의학박사 학위논문

Hormone receptor expression and clinicopathological characteristics of co-occurred thyroid cancer and breast cancer

동시발생한 갑상선암과 유방암의 호르몬 수용체 발현과 임상병리학적 특성

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Abstract

Hormone receptor expression and clinicopathological characteristics of co-occurred thyroid cancer and breast cancer

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There is growing interest in the high risk of co-occurrence of thyroid cancer and breast cancer, and it is speculated that there may be a hormonal link between the two cancers. Both thyroid and breast cancers express estrogen receptors (ERs) and thyroid hormone receptors (TRs), and identifying the expression of these hormone receptors in both cancers may provide a clue to understanding the hormonal link. Therefore, this study aims to identify the expression of hormone receptors in the co-occurred thyroid cancer and breast cancer. In particular, since antiestrogen and levothyroxine are used to treat breast and thyroid cancer treatment respectively, I investigated the association between receptor positivity and previous cancer

treatment as well as clinicopathological characteristics. In addition, the expression of sodium iodide symporter (NIS) is also investigated in breast cancer, since it may mediate the effect of radioactive iodine (RAI) therapy for thyroid cancer and influence the risk of subsequent breast cancer.

Here, immunochemical staining for hormone receptors was performed on thyroid and breast tissues of 99 co-occurred thyroid and breast cancer patients, 92 thyroid cancer controls, and 147 breast cancer controls. Since NIS correlated with ERα and TRα and NIS positive breast cancer showed a good prognosis, the Cancer Genome Atlas (TCGA) data analysis was performed to assess the association between solute carrier family 5 member 5 (SLC5A5) and estrogen receptor 1 (ESR1) or thyroid hormone receptor alpha (THRA) and the prognostic value of SLC5A5.

The expression of ERα was higher and both ERβ and TRβ were lower in thyroid cancer co-occurred with breast cancer than in the thyroid cancer controls. High ERα positivity was associated with previous antiestrogen therapy and menopause at the time of thyroid cancer diagnosis. On the other hand, both ERα and TRα expressions were increased in breast cancer that co-occurred with thyroid cancer, which did not show any association with previous thyroid cancer treatment or clinicopathological features. NIS expression was more frequently observed in breast cancer after thyroid cancer than in the breast cancer controls, and was associated with prior RAI therapy and ERα positivity. NIS-positive breast cancer had a better prognosis than NIS-negative breast cancer. However, in TCGA analysis, SLC5A5 showed a weak positive association with THRA but not with ESR1.

This study demonstrated high expression of ERα in both thyroid and breast cancers. ERα positivity in thyroid cancer was associated with antiestrogen treatment or menopause, while ERα positivity in breast cancer did not show any association with treatment or clinical characteristics. The increased expression of hormone receptors in thyroid and breast cancer tissues may suggest the influence of hormones in the co-development of cancer. Moreover, NIS expression in breast cancer was associated with hormone receptors and the prognosis of subsequent breast cancer. This suggest that NIS expression may also be involved in breast carcinogenesis and subsequent breast cancer risk associated with RAI therapy.

- This doctoral dissertation is based on the following published paper in Part II.
- Increased expression of thyroid hormone receptor alpha and estrogen receptor alpha in breast cancer associated with thyroid cancer. Eur J Surg Oncol. 2021 Jun;47(6):1316-1323.

Key words: Estrogen Receptor, Thyroid Hormone Receptor, Sodium iodide symporter, Thyroid Cancer, Breast Cancer, Immunohistochemistry

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List of Abbreviations

Estrogen receptor (ER)

Thyroid hormone receptor (TR)

Sodium iodide symporter (NIS)

Radioactive iodine (RAI)

the Cancer Genome Atlas (TCGA)

Standardized incidence ratios (SIR)

Surveillance, Epidemiology, and End Results (SEER)

Progesterone receptor (PR)

Odds ratio (OR)

Estrogen-responsive element (ERE)

Mitogen-activated protein kinase (MAPK)

Phosphoinositide 3-kinase (PI3K)

Activator protein-1 (AP-1)

Papillary thyroid carcinoma (PTC)

Estrogen Receptor 1 (ESR1)

Estrogen Receptor 2 (ESR2)

Epidermal growth factor (EGF)

Insulin-like growth factor 1 (IGF-1)

Hypoxia-inducible factor 1α (HIF-1α)

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)

Thyroid hormone response elements (TRE)

Triiodothyronine (T3)

Runt-related transcription factor 2 (RUNX2)

Cyclophosphamide, methotrexate and 5-fluorouracil (CMF)

Acute myeloid leukemia (AML)

Selective estrogen receptor modulator (SERM)

Radioactive iodine (RAI)

Thyroid Stimulating Hormone (TSH)

Free thyroxine (FT4)

Triiodothyronine (T3)

Phosphatase and tensin homolog (PTEN)

Body mass index (BMI)

Extrathyroidal extension (ETE)

Aromatase inhibitor (AI)

Tamoxifen (TMX)

Upregulates endothelial nitric oxide synthase (eNOS)

Follicular thyroid cancer (FTC)

v-raf murine sarcoma viral oncogene homolog B (BRAF)

Protein kinase B (AKT)

Invasive ductal carcinoma (IDC)

Introduction

1. Increased risk of developing thyroid cancer after breast cancer

As life expectancy increases with the advancement of cancer treatment, the incidence of second primary cancer after breast cancer is also increasing (1). During the follow-up period of 8.9 years of 376,825 breast cancer patients, the standardized incidence ratios (SIR) for second cancer was 1.15 (95% confidence internal [95% CI] 1.14–1.17) (2). Evans *et al* (3) found that the risk of second cancer after breast cancer was higher in those under 50 years of age, in particular, the risk for second thyroid cancer was higher than that of all other cancers. Retrospective analysis of the Surveillance, Epidemiology, and End Results (SEER)-9 database between 1992 and 2011 showed an increased SIR for second thyroid cancer of 1.22 (95% CI 1.14–1.31) among breast cancer survivors (4). The risk of second thyroid cancer was particularly high within 3 years after breast cancer diagnosis and in estrogen receptor (ER) or progesterone receptor (PR) positive breast cancer (4).

In 2015, two meta-analyses reported an increased risk of thyroid cancer among breast cancer survivors (5, 6). Nielson *et al* (6) reported an odds ratio (OR) of 1.55 (95% CI 1.44–1.67) from 19 cohorts with 956,672 breast cancer patients and 611 second thyroid cancers with an SIR of 0.8–3.7 during the mean follow-up period of 5.4–10.5 years. Joseph *et al* (5) showed an SIR of 1.59 (95% CI 1.28–1.99) from 10 studies with 592,498 patients between 1971 and 2003. Surveillance bias has been considered to cause an increased risk of subsequent thyroid cancer (7). However, the biological association between breast and thyroid cancers also has been

suggested, considering the risks of secondary cancer are bidirectional and their common hormonal risk factors and treatment effect (6, 8, 9).

2. The role of estrogen and hormone receptors in the increased risk of thyroid cancer co-occurred with breast cancer

Both thyroid and breast cancer are most prevalent in patients in their late 40s to early 50s, the transitional period of menopause (10, 11). It is well known that estrogen is a risk factor for breast cancer (12). Menstrual and reproductive factors such as late age at menopause, early menarche, and nulliparity increase the risk of breast cancer (13, 14). Estrogen also plays a role in the development of thyroid cancer (15). In several epidemiologic studies, late menopause, early menarche, and nulliparity are associated with increased the risk for thyroid cancer (16-18); in cell experiments, estrogen stimulates cell proliferation in thyroid cancer cell lines (19, 20).

Estrogen and ERs are involved in carcinogenesis through genomic or non-genomic actions in thyroid and breast cancer (21). In a classical pathway, estrogen binds to an ER and translocates into the nucleus, and assists transcription near estrogen-responsive elements (EREs), thereby activating several target genes (22). Alternatively, estrogen binds to ERs in the membrane and directly binds to the mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K) pathways, resulting in several protein-kinase cascades or the activation of transcription factors such as activator protein-1 (AP-1) (21). Since both MAPK and

PI3K are important oncogenic pathway in papillary thyroid carcinoma (PTC), their activation is presumed to be responsible for the mechanism of developing PTC by ERs (15, 23).

ERs have several isoforms, including ERα, ERβ, and those produced by alternative RNA splicing (19). ERα and ERβ are encoded by estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) genes and have different distribution and biological actions (24). In several human cancers, ERα activates oncogenic pathways, promoting gene transcription involving cellular proliferation and growth; while ERβ plays a role in anti-tumor activity (25). Although the role of ER in thyroid cancer is not well understood as in breast cancer, ERα promotes cell proliferation (26), and ERβ supports apoptosis in thyroid cancer cells (27). The unbalanced distribution of ERα/ERβ and overexpression of ERα are also involved in carcinogenesis (28). Estrogen can stimulate cell proliferation by upregulating ERα in both benign and malignant thyroid glands (19). However, unliganded ER, even without estrogen, can activate several pathways which include the epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) mitogenic pathways (29). Recently, a hypothesis has been raised that hypoxia and inflammation mediate a crosstalk between ERα and hypoxia-inducible factor 1α (HIF-1α) or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), resulting in tumor progression (30-32). *In vitro*, ERα promotes thyroid carcinogenesis via a disrupted thyroid redox homeostasis (33).

ERs in the thyroid tissue were first discovered in the early 1980s (34). However,

the results of ER staining of thyroid tissues are somewhat inconsistent until 2010, since there were technical problems of staining methods or the use of various monoclonal or polyclonal antibodies (15). Based on studies since 2010, ERα expression was more frequent at the positive rate of 20–60% in thyroid tumor compared to a low expression of 5–10% in the surrounding normal tissues (35-39). Many studies have reported a higher rate of ERβ positivity than ERα in thyroid tissues (35, 36, 40). In contrast to ERα, the positive rate of ERβ tended to be higher in the benign thyroid lesions, showing 67–96% positivity and 60–80% in cancer lesions (15, 28, 35).

Thyroid hormone receptors (TR) also belong to the nuclear receptor superfamily and have two representative isoforms: TRα and TRβ (41). In the early 1980s, the presence of TRs in human cancer tissues was revealed (42). In addition to ERs, TRs can also play a role in the thyroid and breast carcinogenesis. By dimerization with retinoic acid receptors, TRs can modulate transcriptional activity for several genes by binding to thyroid hormone response elements (TRE) (43). Triiodothyronine (T3) binds to integrin αγβ3 and activates the MAPK pathway in thyroid cancer (44). The activation of the MAPK pathway results in the upregulation of TRα and HIF-1α, promoting carcinogenesis (45). Recently, a novel mechanism for the role of TRβ was identified as that of a tumor suppressor in association with runt-related transcription factor 2 (RUNX2) (46), the transcription factor responsible for cellular differentiation in osteoblasts and chondrocytes. RUNX2 has emerged as a major player in cancer metastasis (47) and mediates oncogenic transcription in both breast

and thyroid cancer cells (9, 48). TRβ binds to a TRE motif and represses RUNX2 promoter activity in a T3-dependent manner or its unliganded form (49). Several lines of evidence suggest the possibility that hormone receptors play a role in the co-occurrence of breast and thyroid cancers; therefore, it would be worthwhile to investigate at their expression in both cancers.

3. Effect of breast cancer treatment on following thyroid cancer

As the follow-up period of primary cancer increases, concerns about the risks of cancer treatment for secondary cancer have been raised. There are various adjuvant treatments for breast cancer, which have been developed by extensive investigations and clinical studies to understand the complexity of breast cancer.

In 2000, the National Institutes of Health Consensus Development Conference recommended that adjuvant chemotherapy be considered for breast cancers sized ≥1cm (50). Over the decades, the mainstream of adjuvant chemotherapy has shifted from a cyclophosphamide, methotrexate and 5-fluorouracil (CMF) regimen in the 1970s to anthracycline-based regimens in the 1990s-2000s with an increased risk of acute myeloid leukemia (AML) associated with the CMF regimen (51). For taxane, SEER database analysis (52) and large adjuvant trials (53-55) demonstrated that there was no increased risk of secondary leukemia in patients treated with paclitaxel or docetaxel.

Antiestrogen therapy emerged in the 1960s, following the identification of ER, and is currently considered the standard treatment for ER-positive breast cancer (56).

Over the last 30 years, tamoxifen has been the drug of choice for adjuvant hormone therapy for breast cancer (57). However, epidemiologic evidence suggested that the risk of endometrial cancer is higher in breast cancer patients treated with tamoxifen (58-60). In addition, the duration of tamoxifen use was positively correlated with the risk of secondary endometrial cancer (58). Tamoxifen-related endometrial cancer exhibited uncommon cell types, Mullerian and mesodermal-mixed endometrial tumors, rather than common adenocarcinoma, as seen in the general population (58, 60). The risk of endometrial cancer was relatively low after using other selective ER modulators (SERM), such as raloxifene and toremifene (61-63). Meanwhile, a population-based study showed an association between tamoxifen or aromatase inhibitors and melanoma (64); the risk of melanoma was higher in patients treated with antiestrogen than the general population (64).

The risk of thyroid cancer can also be considered in association with adjuvant therapy for breast cancer. *In vitro* and *in vivo*, tamoxifen inhibits the progression of human follicular and papillary thyroid cancer cells (65). However, there are few epidemiologic studies on the risk of second thyroid cancer due to breast cancer treatment since the incidence is too low to prove causation, and the composition of adjuvant therapy is too complex to unify the treatment group. For radiation therapy, two studies showed no increased risk of second thyroid cancer (66, 67). In general, the latency period of thyroid cancer is 20–30 years from radiation exposure (68), so it is difficult to explain the risk of thyroid cancer by radiation therapy to treat breast cancer alone.

4. Increased risk of developing breast cancer after thyroid cancer

According to the SEER data from between 1973 and 2002, the risk of secondary cancer increased by 9% in 30,278 thyroid cancer patients with a median follow-up period of 103 months (69). The risk was greater in patients treated with radioactive iodine (RAI) therapy or those within 5 years of diagnosis (69). Another study based on the SEER data from 1973–2008 with 52,103 thyroid cancer patients also showed a 9% increased risk of second cancer, with the most common sites being the salivary gland and kidney (70). The incidence rate of secondary cancer was 13% in the SEER 13 data, with 75,992 thyroid cancer patients from 1992–2013 (71). The risk of secondary cancer was greater with RAI therapy, and the increased risks were observed in salivary gland cancers and leukemia, while the most common cancer site, irrespective of RAI therapy, was bone and joint (71). A multinational record linkage study reported the risk of secondary cancer as 31% in 39,002 thyroid cancer patients with 25 years of follow-up (72). This study showed a high risk of breast cancer, which increased with follow-up duration (72).

Nielson *et al.* (6) presented an odds ratio (OR) of 1.18 (95% CI 1.09–1.26) from 5,791 secondary breast cancer and 44,879 thyroid cancer patients from 18 cohorts. Joseph *et al.* (5) reported an SIR for secondary breast cancer of 1.24 (95% CI 1.16–1.33) from 17 studies with 223,782 thyroid cancer patients between 1984 and 2013. In two meta-analyses, the risk of secondary breast and thyroid cancers increased, but the risk of secondary breast cancer was slightly lower than that of secondary

thyroid cancer (5, 6).

