ANTICANCER RESEARCH *40*: 6417-6428 (2020)
doi:10.21873/anticanres.14663

Thyroid Hormones, Silencing Mediator for Retinoid and Thyroid Receptors and Prognosis in Primary Breast Cancer

BENJAMIN NISMAN[1#], TANIR M. ALLWEIS[2,3#], EINAT CARMON[4], LUNA KADOURI[1], BELLA MALY[5], OFRA MAIMON[1], AMIHAY MEIEROVICH[1] and TAMAR PERETZ[1]

[1]*Department of Oncology, Hadassah and Hebrew University Medical Centre, Jerusalem, Israel;*
[2]*Department of Surgery and Breast Health Center, Kaplan Medical Centre, Rehovot, Israel;*
[3]*Faculty of Medicine, Hebrew University, Jerusalem, Israel;*
[4]*Department of Surgery, Hadassah and Hebrew University Medical Centre, Jerusalem, Israel;*
[5]*Department of Pathology, Hadassah and Hebrew University Medical Centre, Jerusalem, Israel*

Abstract. *Background/Aim: Silencing mediator of retinoid and thyroid receptors (SMRT) is a nuclear corepressor in thyroid and estrogen hormones pathways. The aim was to evaluate SMRT expression in relation to thyroid hormone levels and prognostic markers in breast cancer (BC). Patients and Methods: Serum and tumor tissues were obtained from 36 patients with benign breast disease (BBD) and 79 BC patients. SMRT expression was determined by immunohistochemistry. Free-triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) were measured in serum. Results: Higher FT4, lower FT3/FT4 ratio and higher expression of SMRT were found in BC compared to BBD (for all p<0.001). In BC, increased SMRT expression was associated with lower FT3 (p=0.028), higher tumor grade (p=0.031), increased KI67 proliferation index (p=0.015), higher risk of recurrence (p=0.014) and shorter disease-free survival (p=0.006). In multivariate analysis, SMRT overexpression and below-median levels of TSH were independent prognostic factors in BC. Conclusion: Elevated FT4 and decreased FT3/FT4 in BC patients suggest a role for thyroid hormones in malignant transformation. SMRT tumor overexpression is associated with lower FT3 levels, tumor proliferative activity and an aggressive clinical course.*

[#]These Authors contributed equally to the present study.

Correspondence to: Benjamin Nisman, Department of Oncology, Hadassah and Hebrew University Medical Centre, P.O. Box 12000, Jerusalem 91120, Israel. Tel: +972 508946713, e-mail: nisman@hadassah.org.il

Key Words: Breast cancer, thyroid gland, TSH, FT3, FT4, silencing mediator for retinoid and thyroid receptors, prognosis.

Epidemiological studies of the relationship between thyroid function and breast cancer (BC) have yielded conflicting results. Some population-based cohort studies have cited an increased risk among women with hyperthyroidism and decreased risk among women with hypothyroidism (1, 2). Other studies found an increased risk of BC with an earlier diagnosis of hypothyroidism (3, 4). Findings from a recent population-based, case–control study suggested that women with a history of hyperthyroidism or hypothyroidism might have increased risk of developing BC (5).

Thyroid function in patients with benign breast disease (BBD) and BC was evaluated based on serum levels of hormones such as thyroid-stimulating hormone (TSH), free thyroxine (FT4) and free triiodothyronine (FT3). Findings are conflicting (6-10), and to date, there are no conclusive data.

Transcriptional activation by thyroid hormones (TH) involves their binding to specific nuclear thyroid hormone receptors (THRs), acting mainly as a complex with retinoid X receptors (RXRs) (11). The presence of TH allows the cooperation of the complex with coactivators (12). In the absence of TH, the complex interacts with nuclear receptor corepressors, one of which is the silencing mediator for retinoid and thyroid receptor (SMRT), facilitating transcriptional repression through recruitment of histone-modifying enzymes to induce local chromatin condensation and transcription repression (13-15). The classical model of TH action suggests a relationship between ligand availability for THR and SMRT expression. *In vivo* experiments have not revealed any role of SMRT in TH-regulated pathways (16, 17). In a global SMRT knockout mouse TH levels were not affected (16), and SMRT was not involved in determining the set point of the hypothalamic-pituitary-thyroid (HPT) axis (17). However, to the best of our knowledge, there is no available information on the relationship between TH serum levels and tumor expression of SMRT.

Considerable attention has been focused on the relationship between SMRT expression and resistance to endocrine therapy (18, 19). SMRT was characterized as a co-repressor, interacting with the estrogen receptor (ER) in the presence of tamoxifen (20). In some studies, the decrease in the stability and levels of SMRT was associated with resistance to tamoxifen (19), while others suggested that increased stability of SMRT and its overexpression in BC may be involved in acquiring resistance to anti-hormonal treatment (18).

Regarding the prognostic significance of SMRT, there are indications for the protective role of SMRT, and its diminished expression was suggested to drive BC initiation and progression (21). In a study of patients with ER-positive BC, higher levels of SMRT mRNA were associated with a better outcome (22). However, in other studies, elevated expression of SMRT was associated with a poorer prognosis (18, 23, 24). The aim of this study was to evaluate the associations of tumor SMRT nuclear expression with thyroid hormones and prognostic markers in BC.

