n (%)	p	n (%)	p
RAI-treatment								
BC control	24/30 (80.0)	0.369*	17/35 (48.6)	<0.001*	7/31 (22.6)	0.044*	1/35 (2.9)	0.028*
BC after TC								
No RAI	16/17 (94.1)	0.196 [†]	16/18 (88.9)	0.005 [†]	10/18 (55.6)	0.030 [†]	3/18 (16.7)	0.074 [†]
RAI	14/17 (82.4)	0.845 [†]	17/19 (89.5)	0.003 [†]	7/18 (38.9)	0.326 [†]	4/18 (22.2)	0.024 [†]
Thyroxine-treatment								
BC control	24/30 (80.0)	0.369*	17/35 (48.6)	<0.001*	7/31 (22.6)	0.044*	1/35 (2.9)	0.028*
BC after TC								
Free T4 $\leq$ 1.80 ng/dL	13/14 (92.9)	0.283 [†]	14/15 (93.3)	0.003 [†]	7/14 (50.0)	0.069 [†]	1/15 (6.7)	0.533 [†]
Free T4>1.80 ng/dL	17/20 (85.0)	0.655 [†]	19/22 (86.4)	0.004 [†]	10/22 (45.5)	0.082 [†]	6/21 (28.6)	0.106 [†]

BC: breast cancer, ER: estrogen receptor, RAI: radioactive iodine, T4: thyroxine, TC: thyroid cancer, TR: thyroid hormone receptor

*p were calculated from the comparison between total subjects of BC after TC and BC controls; [†]p were from the comparison of indicated subgroup of BC after TC and BC controls; [†]p were from the comparison of two subgroups of BC after TC. Chi square test or Fisher's exact test were used for ER α or TR α positivity depending on the history of RAI or thyroxine treatment.