5. The role of thyroid hormone and hormone receptors in the increased risk of breast cancer co-occurred with thyroid cancer

Many epidemiological studies have shown an increased risk of breast cancer in patients with hyperthyroidism (73-76), although some have yielded conflicting results (77, 78). In experimental studies, thyroid hormone stimulates breast cancer cell proliferation (41, 79-81); thyroid hormone binds to TR and activates the PI3K and MAPK pathways (41, 80, 81). The activated MAPK pathway can then upregulate TRα and HIF-1α, which promotes carcinogenesis (45). In most cases, TRβ acts as a tumor suppressor, but T3-bounded TRβ can activate the MAPK pathway (45). In the absence of T3, TRβ represses PI3K and RUNX2, which regulates oncogenic activity (9, 48).

There are limited data on the expression of TRs in breast cancer. Conde *et al.* (41) reported that TRα positivity was 0% in normal breast tissues and 65–92% in breast cancer tissues, and TRβ positivity was 2–20% in normal breast tissues and 25–33% in breast cancer tissues (41). Jerzak *et al.* (82) showed that the positive rate of TRα1 was 74%, and that of TRα2 was 40% in 130 breast cancer cases. TRα2 positive breast cancer was associated with high ER expression and a good prognosis (82). Charalampoudis *et al.* (83) demonstrated a lower TRα positivity in breast cancer tissue than adjacent normal breast tissue in 41 women with invasive ductal carcinoma and no thyroid disease. Partial loss of TRα was observed in large and

high-grade breast cancers (83). However, there are no studies to date on TRs in breast cancer co-occurred with thyroid cancer.

The role of estrogen and ER in breast cancer development and the expression of ER in breast cancer are very well-known. Although ERα promotes breast cancer cell proliferation (26), there are few studies on the expression of ER in breast cancer co-occurred with thyroid cancer (84-86). Based on the surgical pathology records, 70–89% ER positivity has been reported, which was higher than 55–69% in the breast cancer control group (84-86).

6. Effect of thyroid cancer treatment on following breast cancer co-occurred with breast cancer

Thyroid cancer treatments after thyroidectomy involve thyroid hormone suppression in most patients and RAI therapy in intermediate-high risk patients. Hyperthyroidism can be a risk factor for breast cancer (73-76). Therefore, it is necessary to investigate whether the risk of secondary breast cancer is due to levothyroxine use depending on the dose and administration period of levothyroxine. A recent study showed an increased risk of breast cancer in patients who had been taking levothyroxine (adjusted OR 1.24, 95% CI 1.15–1.33, $P \leq 0.001$), and a higher risk in long-term users for more than 1 year (adjusted OR 1.26, 95% CI 1.12–1.41, $P \leq 0.001$) compared with short-term users for less than 1 year (adjusted OR 1.22, 95% CI 1.11–1.35, $P \leq 0.001$) (87).

There are concerns about the potential carcinogenic effects of RAI therapy because

the salivary gland, stomach, small intestine, bladder, and mammary glands are organs that concentrate or eliminate iodine (17, 88). However, quiescent breast tissue does not concentrate radioisotopes significantly in a ^{131}I whole-body scan (89). Most cohort studies and meta-analyses have reported that RAI therapy for thyroid cancer is not associated with breast cancer risk (86, 88, 90-92).

7. Effect of RAI therapy and NIS expression in breast cancer developed after thyroid cancer

Ahn *et al.* (93) showed that the incidence of breast cancer did not increase in thyroid cancer patients who received RAI therapy, and the risk of recurrence was lower than in patients with no RAI. Moreover, the risk of breast cancer was lower in the higher-dose RAI group (93). The existence of sodium iodide symporter (NIS) in breast cancer may explain the effect of RAI on the risk of breast cancer. However, no studies have demonstrated NIS expression in breast cancer co-occurred with thyroid cancer. NIS is a membrane-bound glycoprotein and is primarily expressed in the thyroid tissues (94). It mediates highly selective iodide uptake for thyroid hormone synthesis, making RAI an effective treatment for thyroid cancer. Although the expression level is low, the presence of NIS has been detected in several extrathyroidal tissues, including breast tissues, particularly in lactating mammary glands (95). However, the positive rate of NIS has been variously reported; NIS positivity is in the range of 0–80% in normal breast tissue and 20-90% in breast cancer tissue (95-100), which may be due to differences in antibodies and

immunochemical staining methods (101). Studies before 2010 (95-97) used polyclonal antibodies for NIS, showing high positivity rates of 76–88% in breast cancer tissues compared with 0–80% in normal breast tissue. Meanwhile, studies after 2010 (98-100) used monoclonal antibodies and reported 29-75% of NIS positivity in normal breast tissue, comparable to 25-91% in breast cancer tissues.

For the regulatory mechanism of NIS in the breast, estradiol, prolactin, or oxytocin has been suggested (95, 102). Thyroid-stimulating hormone (TSH) or thyroid hormone, which are known as the primary regulators in thyroid tissues (103, 104), have shown little effect (105). Recently, experimental studies (99, 104, 106, 107) suggested that ERα is also an important regulator, although studies in humans are lacking. In anticipation of the future therapeutic application of RAI treatment for breast cancer, NIS regulation in breast cancer is now under active investigation (97, 108, 109).

8. Hypothesis

First, I hypothesized that the expression of hormone receptors (ER, TR) and NIS would be increased in the co-occurrence of breast and thyroid cancer, as these are shared antigens related to the risk of cancer in both the breast and thyroid gland.

Second, I hypothesized that cancer treatment would affect the expression of hormone receptors in thyroid or breast cancer tissue. Antiestrogen therapy is used to treat breast cancer, and levothyroxine is prescribed for thyroid cancer, and these hormonal treatments may be related to the increased risks of the two cancers. In

addition, radiation therapy may also be associated with the risk of subsequent cancers.

Third, I hypothesized that NIS expression could be different in breast cancer after thyroid cancer associated with RAI therapy and may influence the risk of developing breast cancer. Since ER has been suggested as an NIS regulator in previous cell experiments, I also hypothesized that NIS expression could be associated with hormone receptor positivity. Furthermore, I also evaluated the prognostic value of NIS in breast cancer, considering the possibility of mediating the effect of RAI therapy on the breast. Although the functionality was not evaluated, identifying the expression and prognostic value of NIS may help to understand the potential of RAI therapy for breast cancer.

9. Aims of the study

In Chapter I, I aimed to investigate the expression of hormone receptors in thyroid cancer co-occurred with breast cancer. I examined the association of hormone receptor positivity in thyroid cancer with breast cancer treatment or clinicopathological features.

In Chapter II, I aimed to evaluate the expression of hormone receptors in breast cancer co-occurring with thyroid cancer. Hormone receptor positivity in breast cancer was evaluated for its association with RAI therapy, suppressed TSH levels in thyroid cancer patients, and other clinicopathological factors.

In Chapter III, I aimed to determine the expression and the prognostic significance

of NIS in breast cancer after thyroid cancer. I also identified the association between thyroid cancer treatment, hormone receptor positivity, and NIS. Further, the Cancer Genome Atlas (TCGA) analysis was performed to validate the association with ERα/TRα positivity and the prognostic value of NIS.

Materials and Methods

Patients and tissue microarray

This study included 99 patients diagnosed with breast cancer among 6150 female patients with papillary or follicular thyroid cancer followed up at Seoul National University Hospital between 1973 and 2012 (Figure 1). There were 99 patients with available hematoxylin and eosin-stained paraffin specimens for the breast or thyroid tissues. Appropriate core tissues with a diameter of 2 mm were selected for the construction of a tissue microarray. The tissue microarray was constructed for both thyroid and breast cancer and contains a pair of normal and cancer tissues for each case.

First, the expression and association of hormone receptors in thyroid cancer tissues were investigated. Fifty-seven cases of thyroid cancer co-occurred with breast cancer (TC+BC cases) were analyzed with 92 TC controls. TC control cases were selected among thyroid cancer patients who underwent surgery in Boramae hospital between 2011 and 2013. Clinicopathological data of thyroid cancers were retrospectively obtained, including the following: age at diagnosis, body mass index (BMI), postmenopausal status, tumor size, pathologic type, extrathyroidal extension (ETE), lymph node metastasis, follow-up period, and recurrence. Among 57 TC+BC cases, 15 patients were diagnosed with thyroid cancer after breast cancer with at least a 2 year latency period (BC→TC cases). Information on the adjuvant treatment of breast cancer was also gathered for BC→TC cases. For the rest, 42 were diagnosed with thyroid cancer and breast cancer at the same time or were

diagnosed with breast cancer after thyroid cancer (TC→BC cases).

Second, the expression and association of hormone receptors in breast cancer tissues were evaluated. There were 75 cases with breast cancer co-occurring with thyroid cancer (BC+TC cases) and 147 BC controls. As BC controls, 35 cases were selected from breast cancer patients who received surgery in Seoul National University Hospital between 2010 and 2012, and 112 were from Boramae hospital between 1999 and 2005. Clinicopathological data on breast cancer included the following: age at diagnosis, BMI, postmenopausal status, parity, type of surgery, pathologic type, pathologic stage including tumor size, lymph node metastasis, and distant metastasis, adjuvant therapy, follow-up period, and recurrence. To investigate the effect of thyroid cancer treatment, 39 breast cancer patients diagnosed after thyroid cancer with at least a 2 year latency period (TC→BC cases) were compared with BC controls. Additional information was obtained for RAI therapy and suppressed TSH levels. Thirty-six patients were diagnosed with breast cancer and thyroid cancer at the same time or were diagnosed with thyroid cancer after breast cancer (BC→TC cases).

Third, the expression of NIS in breast cancer tissue was examined in 39 TC→BC cases (mean age 51.1 ± 9.6 years) and 35 age-matched BC controls (mean age 51.4 ± 11.8 years) who were selected from Seoul National University Hospital between 2010 and 2012. Additional information on RAI therapy and thyroid hormone suppression was thoroughly gathered, including total RAI dose, latency period from the day of first RAI therapy to the time of breast cancer diagnosis, median doses of

levothyroxine, and prescription duration of levothyroxine. All patients, except one, were prescribed levothyroxine 50−250 μg/day (median 150 μg/day) for 1.8−20.4 years (median 4.4 years) until their breast cancer surgery. One patient was administered methimazole for Graves' disease, which developed in the residual lobe after a thyroid lobectomy at the time of breast cancer diagnosis. Twenty-seven patients underwent total thyroidectomy, and 20 received RAI therapy with a median dose of 90 mCi (range 60−190 mCi), 1−3 times with TSH stimulation. Laboratory data for thyroid function were obtained during the latency periods, which was the entire follow-up period for thyroid cancer before breast cancer surgery. The representative value for free thyroxine (FT4) was determined as the mean of all measurements.

Additionally, TSH before RAI therapy was determined for the mean, median, and last values. An immunometric assay was used to determine the concentrations of FT4 and TSH in the blood, as well as total triiodothyronine (T3) (Abbott, North Chicago, IL, USA). The normal ranges for TSH, FT4, and total T3 were 0.4−4.1 uIU/mL, 0.70−1.80 ng/dL, and 87−184 ng/dL, respectively.

The study was approved by the Institutional Review Board of Seoul National University Hospital (H 0912-009-302, H 1303-109-476, 16-2016-140, and 16-2017-57). This study conformed to the principles of the Declaration of Helsinki.

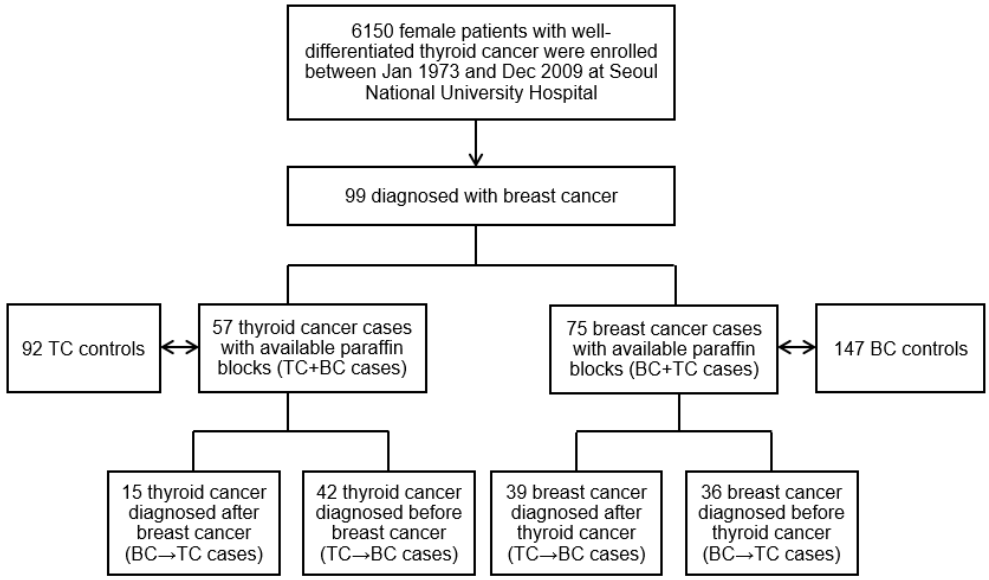

Figure 1. Flows of patients with thyroid and breast cancer.

Immunohistochemical staining

The BenchMark XT automated immunohistochemistry slide staining system (Ventana, Tucson, AZ, USA) was used for immunohistochemical staining using the following antibodies: ERα (Thermo Scientific; no dilution), ERβ (Biogenex, San Ramon, CA, USA; dilution of 1:300), TRα (Thermo Scientific; dilution of 1:600), TRβ (Santa Cruz Biotechnology, Dallas, TX, USA; dilution of 1:800). Two expert histopathologists (H.S. Min and Y.A. Kim) and one professional endocrinologist independently evaluated the expression (Y.A. Kim). The Allred score (range, 0 to 8 depending on the proportion and staining intensity) was used to assess the expression of ERs or TRs. Cases with a score of 2 or higher were considered positive. Representative images of each hormone receptors in thyroid cancer tissues are shown in Figure 2.

In breast cancer tissues, the expression of ERs or TRs was also evaluated using the Allred score, and the representative images are shown in Figure 3. The Ki-67 proliferation index in breast cancer was considered positive if the positively stained cells are more than 14 percent. Phosphatase and tensin homolog (PTEN) was stained both in the nucleus and cytoplasm, and the staining was interpreted through comparison with the adjacent normal epithelium and stroma, which acted as internal positive controls. PTEN staining was graded as 1 (weak positive staining relative to normal epithelium), 2 (positive staining with a strength equal to normal epithelium), and 0 (no immunoreaction), which represents PTEN loss. Immunohistochemical staining of NIS was performed using mouse monoclonal anti-NIS antibody (Thermo

Scientific, Waltham, MA, USA; dilution of 1:200). NIS showed only cytoplasmic staining, and its expression was defined as either negative or positive. The representative images for the expression of NIS in the normal breast and breast cancer tissues are shown in Figure 4.