Patients and Methods

This study, which was approved by the Institutional Ethical Review Board, included 115 women who underwent surgery for breast lesions between May 2004 and December 2010. All had surgery and were followed at Hadassah University Hospital, Jerusalem, Israel. All patients signed an informed consent form. There were 36 patients with benign breast disease (BBD) and 79 patients with invasive breast cancer. Patients with a prior history of malignancy or metastatic breast cancer and patients who had preoperative neoadjuvant chemotherapy, hormonal therapy or thyroid hormone replacement therapy were excluded.

Serum samples were obtained from all patients preoperatively, aliquoted and stored at –80˚C until analysis. Thyroid function tests including FT4, FT3 and TSH were performed by using the commercial IMMULITE kits, which are solid-phase, two-site chemiluminescent immunometric assays (Immullite, DPC, USA). The normal ranges in our lab were 0.35-4.50 mIU/l for TSH, 3.50-6.50 pmol/l for FT3, and 9.0-23.0 pmol/l for FT4.

Tissue samples were obtained from mastectomy, lumpectomy or wide local excision specimens, embedded in a 10% formalin solution shortly after surgical resection, and used for tissue microarray construction. The expression of SMRT in these samples was examined using immunohistochemistry on tissue microarray sections, containing specimens from patients with BC and BBD. Two cores from representative areas (BC or BBD) were deposited in a paraffin block using a semi-automated tissue arrayer.

The expression of SMRT was detected by immunohistochemistry, with the commercially available SMRT antibody (Product code sc-13554, antibody1542/H7 Santa Cruz Biotechnology, Santa Cruz, CA, USA). This antibody was raised against amino acids 994-1005. SMRT immunohistochemical (IHC) staining used the modified version of the H-score method. The staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong) (Figure 1). A nuclear staining score of 3 was defined as overexpression.

Statistical analysis. FT4, FT3 and TSH concentrations were evaluated both as continuous variables, and as categorical variables. Numeric variables are presented as median and interquartile range (IQR). Non-parametric Kruskal–Wallis and Mann–Whitney tests were used for comparisons. Associations between categorical variables were evaluated with the Fisher's exact test. Pearson's coefficients are presented as a measure of correlation between numeric variables. The clinical end-point was recurrence-free survival (RFS), measured from the date of surgery to the date of the last follow-up or disease recurrence.

Patients who developed a second unrelated malignancy, and those who died due to unrelated causes, were censored. Recurrence was defined as any first recurrence of disease either local or at distant sites, and calculated using the Kaplan–Meier method. Cox proportional hazards model was used to test the statistical independence and significance of predictors on disease-free survival (DFS). Statistical calculations were performed using SPSS, version 17 for Windows (SPSS Inc., Chicago, IL, USA). A value of $p<0.05$ was considered significant.

Results

The BBD group consisted of 36 patients (range=19-81 years, median 41, IQR=30-50). This group included patients with fibroadenoma, papilloma, and epithelial ductal and lobular hyperplasia. The BC group consisted of 79 women (range=24-83 years, median 56, IQR=46-68). Seventy-three patients (92%) had invasive ductal carcinoma and 6 patients (8%) had invasive lobular carcinoma. Thirty-two patients (41%) were diagnosed with grade I/II and 43 cases (54%) were diagnosed with grade III BC. T1 (≤2.0 cm) tumors were seen in 46 patients (58%), and T2-T3 (>2.0 cm) in 33 patients (42%). Thirty-six were node-positive (46%) and 43 patients (54%) were node-negative. Sixty-five were ER positive (82%), 58 cases were progesterone receptor (PR) positive (73%) and 18 cases were Her2/neu positive (23%). Nine patients (11%) had triple-negative (TN) BC. Of the 79 women who underwent surgery for BC, 14 (17.7%) developed a recurrence. Median follow-up was 132 months.

FT3, FT4 and TSH in BBD and BC. In the total cohort of 115 patients there was no significant correlation of FT4 and TSH levels with age (r=0.02, $p=0.865$ and r=0.05, $p=0.628$), while FT3 levels were reversely correlated with age (r=–0.43, $p<0.001$). The median serum level of FT4 (Table I) was significantly higher in BC than in BBD (18.53 *vs*. 16.73 pmol/l, $p<0.001$). No patients in this study had abnormally low FT4 levels (<9.0 pmol/l), whereas the rate of patients with abnormally elevated FT4 levels (>23.0 pmol/l) was higher in BC patients than in patients with BBD (15.2% *vs*. 0%, $p=0.017$). When stratifying patients by age (below and above 45 years) the difference in median levels of FT4 between BBD and BC subgroups remained significant ($p=0.004$ and $p=0.009$). FT4 above the median (>18.53 pmol/l) significantly predicted BC, after adjustment for age (OR=7.8, 95%CI=2.5-24.5.5, $p<0.001$).