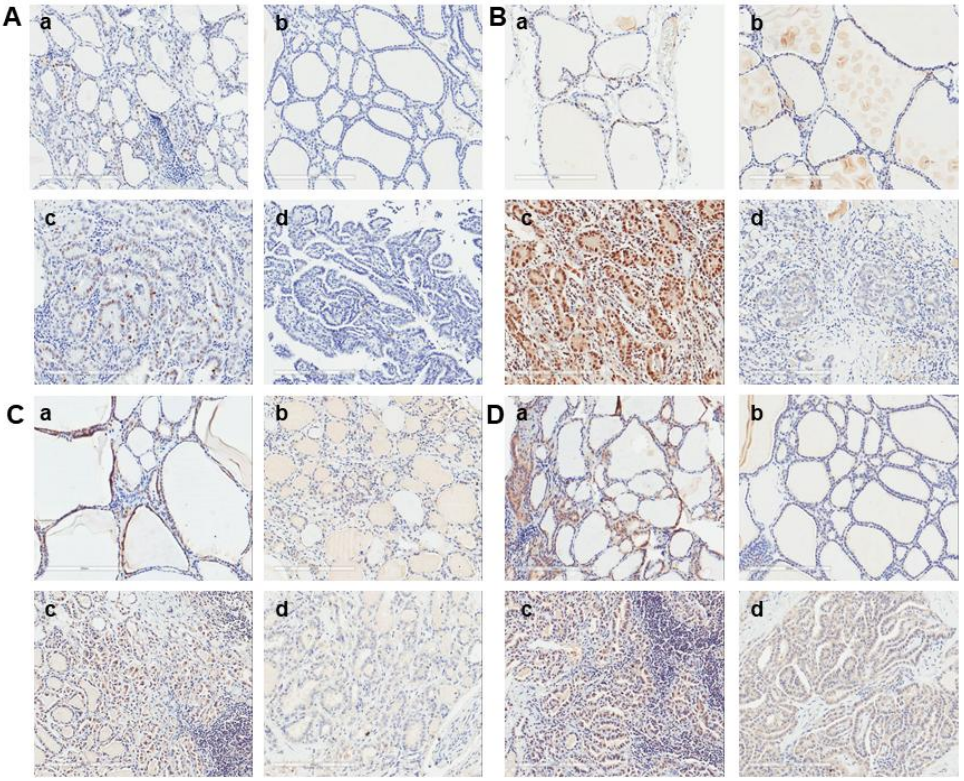

Figure 2. Immunohistochemical staining of ERα (A), ERβ (B), TRα (C), and TRβ (D) in normal thyroid and thyroid cancer tissues. Representative images for hormone receptor positive (a,c) and negative (b,d) both in normal tissues (a,b) and cancer tissues (c,d) (X200 magnification)

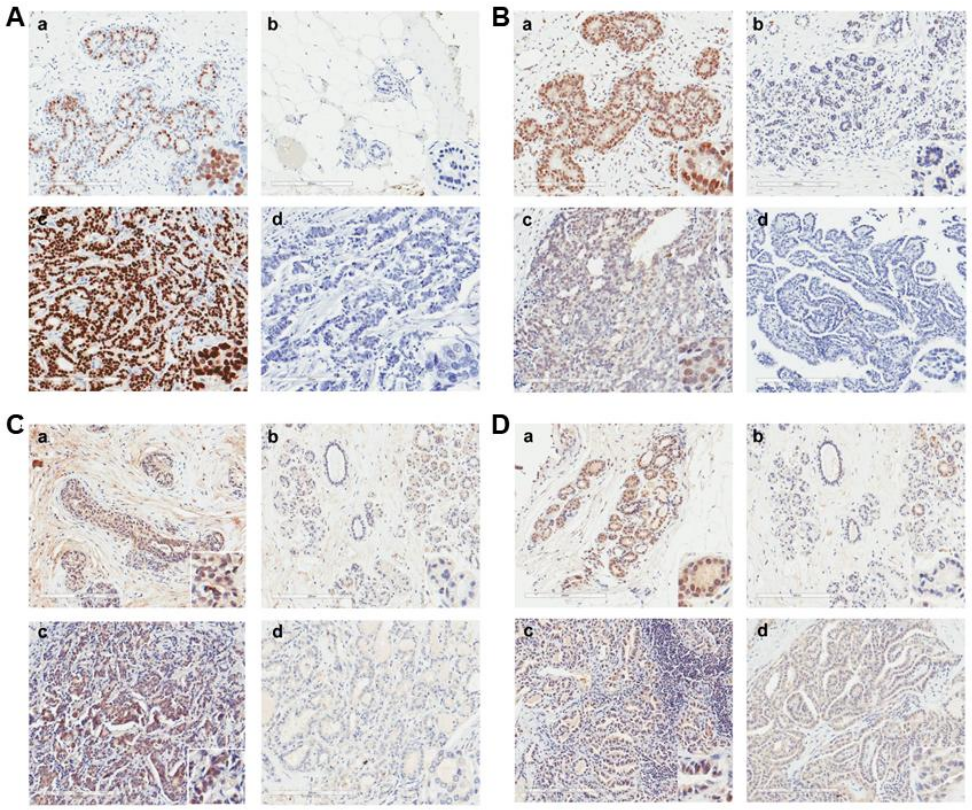

Figure 3. Immunohistochemical staining of ERα (A), ERβ (B), TRα (C), and TRβ (D) in normal breast and breast cancer tissues. Representative images for hormone receptor positive (a,c) and negative (b,d) both in normal tissues (a,b) and cancer tissues (c,d) (X200 magnification)

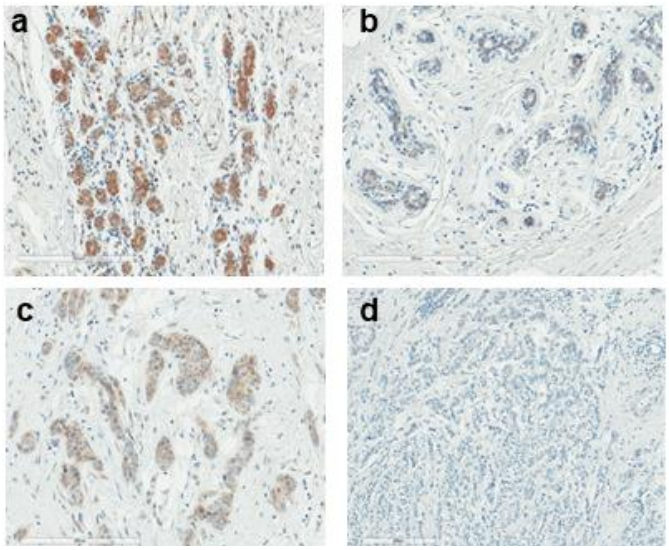

Figure 4. Immunohistochemical staining of NIS in normal breast and breast cancer tissues. NIS-positive (a) and negative (b) in normal breast tissue and NIS-positive (c) and negative (d) in breast cancer tissue (X200 magnification).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 19.0; SPSS, Inc., Chicago, IL, USA). Chi square or Fisher's exact test was used to compare dichotomous variables, as appropriate. For continuous variables, analysis of variance, independent Student's *t-test*, or Mann-Whitney *U* test were used. To investigate the association between markers and clinical factors, continuous variables including age at diagnosis and tumor size were dichotomized according to their median cutoff values. Regarding the level of thyroxine suppression, ER, TR, and NIS positivity in breast cancer was compared according to the TSH tertile of TC→BC cases. For TCGA analysis, the expression data was transformed by $\log_2(x+1)$. The prognosis of thyroid cancer patients was analyzed by Kaplan-Meier methods according to the mRNA expression levels of ESR1, ESR2, THRA, and THRB divided by two groups according to median values. Spearman method was used for correlation between SLC5A5 and ESR1/THRA to validate the association observed between NIS and ER/TR positivity in breast cancer. Kaplan-Meier estimation with Log Rank test was used to calculate time-dependent survivals in breast cancer according to NIS positivity and low/high mRNA expression levels of SLC5A5. *p* value less than 0.05 was considered statistically significant.

Chapter I. Expression of hormone receptors in thyroid cancer co-occurred with breast cancer

Results

Patients' characteristics

The clinical characteristics of thyroid cancer patients are shown in Table 1. The age at diagnosis was 48.4 years and 51.3 years in the TC+BC cases and TC controls, respectively ($p = 0.139$). In the TC control group, ETE was more frequent (76.1 vs. 52.7%, $p = 0.006$), and the follow-up period was shorter (3.3 vs. 7.6 years, $p < 0.001$). The difference in the rate of ETE and follow-up period seems to be due to the difference in the diagnosis time. Because the TC controls included more recent cases diagnosed in 2011–2013, the follow-up period is shorter, and the rate of ETE is higher with different surgical methods compared with the TC+BC cases. In the TC+BC group, the number of BC→TC cases was small (n = 15), and the follow-up period was shorter ($p = 0.004$) than in the TC→BC group (n = 42). These differences may be due to the collecting method of breast cancer cases diagnosed from thyroid cancer patients during follow-up. Otherwise, there was no difference between groups. Compared with the TC controls, BC→TC cases had a lower rate of PTC ($p = 0.013$), and TC→BC cases showed a lower PTC ratio ($p = 0.036$) and a longer follow-up period ($p < 0.001$).

Table 1. Clinicopathological characteristics of thyroid cancer patients

	TC+BC cases			TC controls (n = 92)	p^*	$p^\dagger$
	BC→TC (n = 15)	TC→BC (n = 42)	Total (n = 57)			
Age at diagnosis, yrs	53.5 ± 8.3	47.0 ± 10.2	48.4 ± 10.3	51.3 ± 12.5	0.272	0.139
BMI, kg/m^2	25.3 ± 2.7	23.9 ± 2.5	24.2 ± 2.7	24.7 ± 4.0	0.960	0.356
PTC, n (%)	14 (93.3%)‡	40 (95.2%)§	54 (94.7%)	92 (100%)	0.779	0.993
Tumor size, cm	1.1 ± 0.7	1.5 ± 1.1	1.4 ± 1.0	1.1 ± 0.6	0.453	0.097
Multifocality, n (%)	3 (20.0%)	15 (37.5%)	18 (32.7%)	43 (46.7%)	0.165	0.120
ETE, n (%)	10 (66.7%)	19 (47.5%)§	29 (52.7%)	70 (76.1%)	0.239	0.006
LN metastasis, n (%)	4 (36.4%)	10 (29.4%)	14 (31.1%)	21 (22.8%)	0.808	0.305
BRAF mutation (%)	12 (92.3%)	28 (75.7%)	40 (80.0%)	27 (71.1%)	0.202	0.449
FU period, yrs	5.7 ± 2.1	8.5 ± 6.0§	7.6 ± 4.1	3.3 ± 0.6	0.004	<0.001
Recurrence, n (%)	0	3 (7.1%)	3 (5.3%)	NA	0.292	NA

TC, thyroid cancer; BC, breast cancer; BMI, body mass index; PTC, papillary thyroid cancer; ETE, extrathyroidal extension; LN, lymph node metastasis; p^* was calculated by the comparison of BC→TC and TC→BC; $p^\dagger$ was calculated by the comparison of TC+BC cases and TC controls; p‡ was calculated by the comparison of BC→TC and TC controls; $p^\S$ was calculated by the comparison of TC→BC and TC controls.

Expression of hormone receptors in thyroid cancer tissue

The degree of hormone receptor positivity is summarized in Table 2. ERα positivity in the tumor tissue was higher in the TC + BC cases (66.7%) than in the TC controls (43.5%, $p = 0.007$). On the other hand, ERβ and TRβ positivity of tumor tissue were lower in the TC+BC cases than in TC controls (76.8 vs. 97.8%, $p < 0.001$ for ERβ and 19.6 vs. 45.1%, $p = 0.002$ for TRβ). Both ERβ and TRβ showed a homogenous pattern in the normal tissues, unlike in the cancer tissues, whereas ERα showed a difference in the cancer tissues but not in the normal tissues. The BC→TC cases showed a higher positive rate of TRα than the TC→BC cases (66.7 vs. 31.7%, $p = 0.031$). Compared with TC controls, BC→TC cases had a higher positive rate of ERα in both normal ($p = 0.024$) and cancer tissues ($p = 0.002$), whereas cancer tissues had lower positive rates in ERβ ($p < 0.001$) and TRβ ($p = 0.021$). TC→BC cases also showed lower expression of ERβ ($p < 0.001$) and TRβ ($p = 0.012$) in cancer tissues compared to TC controls.

Table 2. Expression of hormone receptors in thyroid cancer tissue

	TC+BC cases			TC controls (n = 92)	p^*	$p^\dagger$
	BC→TC (n = 15)	TC→BC (n = 42)	Total (n = 57)			
Normal thyroid						
ERα	3 (20.0%)‡	2 (5.7%)	5 (10.0%)	4 (4.3%)	0.127	0.188
ERβ	14 (100%)	32 (94.1%)	46 (95.8%)	92 (100%)	0.256	0.049
TRα	5 (35.7%)	9 (26.5%)	14 (29.2%)	16 (17.4%)	0.480	0.130
TRβ	8 (57.1%)	20 (58.8%)	28 (58.3%)	74 (80.4%)	0.915	0.009
Thyroid cancer						
ERα	13 (86.7%)‡	25 (59.5%)	38 (66.7%)	40 (43.5%)	0.058	0.007
ERβ	10 (66.7%)‡	33 (80.5%)§	43 (76.8%)	90 (97.8%)	0.489	<0.001
TRα	10 (66.7%)	13 (31.7%)	23 (41.1%)	37 (40.2%)	0.031	1.000
TRβ	2 (13.3%)‡	9 (22.0%)§	11 (19.6%)	41 (45.1%)	0.476	0.002

TC, thyroid cancer; BC, breast cancer; ER, estrogen receptor; p^* was calculated by the comparison of BC→TC and TC→BC; $p^\dagger$ was calculated by the comparison of TC+BC cases and TC controls; $p^‡$ was calculated by the comparison of BC→TC and TC controls; $p^§$ was calculated by the comparison of TC→BC and TC controls.

Hormone receptor positivity according to breast cancer treatment

For the impact of previous cancer treatment on thyroid cancer, receptor positivity in BC→TC cases according to breast cancer treatment is shown in Figure 5-8. As shown in Figure 5, patients treated with antiestrogens (tamoxifen or aromatase inhibitors) had higher expression of ERα (p = 0.015) and ERβ (p = 0.046) than those who did not. On the other hand, there was no significant difference in receptor positivity according to chemotherapy (Figure 6) and radiation therapy (Figure 7).

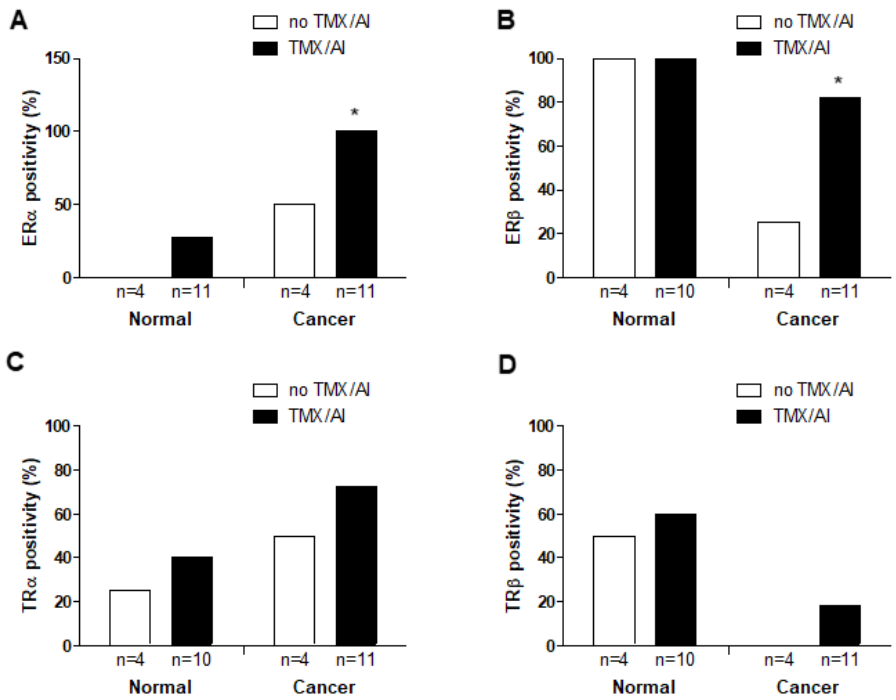

Figure 5. Expression of hormone receptors in thyroid cancer according to antiestrogen treatment for breast cancer. The positive rate of ERα (A), ERβ (B), TRα (C), and TRβ positivity (D) in normal and thyroid cancer tissues in BC→TC patients according to antiestrogen (tamoxifen and/or aromatase inhibitors, TMX+AI) treatment; $^{*}p$ <0.05 between TC controls and TC+BC cases.

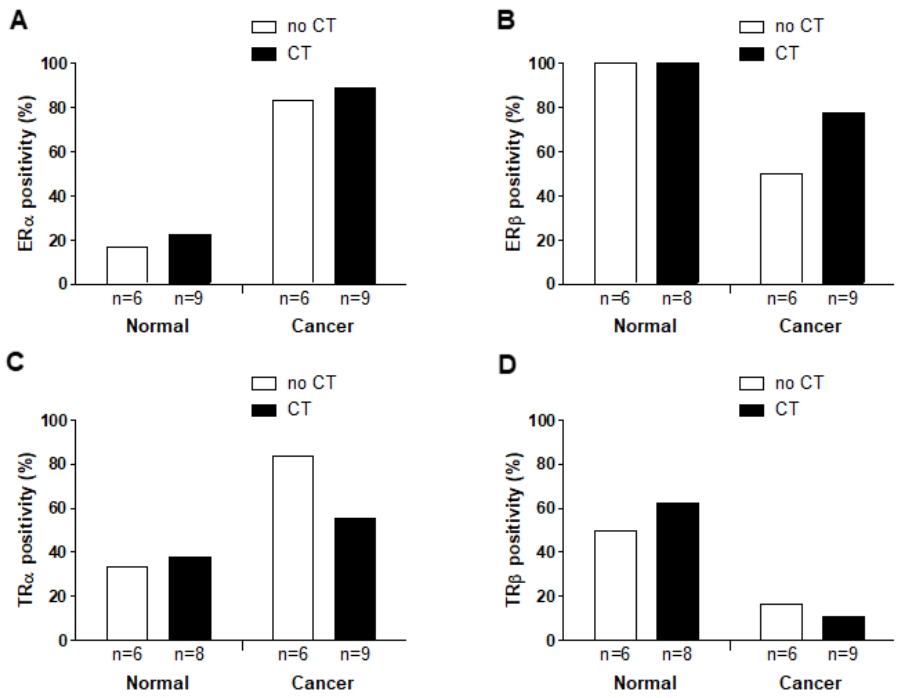

Figure 6. Expression of hormone receptors in thyroid cancer according to chemotherapy for breast cancer. The positive rate of ERα (A), ERβ (B), TRα (C), and TRβ positivity (D) in normal and thyroid cancer tissues in BC→TC patients according to chemotherapy.

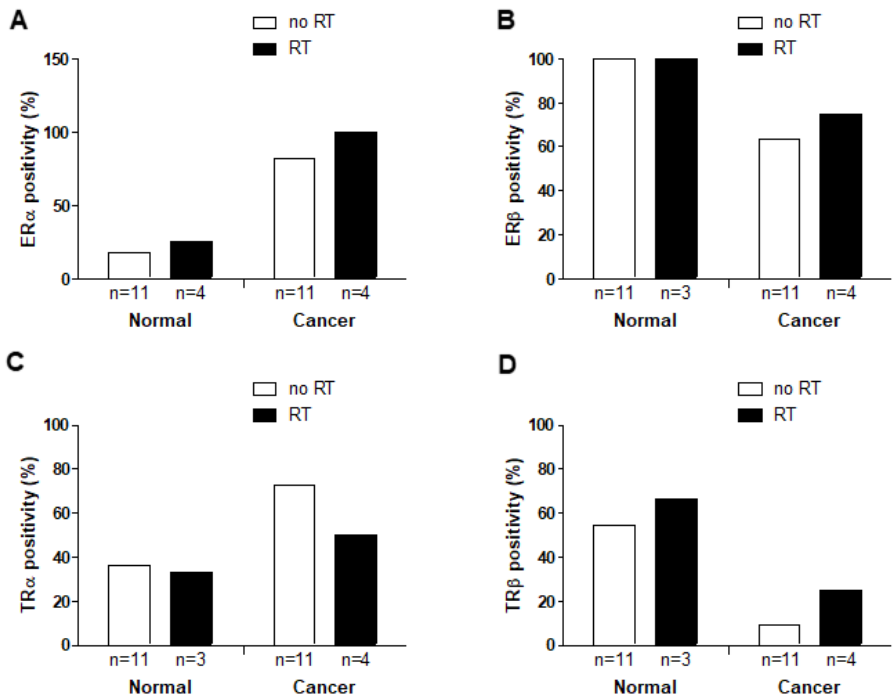

Figure 7. Expression of hormone receptors in thyroid cancer according to radiation treatment for breast cancer. The positive rate of ERα (A), ERβ (B), TRα (C), and TRβ positivity (D) in normal and thyroid cancer tissues in BC→TC patients according to radiation treatment.

Hormone receptor positivity according to clinicopathological characteristics

Considering higher ERα and lower ERβ, TRβ positivity in TC+BC cases than in TC controls, Figure 9 shows their association with clinicopathological features at the time of thyroid cancer diagnosis. A high positive rate of ERα was associated with postmenopausal status (p = 0.037). ERβ loss was more frequently observed in thyroid tumors ≥ 1 cm (p = 0.003) or lymph node metastasis (p = 0.009). TC+BC cases also had a lower TRβ expression in tumors ≥ 1 cm (p = 0.006). Otherwise, there was no significant difference in clinicopathological characteristics according to hormone receptor positivity.

Additionally, the positivity of hormone receptors was investigated according to the presence or absence of v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations (Figure 9). Although the positive rate of ERα seemed to be high when a BRAF mutation was present, it was not statistically significant (p = 0.076). The expression of other hormone receptors did not differ according to BRAF mutations.

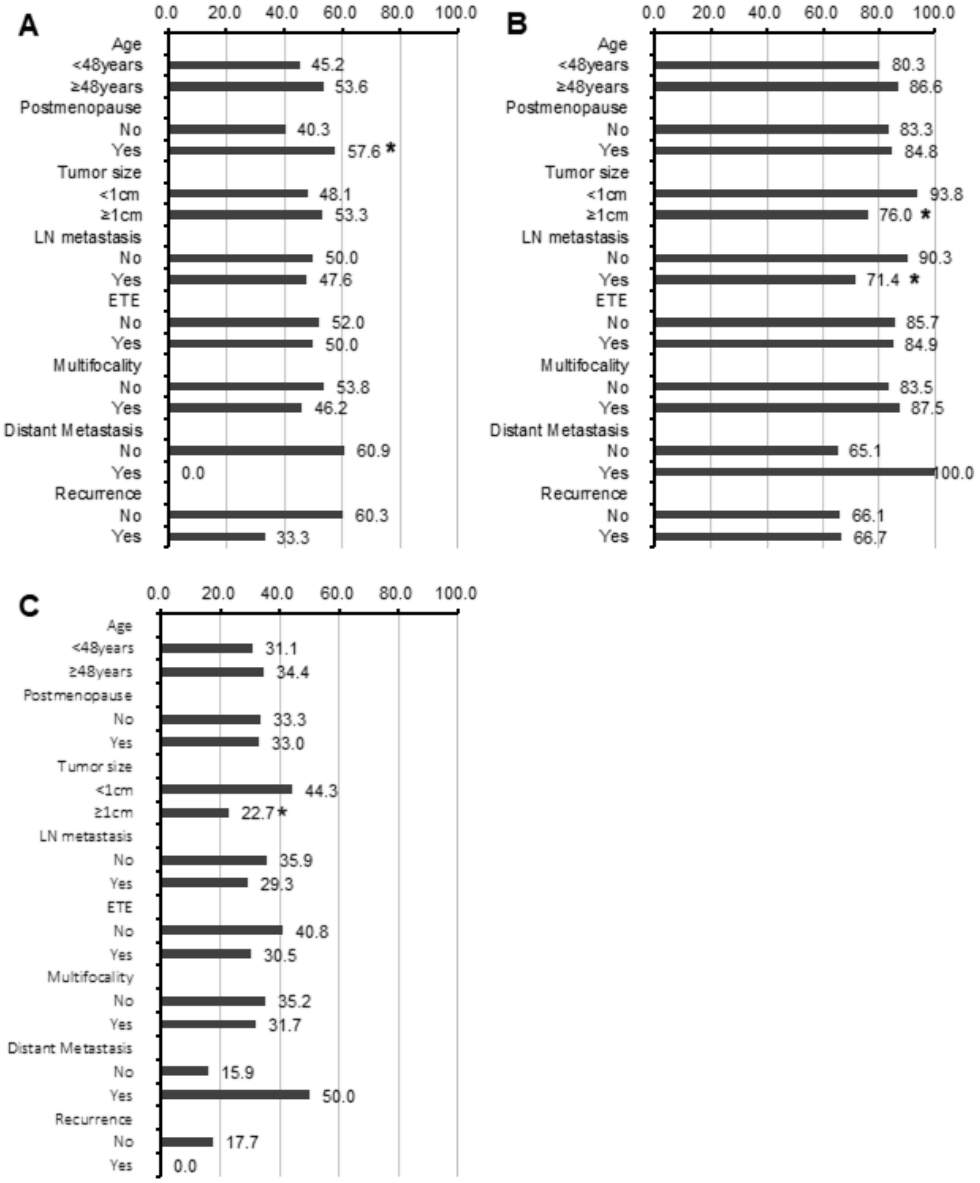

Figure 8. Hormone receptor positivity in the thyroid cancer tissue according to clinicopathological characteristics in the TC+BC cases. The positive rate of (A) ERα, (B) ERβ and (C) TRβ in the tissue of thyroid cancer co-occurred with breast cancer, $^{*}p < 0.05$.

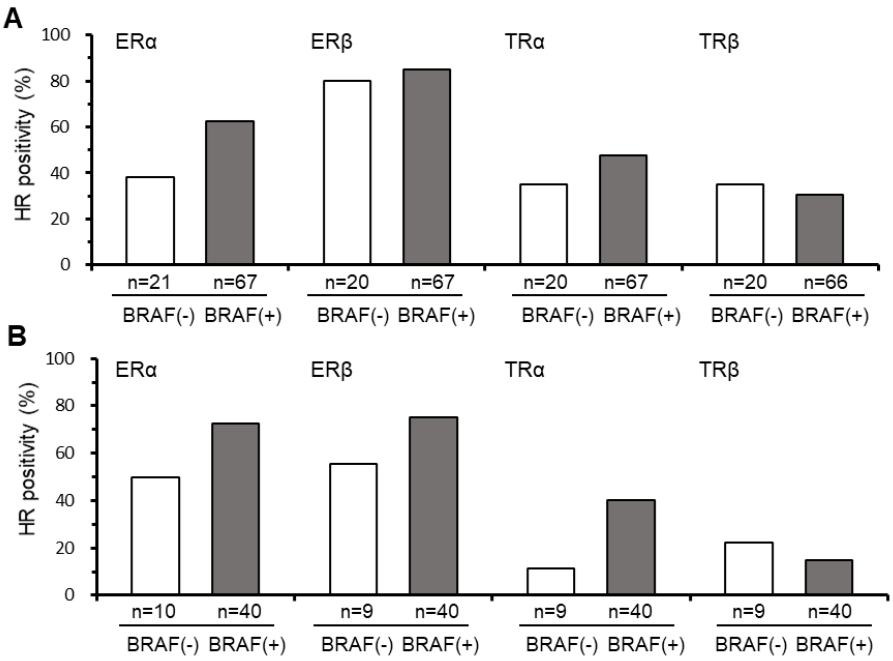

Figure 9. Hormone receptor positivity according to the presence or absence of BRAF mutation in thyroid cancer tissue. ERα, ERβ, TRα, and TRβ in total TC cases (A) and in the TC+BC cases (B).

Prognosis of thyroid cancer patients according to hormone receptor gene expression in TCGA analysis

Since there were differences in the pathological characteristics related to the prognosis of thyroid cancer according to the hormone receptor, it was further analyzed using TCGA data. As a result, there was no difference in the prognosis of thyroid cancer patients according to hormone receptor gene expression (Figure 10).

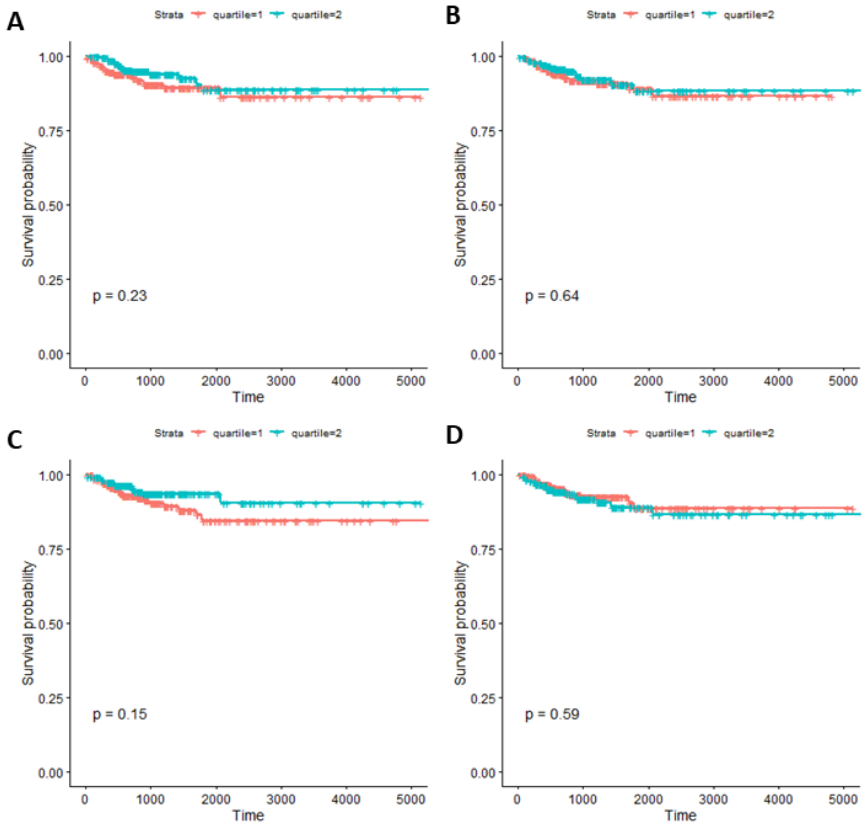

Figure 10. Recurrence-free survival in two groups of thyroid cancer patients divided by median values of mRNA expression of hormone receptors in TCGA data. ESR1 (A), ESR2 (B), THRA (C), and THRB (D).