There was no difference in median baseline values of TSH between patients with BBD or BC before (p=0.169) or after

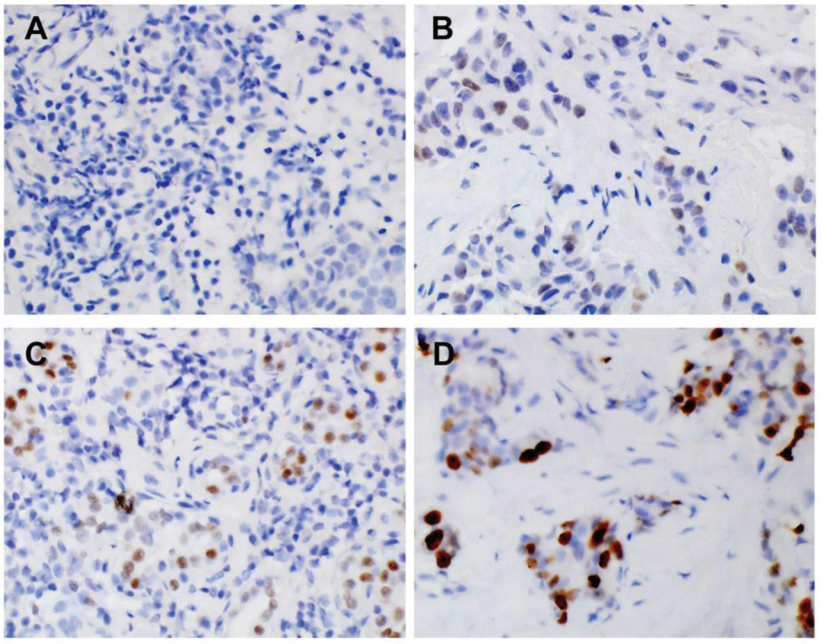

Figure 1. *Representative photographs of immunohistological expression of silencing mediator of retinoid and thyroid hormone receptors (SMRT) in breast cancer specimens. A. Negative (staining score 0); B. Weak (staining score 1); C. Moderate (staining score 2); D. Strong (staining score 3 - overexpression). Original magnification, ×40.*

(p=0.288 and p=0.751) stratifying by age 45 (Table I). The rates of abnormally low (<0.35 mIU/l) and abnormally high (>4.5 mIU/l) basal TSH levels were similar in both groups (0 *vs*. 2.5%, p=1.000 and 5.6 *vs*. 5.1%, p=1.000).

The median serum levels of FT3 were higher in BBD than BC (5.22 *vs*. 4.78 pmol/l, p=0.010). Abnormally low FT3 levels (<3.5 pmol/l) were recorded in 0% and 6.3% of cases with BBD and BC, respectively (p=0.323). Abnormally high serum levels of FT3 (>6.5 pmol/l) were more prevalent in BBD than BC (25.0% *vs*. 7.6%, p=0.016). Lower median FT3 level was detected in elder compared to younger patients both in the BBD and BC groups (4.91 *vs*. 5.85 pmol/l, p=0.011 and 4.62 *vs*. 5.25 pmol/l, p=0.002). After stratification by age, the difference between BBD and BC was not significant (≤45 years, p=0.376 and >45 years, p=0.386).

Significant correlation between serum levels of FT3 and FT4 was found in patients with BBD (r=0.43, p=0.010), but not in BC patients (r=0.01, p=0.916). FT3/FT4 ratio was significantly higher in BBD than in BC (median: 0.32 *vs*. 0.26, p<0.001). After stratification by age, the difference in FT3/FT4 ratio between BBD and BC remained significant both in women below and above 45 years of age (median: 0.35 *vs*. 0.30, p=0.006 and 0.31 *vs*. 25, p=0.024, respectively). FT3/FT4 ratio below the median (0.26) was significantly associated with BC after adjustment for age (OR=10.0, 95%CI=2.2-46.5, p=0.003).

FT3, FT4 and TSH in relation to histopathological characteristics of BC. There was no association of FT3, FT4 and TSH levels with tumor stage, tumor grade, or nodal status (Table II). TSH levels above the median basal level was significantly associated with PR positive BC (1.67 mIU/l *vs*. 0.98 mIU/l, p=0.020). The median basal FT4 in TN BC (ER-negative, PR-negative and Her2/neu negative) cases tended to be higher than in ER-positive (19.31 *vs*. 18.02 pmol/l, p=0.186), PR-positive (19.30 *vs*. 18.00 pmol/l, p=0.082) and Her2/neu-positive cases (19.18 *vs*. 16.47 pmol/l, p=0.073).

FT3, FT4 and TSH in relation to SMRT expression in BBD and BC. Immunohistochemical assessment of breast tissues revealed a positive expression of SMRT, predominately localized to the nucleus (Figure 1). Only nuclear staining of SMRT was considered positive. The rate of cases with negative-weak expression of SMRT (staining score 0-1) was higher in BBD (Figure 2) than in BC (86.1% *vs*. 34.2%, p<0.0001), while strong expression (staining score 3-overexpression) was detected only in BC (0% *vs*. 39.2%, p<0.0001).

In the entire cohort of 115 patients, TSH and FT4 serum levels were not associated with SMRT expression (Table III), while FT3 was negatively associated with the expression of SMRT (negative/weak, moderate, strong: median 5.16, 4.82 and 4.58 pmol/l, p=0.017). Decreased (<3.5 pmol/l) and elevated FT3 levels (>6.5 pmol/l) were significantly associated with up- and down-regulation of SMRT, respectively (p=0.014).