Discussion

This study demonstrated higher ERα positivity with lower ERβ and TRβ expression in thyroid cancer co-occurred with breast cancer. High ERα positivity in the thyroid cancer tissue of TC+BC cases was significantly associated with antiestrogen therapy and menopausal status, which were deprived of estrogen. Therefore, high ERα expression may be a clue to explain the development of subsequent thyroid cancer or only a bystander observed in antiestrogen therapy. On the other hand, ERβ expression was higher in patients treated with antiestrogen, which contrasts with the lower ERβ positivity in the TC+BC cases. ERβ loss was more frequently associated with tumors ≥ 1 cm or positive lymph node metastasis. TRβ loss was also more common in tumors ≥ 1 cm. Although it is not associated with breast cancer treatment, loss of ERβ and TRβ may also play a role in subsequent thyroid cancer development. Since ERβ- or TRβ-negative TC+BC cases had more aggressive tumor biology, loss of ERβ or TRβ may be involved in the progression of thyroid cancer co-occurred with breast cancer.

ERs may be involved in the development of thyroid and breast cancer through shared hormones and pathways. ERα is well known for its cellular proliferative action and promotes breast cancer (110, 111). Several *in vitro* studies showed that ERα can also play a role in thyroid carcinogenesis (79, 106). ERα activates the MAPK pathway, resulting in thyroid and breast carcinogenesis (23, 111). Otherwise, ERα upregulates the endothelial nitric oxide synthase (eNOS) gene and leads to angiogenesis, which is essential for cancer progression (112). Interestingly,

Vannucchi *et al.* (37) showed that BRAF mutations were more common in ERα-positive PTC, although it was not statistically significant with a limited number of cases. In this study, the positivity of ERα appeared to be high in the presence of the BRAF mutation, although it was not statistically significant. Considering the aberrant activation of the MAPK pathway by the BRAF mutation, ERα and BRAF mutations may activate the MAPK pathway synergistically, resulting in PTC propagation (37).

In a previous cell experiment, tamoxifen suppressed cell proliferation in PTC cells (65), which conflicts with the results of this study which found an increased expression of ERα with tamoxifen or antiestrogen treatment. In breast cancer cells, long-term estradiol deprivation increased ERα expression and ER target genes involved in cellular proliferation (113). An adaptive increase in ERα did not increase the transcription of certain genes which mediate differentiated cellular function, resulting in unregulated cellular proliferation (113). However, there is a lack of data for this mechanism of estradiol-deprived ERα adaptation in thyroid cancer cells and whether ERα was increased in thyroid cancer cells due to estradiol deficiency during antiestrogen therapy or menopause.

Unlike ERα, ERβ acts as a tumor suppressor, inducing apoptosis in thyroid tumors (114). Ahn *et al.* (115) showed that ERβ loss in premenopausal women was associated with an increased risk of recurrence in female PTC patients aged < 45 years, suggesting a protective role of ERβ in PTC progression. Heikkila *et al.* (116) also reported that low ERβ expression was correlated with a poor prognosis of

follicular thyroid cancer (FTC). Since ERβ expression was decreased in large tumors or lymph node metastasis, ERβ loss may be responsible for the aggressive biology in thyroid cancer co-occurring with breast cancer.

TRβ is a regulator in cellular growth and differentiation (48). In a mouse model with a dominant-negative TRβ mutant, spontaneous development of FTC is frequently observed with thyroid hormone resistance and elevated TSH levels (93, 117, 118). TRβ downregulates the phosphatidylinositol 3- kinase (PI3K)/protein kinase B (AKT) signaling pathway, thereby reducing the oncogenic action of β-catenin or cyclin D1 (93, 118). TRβ can also attenuate inflammation and NF-kB pathways, which are involved in tumor progression (117). TRβ also plays a role as a tumor suppressor regulating RUNX2 transcription, which is important in cancer metastasis for both thyroid and breast cancer (46, 47). Therefore, TRβ loss may be involved in the progression of thyroid cancer co-occurred with breast cancer, although the mechanism for the decrease in TRβ is unknown.

This is the first study to identify the expression of hormone receptors in thyroid cancer co-occurred with breast cancer. Considering the oncogenic action of ERα and the tumor suppression of ERβ and TRβ, increased ERα expression and loss of ERβ, TRβ may be related to thyroid carcinogenesis associated with breast cancer. Antiestrogen and tamoxifen therapy showed associations with high ERα and ERβ expression. Antiestrogen therapy may upregulate ER expression in thyroid cancer tissue; however, it may not be related to the risk of subsequent thyroid cancer because of the opposite direction of ERβ positivity. Otherwise, enhanced ERα and

the loss of ERβ, TRβ may constitute endogenous expression in concurrent thyroid and breast cancers. Despite uncertain causality, presumptive changes in hormone receptors and the association with antiestrogen therapy may suggest a hormonal link in the co-occurrence of thyroid and breast cancer.

Chapter II. Expression of hormone receptors in breast cancer co-occurred with thyroid cancer

Results

Patients' characteristics

The clinical characteristics of breast cancer patients are shown in Table 3. The BC+TC cases had lower BMI (23.4 vs 24.6, $p = 0.020$) and smaller tumors (2.4 vs 2.7 cm, $p = 0.006$), more ductal carcinoma in situ (DCIS; 15.8 vs. 0%, $p < 0.001$) and less frequent lymph node metastasis (33.3 vs. 47.9%, $p = 0.040$) than the BC controls. They received more mastectomies (42.5 vs. 19.0%, $p < 0.001$), a higher proportion of hormone therapy (79.7 vs. 49.6%, $p < 0.001$) and radiation (62.2 vs. 31.3%, $p < 0.001$), and a lower rate of chemotherapy (64.9 vs. 81.0%, $p = 0.009$). Stage I and Luminal A subtype cancers were more frequent in the BC+TC group (41.8 vs. 18.4%, $p = 0.003$ for Stage I; 68.6 vs. 46.8%, $p = 0.024$ for the Luminal A subtype). Age at diagnosis, BMI, menopausal status, and parity did not differ between the BC+TC cases and the BC controls. In the BC+TC cases, the TC→BC cases had suppressed TSH levels (0.16 vs. 1.51 uIU/L, $p < 0.001$) and a higher proportion of stage IA-IIA cancers (94.1 vs. 63.2%, $p = 0.001$) and DCIS (25.0 vs. 7.7%, $p = 0.040$) than the BC→TC cases. The TC→BC cases also received more radiation therapy (77.1 vs. 48.7%, $p < 0.001$) and less chemotherapy (42.9 vs. 84.6%, $p = 0.009$). The recurrence rate was also lower in the TC→BC cases compared with the BC→TC cases (5.6 vs. 25.6%, $p = 0.026$).

Table 3. Clinicopathological characteristics of breast cancer patients

	BC+TC cases			BC controls (n = 147)	P^*	$P^\dagger$
	TC→BC (n = 36)	BC→TC (n = 39)	Total (n = 75)			
Age at diagnosis, years	48.0 [45.3, 54.8]	46.6 [41.3, 55.7]	47.9 [43.0, 55.0]	49.0 [44.0, 61.0]	0.151	0.124
Body mass index, kg/m^2	23.1 ± 2.6	23.8 ± 2.9	23.4 ± 2.7	24.6 ± 3.7	0.327	0.020
Postmenopause, n (%)	17 (47.2)	12 (30.8)	29 (38.7)	69 (46.9)	0.144	0.240
TSH, mIU/L	0.16 [0.05, 0.30]	1.51 [0.98, 2.82]	0.87 [0.15, 2.12]	2.17 [0.17, 2.67]	<0.001	0.277
Type of surgery, n (%)	23/12	19/19	42/31	119/28	0.175	<0.001
BCS/Mastectomy	(65.7/34.3)	(50.0/50.0)	(57.5/42.5)	(81.0/19.0)		
Tumor size, cm	2.1 [1.0, 3.0]	2.5 [1.7, 3.0]	2.4 [1.3, 3.0]	2.7 [2.0, 3.8]	0.103	0.006
IDC/DCIS/others, n (%)	24/9/3	36/3/0	60/12/3	146/0/1	0.040	<0.001
	(66.7/25.0/8.4)	(92.3/7.7/0)	(80.0/15.8/3.9)	(99.3/0/0.7)		
LN metastasis n (%)	9 (26.5)	15 (39.5)	24 (33.3)	70 (47.9)	0.243	0.040
Distant metastasis n (%)	0 (0)	1 (2.6)	1 (1.4)	6 (4.1)	1.000	0.281
Pathologic stage, n (%)	14/2/1/0	9/15/10/4	23/17/11/4	27/58/32/30	0.001	0.003
IA/IIA/IIB/IIIA	(82.4/11.8/5.9/0)	(23.7/39.5/26.3/10.5)	(41.8/30.9/20.0/7.3)	(18.4/39.5/21.8/20.4)		
Subtype, n (%)	23/5/2/1	25/3/3/8	48/8/5/9	66/33/12/30	0.146	0.024
LA/LB/HER2/Basal	(74.2/16.1/6.5/3.2)	(64.1/7.7/7.7/20.5)	68.6/11.4/7.1/12.9)	(46.8/23.4/8.5/21.3)		
Adjuvant therapy						
Hormone therapy, n (%)	30 (85.7)	29 (74.4)	59 (79.7)	64 (49.6)	0.225	<0.001
Chemotherapy, n (%)	15 (42.9)	33 (84.6)	48 (64.9)	119 (81.0)	<0.001	0.009
Radiation therapy, n (%)	27 (77.1)	19 (48.7)	46 (62.2)	46 (31.3)	0.012	<0.001
Recurrence, n (%)	2 (5.6)	10 (25.6)	12 (16.0)	36 (24.5)	0.026	0.146
Follow up duration, years	10.3 ± 3.6	11.3 ± 4.6	10.8 ± 4.1	10.6 ± 4.4	0.286	0.357

BC, breast cancer; TC, thyroid cancer; TSH, thyroid stimulating hormone; IDC, invasive ductal carcinoma; DCIS, ductal carcinoma in situ; LA, luminal A; LB, luminal B; HER2, HER2-enriched. p^* was calculated by the comparison of BC→TC and TC→BC; $p^\dagger$ was calculated by the comparison of BC+TC cases and BC controls.

Expression of hormone receptors in breast cancer tissue

The expression of hormone receptors in breast cancer tissue is shown in Figure 11. The positive rate of ERα in breast cancer tissues was higher in the BC+TC cases than the BC controls (79.7 vs. 58.7%, $p = 0.002$). TRα positivity was higher in the BC+TC cases than the BC controls in both normal breast tissues (51.5 vs. 23.3%, $p = 0.009$) and breast cancer tissues (21.6 vs. 6.8%, $p = 0.001$). Except for ERα in the BC+TC cases, both ERα and TRα positivity tended to be lower in breast cancer tissues than normal breast tissues. There was no difference in ERβ and TRβ expression between the BC+TC cases and the BC controls in normal and cancer tissues. TRβ positivity was lower in cancer tissues than normal tissues (from 34.5 to 6.8% in the BC controls, $p < 0.001$; from 26.9 to 4.1% in the BC+TC cases, $p < 0.001$).

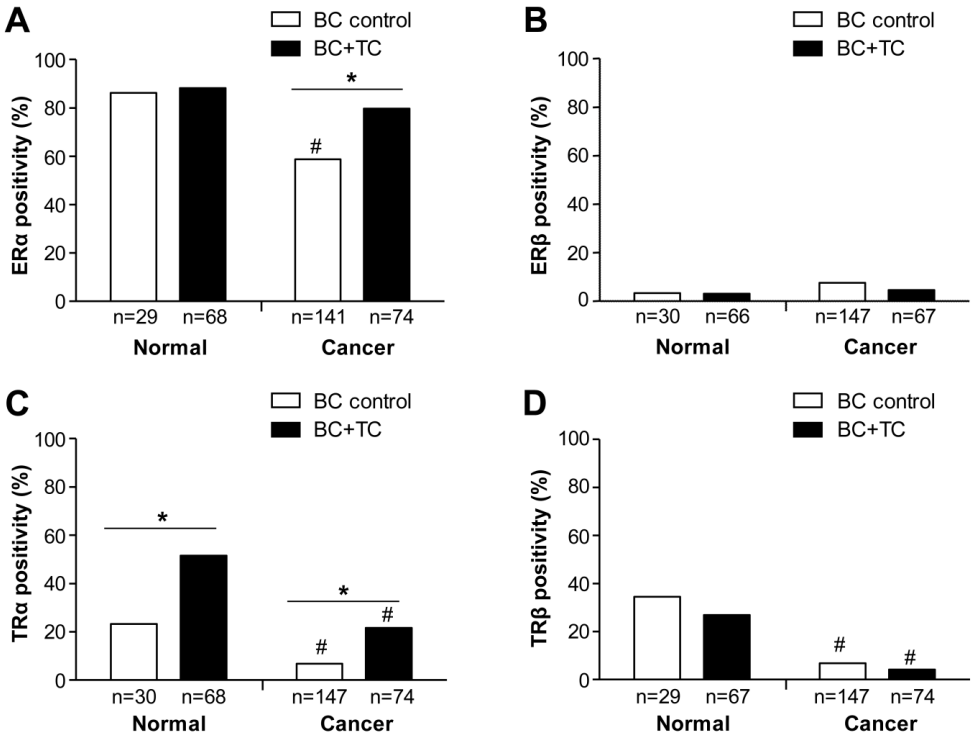

Figure 11. Expression of hormone receptors in breast cancer co-occurred with breast cancer ERα (A), ERβ (B), TRα (C), and TRβ positivity (D) in normal and breast cancer tissues. ***p* <0.05 on comparing the BC+TC and BC control groups; [#]*p* <0.05 on comparing cancer and normal tissues within the group. ER, estrogen receptor; TR, thyroid hormone receptor; BC, breast cancer; TC, thyroid cancer. Reprinted from "Increased expression of thyroid hormone receptor alpha and estrogen receptor alpha in breast cancer associated with thyroid cancer" by Kim YA, Kim YA, Cho SW, Song YS, Min HS, Park IA, Park DJ, Hwang KT, Park YJ, Eur J Surg Oncol. 2021 Jun;47(6):1316-1323. doi: 10.1016/j.ejso.2021.01.015. © 2021 Elsevier Inc.