Table I. *Thyroid function tests in patients with benign breast disease and breast cancer.*

Characteristics	N	BBD	BC	*p*-Value
TSH (mIU/l), median, IQR	115 (36/79)	2.04, 1.16-3.09	1.62, 0.91-2.82	0.169
≤45 years	38 (22/16)	2.61, 1.36-3.18	1.66, 1.37-2.69	0.288
>45 years	77 (14/63)	1.49, 1.08-2.50	1.51, 0.83-2.86	0.751
p-Value		0.189	0.500	
TSH (<0.35 mIU/l)	115 (36/79)	0/36 (0)	2/79 (2.5%)	1.000
TSH (>4.50 mIU/l)	115 (36/79)	2/36 (5.6%)	4/79 (5.1%)	1.000
FT3 (pmol/l), median, IQR	115 (36/79)	5.22, 4.58-6.56	4.78 4.2-5.41	**0.010**
≤45 years	38 (22/16)	5.85, 4.75-6.70	5.25, 4.79-6.06	0.376
>45 years	77 (14/63)	4.91, 4.40-5.33	4.62, 4.06-5.33	0.386
p-Value		**0.011**	**0.002**	
FT3 (<3.5 pmol/l)	115 (36/79)	0/36 (0)	5/79 (6.3%)	0.323
FT3 (>6.5 pmol/l)	115 (36/79)	9/36 (25.0%)	6/79 (7.6%)	**0.016**
FT4 (pmol/l), median, IQR	115 (36/79)	16.73, 14.48-18.02	18.53, 16.22-21.36	**<0.001**
≤45 years	38 (22/16)	16.73, 15.44-17.73	18.98, 16.73-21.11	**0.004**
>45 years	77 (14/63)	15.44, 14.16-18.02	18.02, 15.96-21.36	**0.009**
p-Value		0.631	0.741	
FT4 (<9.0 pmol/l)	115 (36/79)	0/36 (0)	0/79 (0)	-
FT4 (>23.0 pmol/l)	115 (36/79)	0/36 (0)	12/79 (15.2%)	**0.017**
FT3/FT4 ratio, median, IQR	115 (36/79)	0.32, 0.30-0.37	0.26, 0.22-0.32	**<0.001**
≤45 years	38 (22/16)	0.35, 0.31-0.40	0.30. 0.25-0.33	**0.006**
>45 years	77 (14/63)	0.31, 0.29-0.33	0.25, 0.21-0.32	**0.024**
p-Value		**0.017**	**0.046**	

TSH: Thyroid-stimulating hormone; FT3: triiodothyronine; FT4: free thyroxine; IQR: interquartile range. *p*-Values were derived from Mann–Whitney and Fisher's exact tests. Bold values indicate statistical significance.

Table II. *Association between thyroid function tests and histopathological parameters in BC.*

Characteristics	N	TSH (mIU/l) median, IQR	FT3 (pmol/l) median, IQR	FT4 (pmol/l) median, IQR
Tumor stage				
≤2.0 cm	46	1.96, 0.94-2.88	4.80, 4.12-5.43	18.47, 16.15-21.49
>2.0 cm	33	1.51, 0.89-2.67	4.64, 4.29-5.46	18.53, 16.35-21.11
p-Value		0.686	0.919	0.503
Tumor grade				
I-II	32	1.48, 0.87-2.71	4.83, 4.06-5.36	19.18, 18.02-21.36
III	43	1.63, 0.88-2.87	4.67, 4.27-5.55	18.53, 15.44-21.88
p-Value		0.574	0.336	0.336
Nodal status				
Positive	36	1.51, 1.01-2.34	4.71, 4.07-5.54	19.24, 16.02-21.82
Negative	43	1.79, 0.78-3.00	4.80, 4.18-5.36	18.02, 16.73-20.59
p-Value		0.595	0.701	0.737
ER				
Positive	65	1.63, 1.01-2.84	4.78, 4.19-5.40	18.02, 15.96-20.59
Negative	14	1.05, 0.68-2.51	4.73, 4.13-5.82	19.31, 17.57-21.88
p-Value		0.146	0.997	0.186
PR				
Positive	58	1.67,1.07-2.96	4.77, 4.19-5.40	18.00, 15.83-20.11
Negative	21	0.98, 0.73-2.27	4.64, 3.89-5.91	19.30, 17.38-21.88
p-Value		**0.020**	0.756	0.082
Her2/neu				
Positive	18	1.57, 0.81-2.90	4.65, 4.18-5.74	16.47, 15.44-19.31
Negative	61	1.64, 0.97-2.80	4.80, 4.14-5.38	19.18, 17.12-21.75
p-Value		0.896	0.846	0.073

BC: Breast cancer; TSH: thyroid-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; ER: estrogen receptor; PR: progesterone receptor; IQR: interquartile range. *p*-Values were derived from Mann–Whitney test. Bold values indicate statistical significance.