Association between thyroid cancer treatment and hormone receptors

For the possible influence of thyroid cancer treatment, the associations between hormone receptor positivity and thyroid cancer treatment were investigated in the TC→BC cases (Figure 12). There was no difference in the expression of ERα and ERβ according to RAI therapy or the suppressed level of TSH. However, TR expression in the cancer tissues of the no RAI group tended to be lower than in normal tissues (16.7 vs. 52.9%, $p = 0.035$ for TRα; 5.9 vs. 46.7%, $p = 0.013$ for TRβ). In addition, TRα and TRβ positivity tended to be lower in the low TSH group, although it was not statistically significant (TRα: $p = 0.364$ in normal tissues, $p = 0.156$ in cancer tissues; TRβ: $p = 0.300$ in normal tissues, $p = 0.046$ in cancer tissues).

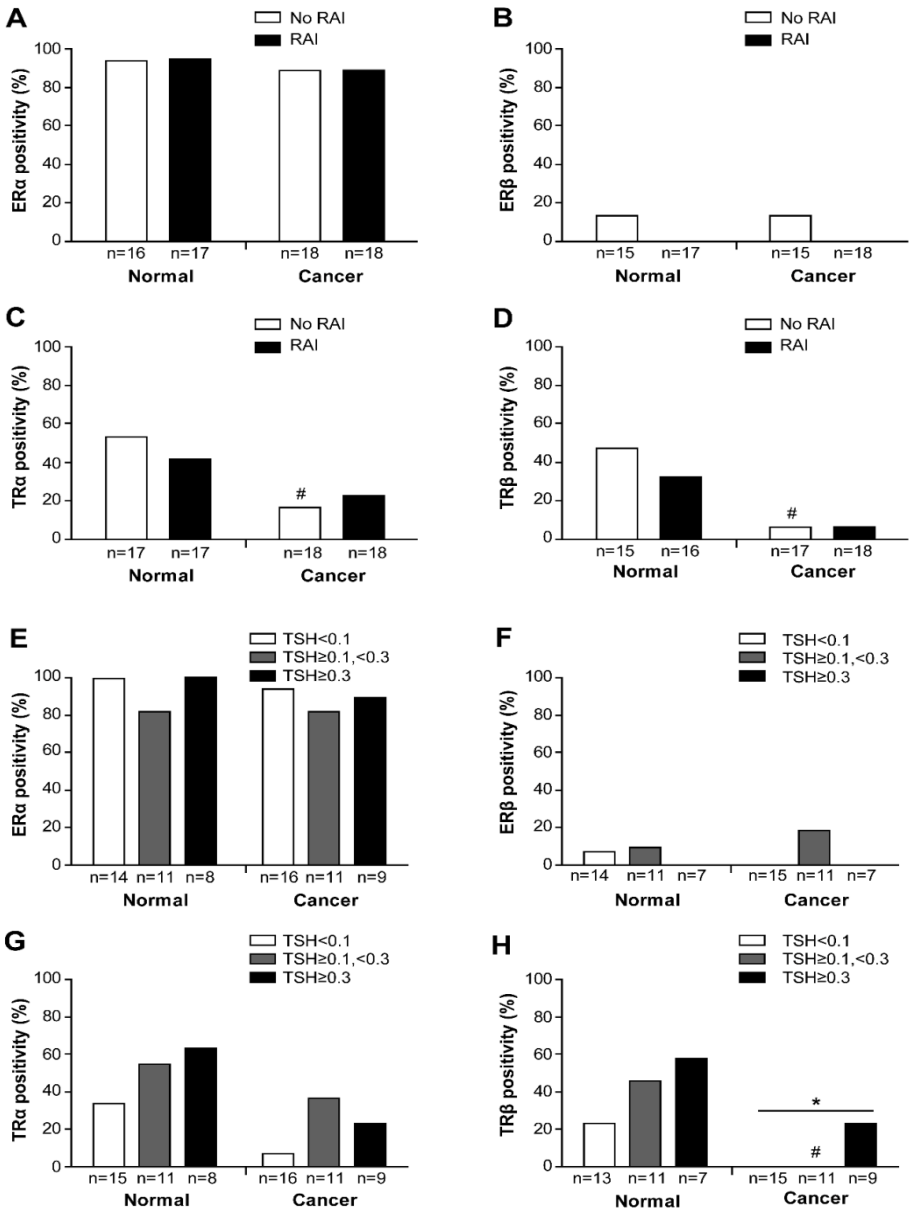

Figure 12. Expression of hormone receptors in the normal and breast cancer tissues according to thyroid cancer treatment hormone receptor positivity according to RAI therapy (A-D) or median serum TSH level (E-H). **p* for trend <0.05 for TR expression according to the TSH level; $^{\#}p$ <0.05 on comparing cancer tissue with normal tissue within the group. Reprinted from "Increased expression of thyroid hormone receptor alpha and estrogen receptor alpha in breast cancer associated with thyroid cancer" by Kim YA, Kim YA, Cho SW, Song YS, Min HS, Park IA, Park DJ, Hwang KT, Park YJ, Eur J Surg Oncol. 2021 Jun;47(6):1316-1323. doi: 10.1016/j.ejso.2021.01.015. © 2021 Elsevier Inc.

Association between clinicopathological features and hormone receptors

In the BC+TC cases, the associations between ERα/TRα positivity in breast cancer tissue and clinicopathological features are shown in Figure 13. In general, ERα-positive breast cancer has a good prognosis, and thus the following associations were observed. The positive rate of ERα was higher in stage I-IIA than in stage IIB-IIIA cancers (85.0 vs. 57.1%, $p = 0.031$). ERα-positivity tended to be higher in the BC+TC cases with a negative Ki-67 index (83.9 vs. 50.0%, $p = 0.045$), in those with patent PTEN (87.3 vs. 53.3%, $p = 0.004$), and those with no recurrence (85.7 vs. 45.5%, $p = 0.002$). Other clinicopathological features did not correlate with either ERα- or TRα-positivity.

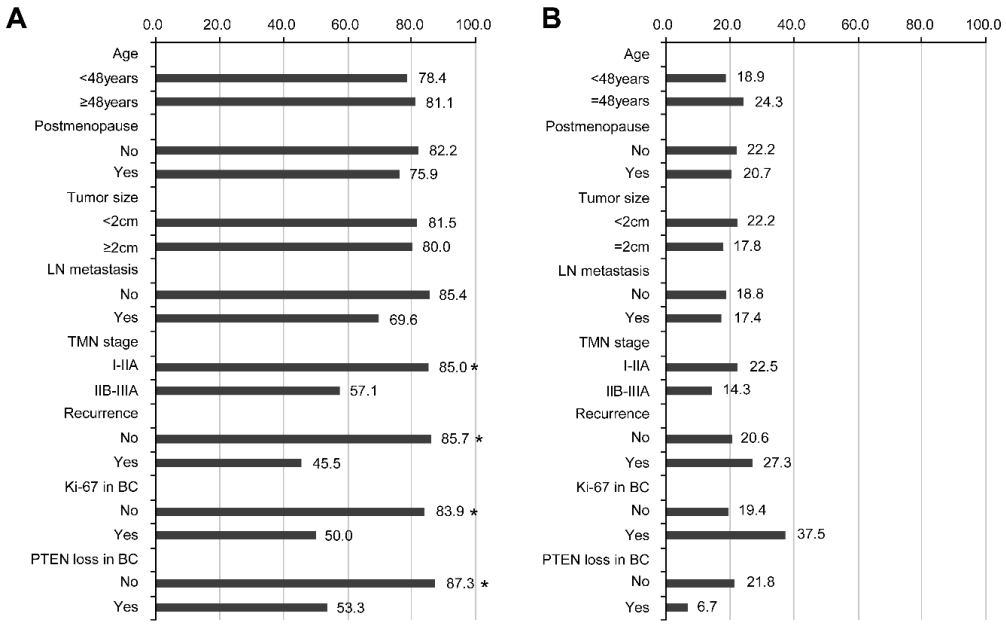

Figure 13. ERα and TRα positivity in the breast cancer tissues according to clinicopathological characteristics in the BC+TC cases ERα (A) and TRα (B) positivity in the breast cancer tissues according to each histologic characteristic in the BC+TC cases. *p <0.05. Reprinted from "Increased expression of thyroid hormone receptor alpha and estrogen receptor alpha in breast cancer associated with thyroid cancer" by Kim YA, Kim YA, Cho SW, Song YS, Min HS, Park IA, Park DJ, Hwang KT, Park YJ, Eur J Surg Oncol. 2021 Jun;47(6):1316-1323. doi: 10.1016/j.ejso.2021.01.015. © 2021 Elsevier Inc.

Discussion

This study showed that the expression of ERα and TRα in breast cancer co-occurring with thyroid cancer (119). Both ERα and TRα positivity tended to be higher in the BC+TC cases than the BC controls. Since any treatment-related factors were not associated with the expression of ER and TR, we assumed the pathophysiologic alteration of hormone receptors according to thyroid and breast cancer progression.

With an increasing interest in the link between breast and thyroid cancer, several studies have reported the positive rate of ER in BC+TC based on surgical pathology (84, 85, 120). There is no data on TR since it is not included in routine breast cancer pathology reports, and this is currently the only study related to it (119). According to the SEER data, ERα-positive breast cancer has been reported more frequently with co-existing thyroid cancer (84), consistent with other studies (86). ERα expression levels were higher in secondary breast cancer in a prospective cohort of primary thyroid cancer patients (120). However, the degree of ERα-positivity was not higher in another retrospective cohort study which selected breast and thyroid cancers based on diagnosis only (85).

Using a tissue microarray, we showed a higher degree of ERα and TRα positivity in breast cancer co-occurring with thyroid cancer. However, as shown in this study, ERα/TRα positivity was not associated with thyroid cancer treatment or any clinicopathological features. Wu *et al.* (87) reported an increased risk of breast cancer associated with levothyroxine use and duration. Thyroxine treatment to

suppress TSH after thyroidectomy might be a reason for the co-occurrence of thyroid and breast cancer. In this study, however, serum thyroxine level due to TSH suppression was not associated with the level of ERα positivity and TRα positivity in the TC→BC group.

The mechanism underlying the increased expression of ERα/TRα remains unclear. In a previous epidemiological study, patients with hyperthyroidism showed an increased risk of breast cancer (73-76). Thyroid hormones may be associated with an increased risk of breast cancer or ERα expression. *In vitro* experiments have shown that the administration of T3 increased the levels of ERα and TRα and promoted BC cell proliferation (79, 110). Thyroid hormone can bind to TR and activates the MAPK pathway, resulting in the proliferation in breast cancer cells (41).

The increased expression of ERα/TRα suggests a possible influence of hormones in breast cancer co-occurring with thyroid cancer, while no effects of prior cancer treatment were observed. Since ERα/TRα expression in breast cancers was not associated with any clinicopathological features, this might be influenced by unchecked hormonal changes or other unrevealed factors. Although no inducing factors for hormone receptors were found, their expression changes in thyroid and breast cancer suggest a hormonal link between the two cancers.

Chapter III. Expression of NIS and the effect of RAI therapy on breast cancer developed after thyroid cancer

Results

Patients' characteristics

Table 4 presents clinicopathological characteristics of the TC→BC cases and the BC controls according to NIS positivity. The TC→BC cases had a higher proportion of stage IA cancers (82.4 vs. 24.0%, p = 0.003) and DCIS (26.5 vs. 0%, p = 0.012) than the BC controls, received less frequent chemotherapy (41.2 vs 92.0%, p < 0.001), and had a longer follow-up period (median 9.9 [8.3-12.2] vs. 8.3 [7.4-9.2] years, p = 0.001). NIS-positive TC→BC cases had more DCIS than NIS-negative TC→BC cases (36.8 vs. 13.3%, p = 0.047). NIS-positive BC controls had smaller tumor sizes than NIS-negative BC controls (2.0 vs. 2.7 cm, p = 0.034). There was no difference in the characteristics of tumor size, breast cancer pathology, lymph node involvement, distant metastasis, or staging.

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Table 4. Clinicopathological characteristics of TC→BC cases and BC controls according to NIS positivity

Characteristics	Total BC (n = 59)		BC control (n = 25)		TC→BC cases (n = 34)	
	NIS positive (n = 24)	NIS negative (n = 35)	NIS positive (n = 5)	NIS negative (n = 20)	NIS positive (n = 19)	NIS negative (n = 15)
Age at diagnosis, years	47.0 (43.0–51.8)	48.0 (46.0–58.0)	43.0 (33.0–50.0)	47.5 (46.0–57.8)	47.0 (43.0–53.0)	48.0 (46.0–59.0)
Body mass index, kg/m^2	23.0 (21.7–25.5)	23.7 (20.8–25.7)	22.3 (21.5–24.1)	23.7 (20.6–25.4)	23.6 (21.6–26.7)	23.9 (21.9–26.2)
Postmenopause, n (%)	8 (33.3)	17 (48.6)	0	9 (45.0)	8 (42.1)	8 (53.3)
TSH, mIU/L	0.2 (0.1–0.3)	0.2 (0.1–2.3)	NA	2.4 (0.1–3.1)	0.2 (0.1–0.3)	0.1 (0.1–0.8)
Type of surgery, n (%)	16/8	17/18	3/2	8/12		
BCS/Mastectomy	(66.7/33.3)	(48.6/51.4)	(60.0/40.0)	(40.0/60.0)	13/6 (68.4/31.6)	9/6 (60.0/40.0)
Tumor size, cm	2.0 (1.0–2.9)	2.5 (1.6–3.5)	2.0 (1.5–2.5)	2.7 (2.0–3.9)†	2.0 (1.0–3.0)	2.1 (1.3–3.0)
[a] BC pathology, n (%)	14/7/3	32/2/1	5/0/0	19/0/1	9/7/3	13/2/0
IDC/DCIS/others, n	(58.3/29.2*/12.5)	(91.4/5.7/2.9)	(100/0/0)	(95.0/0/5.0)	(47.4/36.8†/15.8)*	(86.7/13.3/0)
LN metastasis n (%)	7 (29.2)	13 (37.1)	3 (60.0)	8 (40.0)	4 (21.1)	5 (33.3)
Distant metastasis n (%)	0	0	0	0	0	0
Pathologic stage, n (%)	10/4/2/0	10/10/3/3	1/3/1/0	5/9/3/3	5/1/0/0	9/1/1/0
IA/IIA/IIB/IIIA	(62.5/25/12.5/0)	(38.5/38.5/11.5/11.5)	(20/60/20/0)	(25/45/15/15)	(83.3/16.7/0/0)*	(81.8/9.1/9.1/0)
Subtype, n (%)	12/5/1/2	24/1/3/6	12/0/3/5	2/1/0/2	10/4/1/0	12/1/0/1
LA/LB/HER2/Basal	(60/25/5/10)	(70.6/2.9/8.8/17.6)	(85.7/0/100/71.4)	(14.3/100/0/28.6)	(66.7/26.7/6.7/0)	(85.7/7.1/0/7.1)
Adjuvant therapy						
Hormone therapy, n (%)	18 (81.8)	26 (74.3)	4 (80.0)	12 (60.0)	15 (88.2)	13 (86.7)
[a] Chemotherapy, n (%)	11 (50.0)	24 (68.6)	5 (100.0)	18 (90.0)	6 (35.3)*	8 (53.3)
Radiation therapy, n (%)	16 (72.7)	22 (62.9)	3 (60.0)	12 (60.0)	12 (80.0)	14 (82.4)
FU duration, years	9.2 (8.3–12.2)	8.3 (7.4–9.2)*	8.5 (8.2–10.9)	8.0 (5.4–8.6)	9.5 (8.5–12.5)*	10.7 (7.4–13.7)

BC, breast cancer; TSH, thyroid stimulating hormone; IDC, invasive ductal carcinoma; DCIS, ductal carcinoma in situ; NIS, sodium iodide symporter; TC, thyroid cancer. *p <0.05 on comparing the BC+TC and BC controls; $^{\dagger}p$ <0.05 on comparing NIS positive BC cases and NIS negative BC cases

Positivity of NIS expression in BC+TC cases in comparison with BC controls

The expression of NIS in the TC→BC cases was higher than in the BC controls (64.5 vs. 30.0%, p = 0.016 in normal tissues and 55.9 vs. 20.0%, p = 0.006 in cancer tissues; Figure 14A). Subgroup analysis was performed in only invasive ductal carcinoma patients to exclude the possible confounding effect of the higher proportion of DCIS in the TC→BC cases. Similarly, high NIS positivity was observed in the TC→BC cases, but it was not statistically significant (p = 0.051 for normal and p = 0.139 for cancer tissues; Figure 14B).