Table III. *Distribution of FT3, TSH and FT4 levels by SMRT expression in the total cohort of 115 patients with BBD and BC.*

	SMRT expression			
	Negative/Weak n=58	Moderate n=26	Strong n=31	*p*-Value
FT3 (pmol/l), median, IQR	5.16, 4.56-6.00	4.82, 4.16-5.75	4.58, 4.15-5.25	**0.017**
<3.5	1	0	4	**0.014**
3.5-6.5	45	24	26	
>6.5	12	2	1	
TSH (mIU/l), median, IQR	1.68, 0.92-3.02	2.17, 1.38-3.16	1.51, 0.95-2.70	0.347
<0.35	1	1	0	0.245
0.35-4.5	55	22	30	
>4.5	2	3	1	
FT4 (pmol/l), median, IQR	17.18, 15.44-19.88	18.6, 16.92-21.67	18.0, 14.93-19.56	0.184
<9.0	0	0	0	0.222
9.0-23.0	54	21	28	
>23.0	4	5	3	

SMRT: Silencing mediator for retinoid and thyroid receptor; FT3: free triiodothyronine; BBD: benign breast disease; BC: breast cancer; FT4: free thyroxine; TSH: thyroid stimulating hormone. *p*-Values were derived from Kruskal-Wallis and Fisher's exact tests. Bold values indicate statistical significance.

Most BBD and BC patients with abnormally high FT3 had down-regulated (negative/weak) expression of SMRT (Table IV, 89%, 8 of 9 and 66.7%, 4 of 6, respectively). There were no cases of BBD with low FT3 levels and/or over-expression of SMRT, while in 80% of BC cases (4 of 5) with low FT3 serum levels, the expression of SMRT in the tumor was up-regulated (strong).

SMRT expression in relation to histopathological characteristics in BC patients. In further analysis, FT3, FT4 and TSH serum levels were dichotomized by their median values (4.75 pmol/l, 18.53 pmol/l and 1.62 mIU/l, respectively). Serum FT3 levels were negatively associated with SMRT expression (Table V, p=0.028). In patients with FT3 levels below the median, the negative/weak (down-regulation) and strong (up-regulation) expression of SMRT was found in 20.5% and 51.3% of cases respectively, while in patients with FT3 levels above the median, an opposite direction of changes was found: 47.5% and 27.5% (p=0.01). FT4 and TSH were not associated with SMRT expression (p=0.513 and p=0.513, respectively). Furthermore, the expression of SMRT (Table V) was significantly positively associated with tumor grade (p=0.031), the Ki-67 labeling index (p=0.015) and tumor recurrence (p=0.014). There was no association of SMRT with age, tumor stage, nodal status, ER, PR or Her2/neu status (Table V).

Association of FT3, FT4, TSH and SMRT with prognosis. For prognostic evaluation, SMRT was dichotomized into non-over-expression *versus* over-expression (– *vs*. +). This division was adopted because over-expression of SMRT was a distinctive feature of BC when compared to BBD. Patients with tumors over-expressing SMRT had a shorter DFS (Figure 3A,

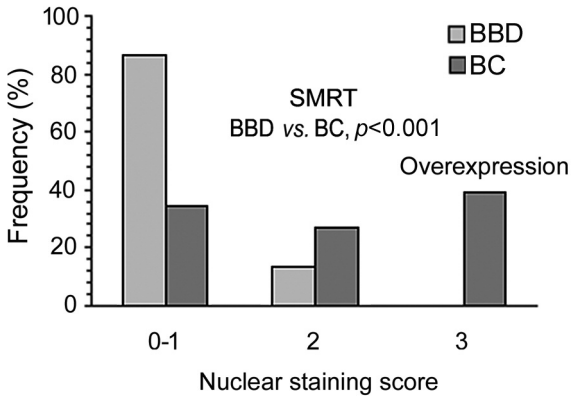

Figure 2. *Distribution of staining score for silencing mediator of retinoid and thyroid hormone receptors (SMRT) in benign breast disease (BBD) and breast cancer (BC).*

LR=7.46, p=0.006). Tumor size (≤2.0 cm *vs*. >2 cm) was also significantly associated with DFS (LR=5.81, p=0.016). ER, PR, Her2/neu and nodal status were not found to be related to prognosis (LR=0.138, p=0.710, LR=0.914, p=0.339, LR=0.355, p=0.551 and LR=2.08, p=0.149 respectively).

There was clear distinction between the patients with above median serum levels of TSH who had a longer DFS than patients with decreased TSH levels (Figure 3B, LR=9.52, p=0.002). Serum levels of FT3 and FT4 were not associated with differences in DFS (LR=0.453, p=0.501 and LR=0.465, p=0.495).

After univariate analysis for the following factors: SMRT, tumor size, TSH, FT3, FT4, ER, PR, Her2/neu and nodal

Table IV. *Distribution of FT3 and SMRT expression in BBD and BC.*

	SMRT expression					
	BBD (n=36)			BC (n=79)		
	Negative/Weak	Moderate	Strong	Negative/Weak	Moderate	Strong
FT3 (pmol/l)						
<3.5	0	0	0	1	0	4
3.5-6.5	23	4	0	22	20	26
>6.5	8	1	0	4	1	1
Total	31	5	0	27	21	31

SMRT: Silencing mediator for retinoid and thyroid receptor; FT3: free triiodothyronine; BBD: benign breast disease; BC: breast cancer.