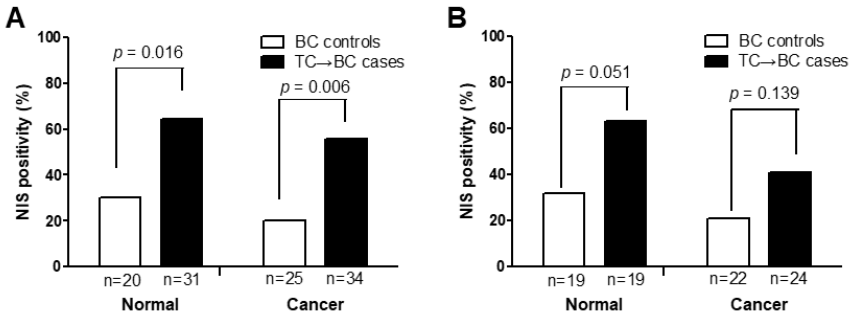

Figure 14. NIS positivity in normal breast tissue and breast cancer tissues. NIS positivity was compared between BC controls and TC→BC cases (A) and only in IDC patients (B).

Effects of prior radioactive iodine treatments or suppressed TSH levels on the expression of NIS in breast cancer tissues

To investigate the effect of thyroid cancer treatments on subsequent breast cancer, the TC→BC cases were divided into subgroups according to RAI therapy or suppressed TSH levels.

Of the 34 TC→BC cases, 18 received RAI therapy (RAI group), and 16 did not (no RAI group). In breast cancer tissues, NIS positivity was higher in the RAI group (72.2%) than in the no RAI group (37.5%, $p = 0.042$) and the BC controls (20.0%, $p = 0.001$; Figure 15A). NIS positivity in the RAI group (68.8%) was similar to that in normal breast tissues compared with the no RAI group (60.0%, $p = 0.611$) but was different from the BC controls (30.0%, $p = 0.021$; Figure 15A).

The effect of RAI on NIS positivity was further analyzed with RAI-related factors such as cumulative dosage of RAI, the latent period from first RAI treatment to the diagnosis of breast cancer, and stimulated TSH levels at the time of RAI treatment. There were no significant differences found between these factors according to the NIS-positivity of the cancer tissues (Table 5).

Next, we evaluated the association between serum TSH levels and NIS expression. There was no significant difference in NIS positivity in the TC→BC cases when divided into three groups according to values in the TSH tertile (mean TSH levels < 0.1, 0.25 uIU/L; Figure 15B). The prescribed dose and duration of levothyroxine were not associated with NIS positivity (Table 5).

Table 5. Factors related to thyroid cancer treatment according to NIS positivity

TC treatment related factors	NIS positive	NIS negative
RAI dose, GBq	110 [60.0−186.5]	90 [60.0−225.0]
Latency period from 1st RAI to BC diagnosis, years	4.1 [3.0−14.5]	6.1 [3.1−18.3]
Stimulated TSH level prior to RAI (mIU/L)		
mean	142.7 [72.2−205.0]	120.1 [73.7−147.9]
median	176.0 [68.0−201.7]	126.3 [68.5−160.9]
last measurement	176 [59.1−201.7]	135.4 [86.8−164.6]
Prescribed dose and duration of thyroxine (LT4)		
LT4 dose, mcg/day	150 [112.5−200.0]	125 [100.0−200.0]
LT4 duration, years	4.3 [3.0−7.4]	4.4 [3.4−5.8]
LT4 dose×duration, mcg/day ×years	10.5 [4.3−12.6]	6.7 [4.0−7.7]

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

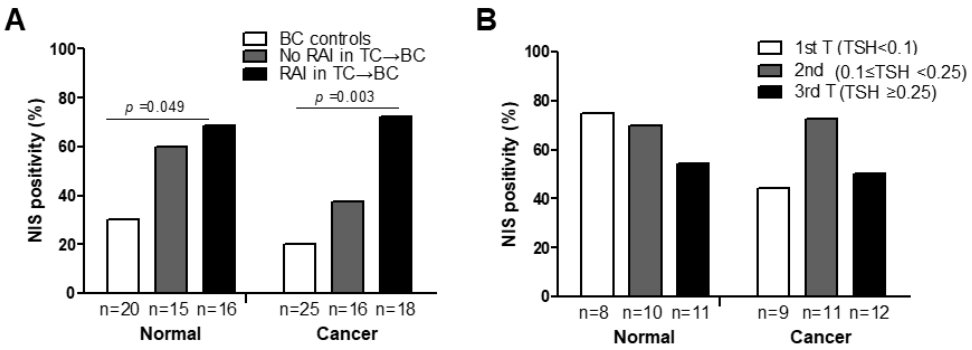

Figure 15. NIS positivity in the no RAI and RAI groups of the TC→BC cases with BC controls (A) and three subgroups according to TSH tertiles (0.1mIU/L, 0.25mIU/L, B).

Association between NIS and hormone receptors

In a previous study (119), ERα and TRα were found to be higher in the TC→BC cases than BC controls. In the total breast cancer cases, including TC→BC cases and BC controls, the positive rate of NIS was significantly higher in ERα-positive breast cancer cases than in ERα-negative breast cancer cases (51.2 vs. 16.7%, p = 0.014; Figure 16). There was no association between NIS and hormone receptors in the BC controls (Figure 17A) or the TC→BC cases (Figure 17B).

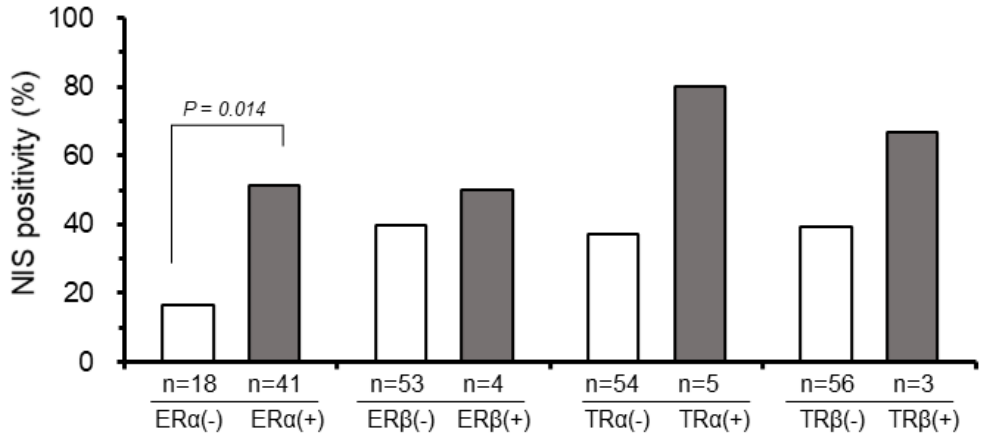

Figure 16. Association between NIS and ERα, ERβ, TRα, and TRβ in breast cancer tissue in total BC cases.

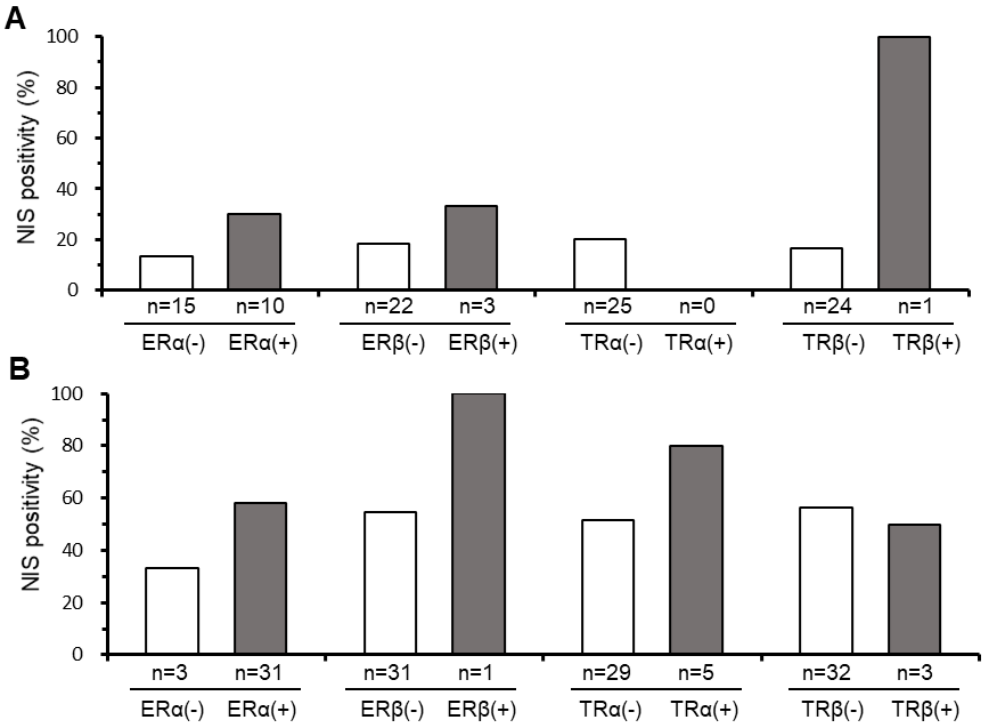

Figure 17. Association between NIS and ER*α*, ERβ, TR*α*, and TRβ in breast cancer tissue in BC controls (A) and BC+TC cases (B).

Next, the associations between NIS and ERα and TRα were investigated using mRNA expression data from 1217 breast cancer patients. A positive association between NIS and thyroid hormone receptor alpha (THRA) was observed, but it was weak (r = 0.074, p = 0.010; Figure 18A). On the other hand, NIS showed a negative correlation between ESR1 (r = -0.054, p = 0.059; Figure 18B) and the mRNA expression level. This was correlated in the opposite direction to the immunohistochemical finding, although it was not statistically significant.

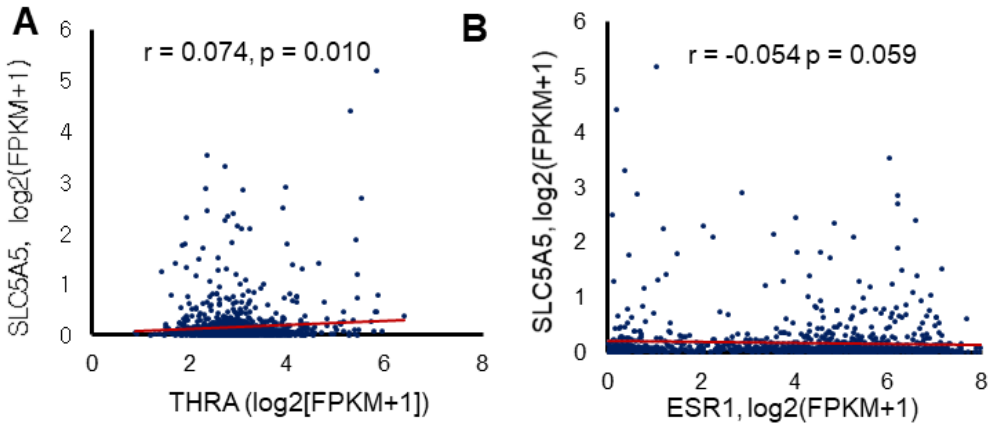

Figure 18. Correlations between SLC5A5 and THRA (A) or ESR1 (B) using mRNA expressional data from the Cancer Genome Atlas (TCGA).

Clinicopathologic characteristics and recurrence free survival according to the NIS positivity

NIS-positive breast cancer had a better prognosis than NIS-negative breast cancer in the total breast cancer cases (Log Rank $p = 0.034$; Figure 19A). The recurrence-free survival rate of breast cancer was not significantly different according to NIS positivity in the TC→BC cases (Figure 19B). In the TCGA data, there was no significant difference in the overall death rates according to low or high mRNA expression of the solute carrier family 5 member 5 (SLC5A5) gene in breast cancer patients (Figure 19C).

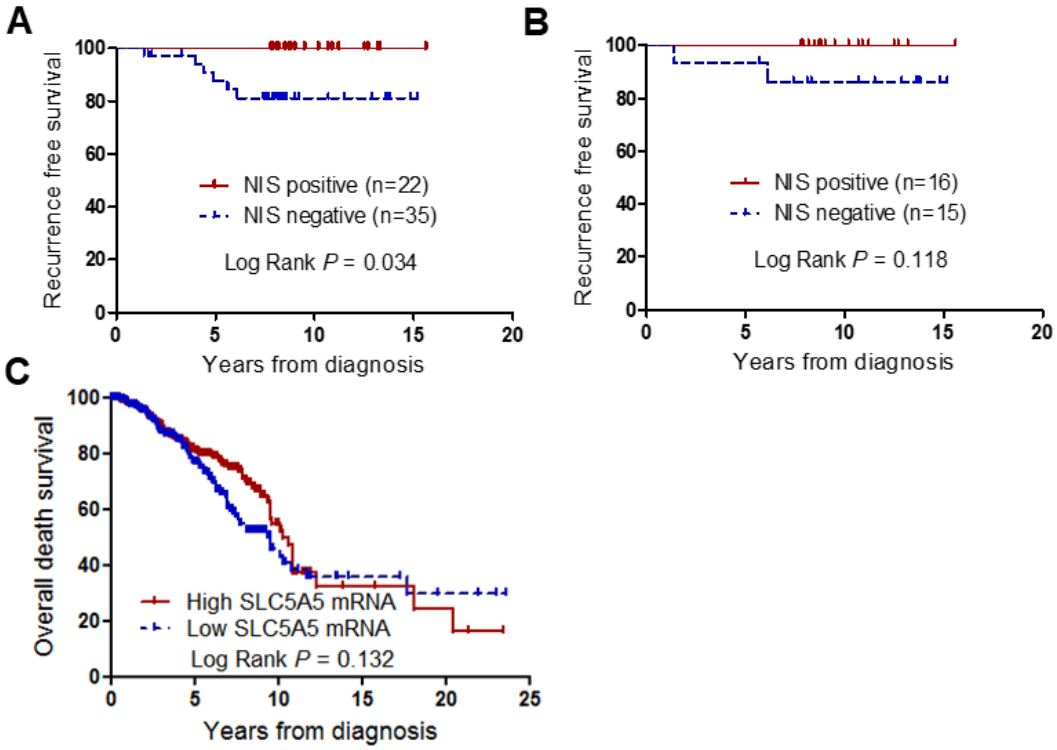

Figure 19. Recurrence-free survival according to NIS positivity or SLC5A5 mRNA expression. Recurrence free survival according to NIS positivity in total BC patients (A), only in BC+TC cases (B). Recurrence free survival in BC patients according to the SLC5A5 mRNA expression in TCGA data (C).

Discussion

This study demonstrated that NIS expression is higher in breast cancer after thyroid cancer and was associated with previous RAI therapy. NIS showed a positive correlation with ERα in total breast cancer cases, which was not reproduced in the TCGA data. Instead, a weak positive correlation between NIS and TRα was revealed by the TCGA analysis. Although the correlations between NIS and hormone receptors were weak, these might be related to their co-stimulation in breast cancer in association with the co-occurrence of breast and thyroid cancer.

In this study, the immunohistochemistry of NIS was analyzed using a monoclonal antibody, and its positivity was as low as 30% in normal breast tissue and 20% in breast cancer tissues in the BC controls. Notably, both positivity was markedly higher in the BC+TC cases (58.6% in normal tissue and 73.4% in cancer tissue). The positive rate of NIS in breast cancer tissue in the RAI group (72.2%) was higher than in the no RAI group of TC→BC cases (37.5%), and BC controls showed a lower positive rate (20%); however, it was independent of the preceding suppression of serum TSH level. This is the first study to show the NIS positivity of TC→BC cases and its association with prior thyroid cancer treatment. Thyroid cancer treatment was not strongly associated with a high NIS positivity in TC→BC cases. Meanwhile, NIS expression was homogenously higher in breast cancer co-occurred with thyroid cancer; increased NIS expression may be more related to the co-occurrence of breast and thyroid cancer than the treatment modalities of thyroid cancer.

Considering the higher positivity of ERα and TRα in breast cancer tissue co-occurring with thyroid cancer (119), associations between NIS and hormone receptors were also investigated. Interestingly, ERα positivity in the breast cancer tissues was associated with NIS expression. However, the positive association between ERα and NIS was reversed in transcriptomic level in the TCGA analysis, although the statistical power was weak. Previous studies of human breast cancer tissue have reported higher NIS positivity rates in ERα-positive breast cancer tissues than in ERα-negative ones (99, 104). NIS positivity was consistent with their endogenous ERα status in several breast cancer cell lines (106). The activation of PI3K increased NIS expression in ERα-positive Michigan Cancer Foundation-7 (MCF-7) cell lines (121, 122). The identification of the TATA box located near EREs in the NIS promoter supported the ERα-dependent transcriptional activation of the NIS gene (106); ERα can activate the PI3K/protein kinase B (Akt) pathway by direct binding in a nonclassical way (123, 124). Because NIS can also be upregulated by the activated PI3K/Akt pathway, it was also proposed as possible crosstalk for the co-activation of ERα and NIS.

The transcriptomic levels from the TCGA dataset supported the positive association between TRα and NIS. However, it was not significant in protein level both for TC→BC cases and in total breast cancer cases with the small number of subjects. Although we could not elucidate this association's mechanism, there are some reports that might support this idea; TH has been suggested for the potential regulator of NIS expression (104, 107). By dimerization with retinoic receptors,

TRs could modulate transcriptional activity for several genes, including NIS (43). In other experimental studies, TR has been reported to activate the human retinoic acid receptor alpha gene promoter in the presence of tamoxifen, which in turn activates the NIS promoter (125, 126), and this could be antagonized by the action of estrogen (125). From these collected results, although they were not wholly consistent, we could postulate that the hormone receptors TRα and ERα may influence NIS transcription. This association may explain the increased NIS expression in breast cancer co-occurring with thyroid cancer, although it is difficult to investigate due to hormone variability and the existence of several isoforms of hormone receptors.

NIS expression also showed a positive correlation with RAI therapy. The molecular mechanism underlying the RAI-related NIS enhancement in breast cancer is poorly understood; however, a previous study reported stable activation of the PI3K/Akt pathway in the mammary glands *in vivo* after external radiation (127-129). Since this pathway is also activated by ERα (123, 124), the activation of PI3K may induce increased NIS expression in ERα-positive MCF-7 cells (121, 122). Another possible mechanism is the activation of p53. In liver cancer cells, the accumulation of p53-family proteins (including p53 and p73) was found to increase endogenous NIS expression (130). *In vitro*, radiation promoted breast cancer cell proliferation through the accumulation of p53 mutants (131). Therefore, breast cancer developed after thyroid cancer may have abundant p53 mutants and an accordingly high level of NIS expression. In addition, it was reported that radiation-induced miRNAs

increased NIS expression by binding to the 3′-untranslated region of the NIS mRNA or upstream regulators such as paired box-8 protein (PAX8) in thyroid cancer cells/tissues (132-134). However, to apply this modulation in breast cancer, further studies are needed.

NIS-positive breast cancer showed a favorable prognosis in the total breast cancer cases, while it was not reproduced in the subgroup analysis and TCGA data. Ahn *et al.* (93) showed a slight reduction in the risk of breast cancer recurrence in thyroid cancer patients treated with high-dose RAI, suggesting a possible protective effect. Although the functionality of NIS was not evaluated, if even a small fraction of NIS is functional, it could potentially concentrate RAI in breast tissues, reducing the risk of subsequent breast cancer. Increased expression of NIS and its association with breast cancer following thyroid cancer may have implications for the future treatment and prevention of breast cancer and the management of thyroid cancer patients.

The main limitation of this study is the limited number of included cases due to the restricted number of double primary breast and thyroid cancer patients. In addition, there exist some missing data due to the retrospective study design. Moreover, the exact localization and functionality of NIS have not been fully elucidated. However, this is the first study demonstrating NIS expression in breast cancer after thyroid cancer and its potential association with RAI therapy or hormone receptors.

NIS expression was increased homogenously in subsequent breast cancer, which may be related to endogenous modulation during the hormone-dependent breast

carcinogenesis. Concerning RAI therapy, a weak but increased expression of NIS was demonstrated, and the prognosis of NIS-positive breast cancer was similar to or better than that of NIS-negative breast cancer. Therefore, the expression and associations of NIS are expected to be clinically meaningful discoveries in the unique condition of metachronous development of breast and thyroid cancer.

Summary and conclusions

In this study, the expression of hormone receptors and NIS in co-occurring thyroid and breast cancers was demonstrated by immunochemical staining. The expression of ERα was increased in both thyroid and breast cancer tissues. In addition, a decrease in ERβ and TRβ was identified in thyroid cancer, and an increase in TRα was confirmed in breast cancer. ERα is presumed to be involved in breast and thyroid cancer development since the oncogenic signaling pathway activated by ERα in breast cancer is similar in thyroid cancer. Furthermore, ERα positivity in thyroid cancer tissue was significantly correlated with previous antiestrogen treatment for breast cancer and menopause, suggesting that an increase in ERα in thyroid cancer is associated with a decreased estrogen status. On the other hand, ERα and TRα positivity in breast cancer tissue was not related to clinical characteristics or thyroid cancer treatment.

NIS expression, which may explain the low risk of breast cancer after high-dose RAI treatment in thyroid cancer patients, was also investigated in this study. NIS expression was increased in breast cancer patients after thyroid cancer and was associated with RAI treatment for thyroid cancer and ERα positivity in breast cancer. In TCGA analysis, NIS showed a weak positive correlation with TRα, but not ERα, and the prognosis of NIS-positive breast cancer was similar to that of the NIS-negative type.

Based on these results, ERα expression was increased in both thyroid and breast cancer tissues, confirming the possibility that ERα-related factors play an important

role in the common pathophysiology of both cancers. These results can also explain the similarities in female-dominated cancer and the age of onset in both cancers. The directional change in hormone receptor expression and its association with cancer treatment suggest the possible influence of estrogen and thyroid hormones. Increased NIS expression was associated with RAI therapy and receptor positivity, and the prognosis of breast cancer after thyroid cancer was comparable. However, the clinical significance of these findings requires further validation.

감사의 글

8년간의 박사 학위 과정에서 많은 가르침을 주신 박영주 선생님, 박도준 선생님, 이가희 선생님께 깊은 감사를 드립니다. 또한 본 연구의 전 과정에 걸쳐 거의 모든 도움을 주신 보라매병원 김영아 선생님께도 깊이 감사드립니다. 선생님께서는 유방암 및 갑상선암 조직 수집, 면역 화학 염색 및 판독과 병리 사진 촬영을 도와주셨습니다. 본 연구의 선행 연구를 해주시고, 연구의 가설을 세우게 도와주신 안화영 선생님, 안지현 선생님, 황보율 선생님과 연구 과정을 도와준 김유형 선생님, 송영신 선생님께도 감사드립니다. 바쁘신 중에도 학위 심사로 연구의 의미를 깊이 있게 해주시고, 논문이 보다 나은 방향으로 개선될 수 있도록 도와주신 임석아 선생님께 감사드립니다. 마지막으로 연구에 몰두하게 도와주고, 곁에서 응원해준 서제현 선생님과 서민규, 서민재에게 감사한 마음을 전합니다.

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국 문 초 록

동시발생한 갑상선암과 유방암의 호르몬 수용체 발현과 임상병리학적 특성

서울대학교 대학원 의학과 중개의학 전공

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갑상선암과 유방암의 동시발생의 위험 증가에 대한 관심이 높아지고 있고, 이에 대한 호르몬 연결고리가 추정되고 있다. 갑상선암과 유방암 모두 에스트로겐 수용체와 갑상선 호르몬 수용체를 발현하므로, 두 암에서 이러한 호르몬 수용체의 발현을 확인하는 것은 호르몬 연결 고리를 이해하는데 도움을 줄 수 있다. 이에 본 연구는 동시발생한 갑상선암과 유방암에서 호르몬 수용체의 발현을 규명하는 것을 목표로 하였다. 특히, 유방암 치료로 항에스트로겐이, 갑상선암 치료로 갑상선 호르몬이 사용되기 때문에, 수용체 발현과 임상병리학적 특징의 연관성

뿐 아니라 이전 암치료와의 연관성도 조사하였다. 추가적으로, 나트륨 요오드 공동 수송체는 갑상선암에 대한 방사성 요오드 요법의 효과를 매개하고 후속 유방암의 위험에 영향을 미칠 수 있기 때문에 유방암에서의 이의 발현도 조사하였다.

본 연구에서는 99 명의 갑상선암과 유방암 동시 발생 환자 및 92명의 갑상선암 대조군, 147명의 유방암 대조군의 갑상선 조직과 유방 조직에서 호르몬 수용체에 대한 면역화학염색을 실시했다. 추가적으로 갑상선암 및 유방암 환자의 예후가 갑상선암보다 유방암에 의해 결정되는 경향을 고려하여, 유방암에서의 에스트로겐 수용체 α, 갑상선 호르몬 수용체 α, 나트륨 요오드 공동 수송체의 예후적 가치를 평가하였다. 나트륨 요오드 공동 수송체는 에스트로겐 수용체 α 및 갑상선 호르몬 수용체 α와 상관 관계가 있고 나트륨 요오드 공동 수송체 양성 유방암에서 좋은 예후를 보였기 때문에 나트륨 요오드 공동 수송체 유전자의 에스트로겐 수용체 α 및 갑상선 호르몬 수용체 α 유전자와의 연관성과 예후적 가치에 대해 TCGA 데이터 분석을 수행하였다.

유방암과 동시발생한 갑상선암에서 갑상선암 대조군에 비해 에스트로겐 수용체 α의 발현은 증가한 반면, 에스트로겐 수용체 β, 및 갑상선 호르몬 수용체 β의 발현은 감소하였다. 높은 에스트로겐 수용체 α의 발현은 이전 항에스트로겐 치료와 갑상선암 진단 당시 폐경 상태와 유의한 연관관계를 보였다. 한편 갑상선암과 동시발생한 유방암 조직에서

에스트로겐 수용체 α 및 갑상선 호르몬 수용체 α 발현은 증가되었고, 이는 이전 갑상선암 치료나 임상병리학적 특징과 연관성을 보이지 않았다. 갑상선암과 동시발생한 유방암의 예후는 유방암 대조군과 유사하였으나, 에스트로겐 수용체 α 및 갑상선 호르몬 수용체 α 모두 음성일 때 예후는 좋지 않았다. 갑상선암 후 발생한 유방암에서 나트륨 요오드 공동 수송체 발현은 유방암 대조군에 비해 증가하였고, 이는 방사성 요오드 치료와 에스트로겐 수용체 α 발현과 연관되었다. 나트륨 요오드 공동 수송체 양성 유방암은 나트륨 요오드 공동 수송체 음성 유방암보다 좋은 예후를 보였다. 그러나 TCGA 분석에서 나트륨 요오드 공동 수송체 유전자의 갑상선 호르몬 수용체 α 유전자와의 약한 연관성을 보였으나 에스트로겐 수용체 α과의 연관성은 보이지 않았으며 예후적 가치도 없었다.

본 연구는 갑상선암과 유방암 모두에서 높은 에스트로겐 수용체 α 발현을 보여주었다. 갑상선암에서 에스트로겐 수용체 α 양성은 항에스트로겐 치료 및 폐경과 관련이 있는 반면, 유방암에서 에스트로겐 수용체 α 양성은 이전 갑상선암 치료 및 임상적 특징과 관련이 없는 것으로 나타났다. 갑상선암과 유방암 조직에서 호르몬 수용체의 발현 증가는 두 암의 동시발생에서 호르몬의 영향을 시사하는 것일 수 있다. 본 연구는 갑상선암과 유방암 조직에서 호르몬 수용체의 발현 증가를 입증하였고, 이는 암의 동시 발생에서 호르몬의 영향을 시사하는

것일지도 모른다. 또한, 유방암에서 나트륨 요오드 공동 수송체 발현은 호르몬 수용체 및 후속 유방암의 예후와 관련이 있었다. 따라서 이는 나트륨 요오드 공동 수송체의 발현 또한 유방암 발생과정에 관여하고 있고, 방사성 요오드 치료와 연관하여 후속 유방암의 위험과 연관됨을 시사하는 것일 수 있다.

- 본 박사학위논문은 다음 출판된 논문을 Part II의 기반으로 하였다.
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주요어: 에스트로겐 수용체, 갑상선 호르몬 수용체, 나트륨 요오드 공동 수송체, 갑상선암, 유방암, 면역화학염색

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