Table V. *Association of SMRT expression with FT3, FT4, TSH and histopathologic characteristics in BC.*

	SMRT expression				
	Negative/Weak	Moderate	Strong	Total	*p*-Value
Age					
≤45 years	6	5	5	16	0.777
>45 years	21	16	16	63	
FT3					
Below the median (<4.75 pmol/l)	8	11	20	39	**0.028**
Above the median (≥4.75 pmol/l)	19	10	11	40	
FT4					
Below the median (<18.53 pmol/l)	14	8	17	39	0.513
Above the median (≥18.53 pmol/l)	13	13	14	40	
TSH					
Below the median (<1.62 mIU/l)	14	8	17	39	0.513
Above the median (≥1.62 mIU/l)	13	13	14	40	
Tumor size					
≤2.0 cm	18	9	19	46	0.230
>2.0 cm	9	12	12	33	
Tumor grade					
Grade 1-2	15	9	8	32	**0.031**
Grade 3	9	12	22	43	
Node					
Negative	14	12	17	43	0.959
Positive	13	9	14	36	
ERα					
Positive	24	17	24	65	0.570
Negative	3	4	7	14	
PR					
Positive	20	16	22	58	0.949
Negative	7	5	9	21	
Her2/neu					
Positive	4	6	8	18	0.441
Negative	23	15	23	61	
KI67					
Positive	2	9	8	19	**0.015**
Negative	25	12	23	60	
Recurrence					
Yes	1	3	10	14	**0.014**
No	26	18	21	65	

SMRT: Silencing mediator for retinoid and thyroid receptor; PR: progesterone receptor; ER: estrogen receptor; BC: breast cancer; FT3: free triiodothyronine; FT4: free thyroxine; TSH: thyroid stimulating hormone. *p*-Values were derived from Fisher's exact test. Bold values denote statistical significance.

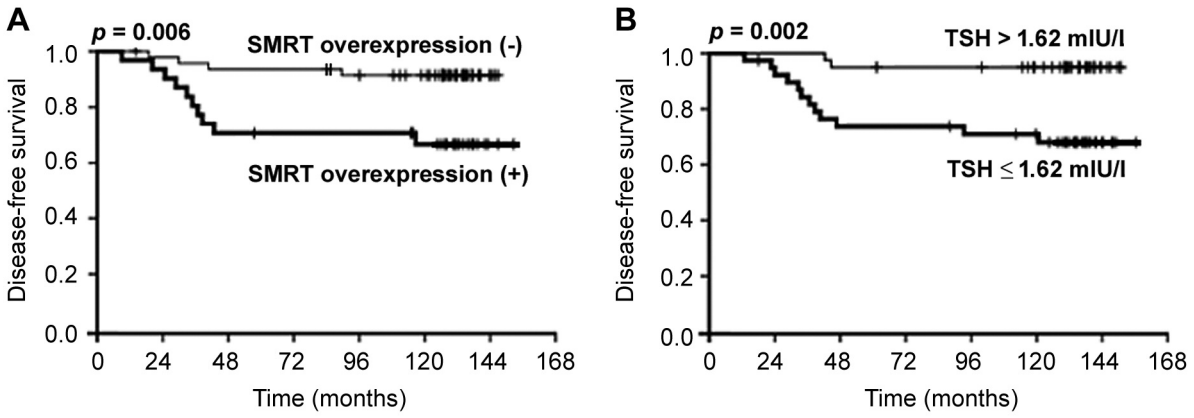

Figure 3. *Disease-free survival according to silencing mediator of retinoid and thyroid hormone receptors (SMRT) expression (A) and thyroid-stimulating hormone (TSH) serum levels (B).*

status, with Log-rank test, three factors, including tumor size, below-median levels of TSH and SMRT over-expression were identified as risk factors for cancer recurrence ($p<0.05$) and included in a multivariate analysis based on Cox regression. FT3 was also included in this model, which demonstrated that tumor stage, nuclear SMRT expression and serum TSH levels were explanatory variables (Table VI), while FT3 did not contribute to this model (HR=2.00, $p=0.268$). The second model including the same four variables was also constructed for 65 patients with ER positive BC who received adjuvant hormonal treatment. In this model, both SMRT and TSH were significantly associated with DFS (SMRT, HR=5.63, 95%CI=1.25-25.3, $p=0.024$ and TSH, HR=11.7, 95%CI=1.43-96.09, $p=0.022$) and the effect of FT3 was not significant (HR=1.5, 95%CI=0.4-6.5, $p=0.604$).

Table VI. *Cox proportional hazards analyses for predictors of disease-free survival.*

Characteristics	Hazard ratio	95%CI	*p*-Value
Tumor size, (≤2.0 cm *vs*. >2.0 cm)	4.10	1.20-14.08	0.025
SMRT overexpression (neg *vs*. pos)	6.39	1.73-23.58	0.005
TSH (>1.62 mIU/l *vs*. ≤1.62 mIU/l)	6.40	1.39-29.37	0.017
FT3 (>4.75 pmol/l *vs*. ≤4.75 pmol/l)	2.00	0.59-6.85	0.268

SMRT: Silencing mediator for retinoid and thyroid receptor; TSH: thyroid-stimulating hormone; FT3: free triiodothyronine.

Discussion

To date, only a handful of studies comparing preoperative TSH, FT4 and FT3 levels in BBD and primary BC have been published. Confirming the data presented in these studies (6-8, 10, 25), we did not find significant differences in preoperative TSH levels in BC patients compared to BBD patients.

Several studies have not found any difference between serum FT4 levels in patients with BBD and BC (6, 10, 25), while others (26-28) found BC to be associated with low levels of FT4. In contrast, we found significantly higher levels of FT4 in BC than in BBD, a finding consistent with earlier studies (7-8). We also found a higher prevalence of cases with abnormally elevated FT4 levels in BC than in BBD. The difference between the two groups retained significance in patients both under and over 45 years of age. These data are in accordance with a recently reported association between elevated serum concentrations of thyroxin and BC in both premenopausal and postmenopausal women in a case-control study (29). Our data are also in line with three independent investigations of pre-diagnosis levels of thyroid hormones in relation to subsequent risk of breast cancer based on a population-based prospective cohort "The Malmo Diet and Cancer Study". In all three studies, the authors reported a significant positive association of elevated serum FT4 levels with BC risk (1, 30, 31). It appears that elevated FT4 levels exist a long time prior to diagnosis, and could be detected at the time of BC diagnosis. In our study, FT4 was found to be a predictor of BC after adjustment for age (OR=7.8, $p<0.001$). Association of elevated FT4 levels with BC risk is explained by the proliferative effects of this hormone on breast tissue through the same signalling cascade utilized by estrogens (32, 33). In human breast cancer cells lines, T4 was shown to stimulate the transcriptional activity of ERα *via* non-genomic activation of MAPK and MAPK-dependent phosphorylation of ERα (32, 34).

With respect to T3, epidemiological studies have also produced contradictory data. Positive association of prospectively measured T3 levels with BC was reported in

postmenopausal women (35). Ditsch *et al*. (7) also reported higher FT3 levels in BC compared to patients with BBD. Other studies (8, 25, 36), however, did not find differences in FT3 levels in BBD and BC. In our study, serum FT3 significantly reversely correlated with age, and lower levels were found in older women with both BBD and BC. The difference in FT3 between BBD and BC groups was significant only when analysing the whole cohort. After stratification by age the difference was not significant.

We confirmed the findings of a previous study (8), reporting a stronger association of serum FT4 levels with BC compared with FT3. These findings were explained by the shorter half-life and the circadian rhythm of FT3 (37), making it a less reliable marker of thyroid function compared to FT4, which is also less dependent on age and menopausal status.

In this study, we found that the FT3/FT4 ratio inversely correlated with age, with significantly lower values detected in older patients with BBD and BC. These data confirm the findings of a study reporting a decrease in FT3 levels and FT3/FT4 ratio with aging (38). The authors related these findings to decreased conversion of thyroxine (T4) to triiodothyronine (T3). They suggested that reduced activity of deiodinase is a protective mechanism against excessive TH levels and considered it a part of the aging process. Meanwhile, we found that FT3/FT4 ratio was lower in BC patients than in patients with BBD both below and above age 45, suggesting that malignant transformation could contribute to the age-related reduction in the conversion of FT4 to FT3. In addition, FT3/FT4 ratio was significantly associated with BC after adjustment for age (OR=10.0, p=0.003) and could be considered a risk factor for BC.

Increased levels of FT4, decreased ratio of FT3/FT4 and weaker correlation between FT3 and FT4 in BC compared to BBD indicate the difference in controlling TH homeostasis in malignant transformation, and suggest the involvement of specific iodothyronine deiodinases in regulating this process. Recently, Brandt *et al*. (39) were able to identify a SNP (rs2235544) in the gene for deiodinase type 1, which was associated with both elevated FT4 levels and breast cancer risk. Reduced expression of deiodinase type 3 mRNA in tumoral breast glandular tissue compared to normal tissue was also reported (40).

Using immunohistochemistry on tissue microarray sections, we found significantly higher expression of SMRT in patients with BC compared to those with BBD. Analysis of SMRT expression distribution showed that over-expression of SMRT was prevalent in BC but not in BBD (39.2% *vs*. 0%, p<0001), suggesting a potential role of SMRT in malignant transformation. The increased SMRT mRNA expression was reported in tumor samples with intraductal carcinomas compared to the normal mammary gland tissue (41). The authors related SMRT up-regulation with breast cancer development.

It is widely accepted that SMRT is subject to extensive alternative mRNA splicing events, which generate a diverse series of distinct co-repressor variants (42, 43). Zhang *et al*. (18) revealed a splice variant of *SMRT* with a deletion of a site responsible for proteasome-mediated degradation in BC cell lines as well as in tumor tissues. The authors suggested that this could be one of the explanations for the over-expression of SMRT in BC. Another mechanism regulating the stability of SMRT was associated with the activation of peptidyl-prolyl isomerase, which together with the cyclin-dependent kinase Cdk2 has been shown to promote degradation of SMRT (44). It can be speculated that a disturbance in the degradation mechanism in BC may be the reason for the observed increased expression of SMRT in ours and other studies (18, 23, 24).

In experiments on synchronized A549 cells, the expression of SMRT increased when entering the S-phase, suggesting that SMRT may play a role in cell cycle progression (45). In ERα-positive MCF-7 breast cancer cells, SMRT promoted the E2-dependent proliferation and activation of ER target genes involved in the proliferation processes, such as cyclin D1, thus controlling the G_1/S transition (46, 47). E2-dependent recruitment of SMRT to the regulatory regions of the PR was also reported. It was suggested that SMRT promotes breast tumorigenesis, at least in part, through amplifying ER target gene expression (48). Depletion of SMRT inhibited growth of ER-positive cells (49). SMRT was also reported to promote cell growth through inhibition of apoptotic and co-activation of antiapoptotic gene expression. These processes were ER independent (49). Our finding of an association between SMRT expression and a higher Ki-67, a validated marker of breast cancer proliferation reflecting mitotic activity, is in accordance with these data.

Our findings are also in agreement with data reported by Green *et al*. (23), who found the association of high SMRT expression with poor DFS, using a SMRT antibody against amino acids 994-1005, which was the same antibody used in our study. Later, Smith *et al*. (24) reported the significant relation of higher SMRT expression [using a different antibody, raised against another amino acids sequence (1366-1473aa) of SMRT] with poor DFS only in BC patients not receiving adjuvant hormonal treatment. In our study, the association of SMRT with survival was significant for patients with ER positive BC who received adjuvant hormonal treatment. The discrepancy between the results of this and our study might be related to the use of different antibodies, which may identify different variants of the corepressor. Different splice variants may differ significantly in their molecular architecture, biological potential and demonstrate functional differences (46, 50). They could also differ notably in their affinity for different nuclear hormone receptors (42). Zhang *et al*. (18) found a new splice variant of SMRT in BC tissue. The expression of this isoform

positively correlated with tamoxifen resistance and proliferative signaling. High expression level of the co-repressor in patients who received adjuvant tamoxifen was associated with a worse DFS. The results of this and our study support the association of high expression of SMRT protein in patients with BC with resistance to hormonal treatment and poor outcomes.

According to the classical model of TH action in unliganded state, SMRT is associated with thyroid receptors and retinoid acid receptor (RAR). Upon natural ligand triiodothyronine (T3) binding, SMRT is released from this complex and replaced by the coactivator (13). Ubiquitination of SMRT for proteosomal degradation favors the exchange of the corepressor for the coactivator (51). This model assumes the relationship between T3 availability and expression of SMRT. In accordance with this model, we found a significant reverse association between FT3 levels and SMRT expression in both BC and BBD. Among these patients, overexpression of the co-repressor was detected in 80% of cases with abnormally low FT3 levels (<3.5 pmol/l), while in 80% cases with abnormally high FT3 levels (>6.5 pmol/l) the expression of SMRT was down-regulated.

In our study, patients receiving levothyroxin for primary hypothyroidism were excluded. This may be the reason for the low prevalence of cases with low FT3 in BC patients, and their lack in the smaller group of patients with BBD. Concerning BBD, in consistence with the negative relationship between FT3 and SMRT, we did not find cases with SMRT over-expression, while 89% of patients with elevated FT3 levels had down-regulated SMRT. Thus, a similar pattern of relationship between FT3 and SMRT was observed in patients with BBD and BC patients, and notably, SMRT over-expression was absent in BBD. Although all these data show a clear association of FT3 levels and tumor SMRT expression both in BBD and BC, it is not possible to conclude on a causal relationship between them.

The involvement of SMRT in the central regulation of TH signaling through interaction with THRs has long been hypothesized (52). However, in mouse models, SMRT was not involved in TH-regulated pathways (16). It is not clear whether these data could be applied to the tumor expression of SMRT. It seems more likely that FT3 has a modulating effect on SMRT expression both in benign and malignant tissues.

Taking into account the significant negative association between FT3 and SMRT, the noticeable effect of FT3 on prognosis could be expected. However, the association of FT3 with DFS was weak, and in multivariate analysis the SMRT over-expression was the explanatory variable. It appears that serum FT3 could exert some modulating effect on tumor expression of SMRT, which however remained a crucial factor in disease progression.

Our data showed improved prognosis for patients with above-median levels of TSH. To the best of our knowledge, no studies have reported the effect of TSH levels on prognosis in primary BC. Groot *et al*. (53) investigated the predictive value of thyroid function on pathological complete response (pCR) in BC patients receiving neoadjuvant chemotherapy, and reported that high TSH was significantly associated with pCR only in univariate analysis.

In the current study, TSH was an explanatory variable along with SMRT. The analysis of the association of TSH with pathohistological characteristics revealed higher levels of this hormone in PR positive BC. This finding could explain at least in part the improved prognosis of patients with elevated TSH levels. The importance of PR on the activation of steroid hormone dependent genes involved in cell proliferation and breast cancer pathology is well established (54). Several studies related PR expression to prognosis in BC, and improved survival is seen in patients with a PR-positive status, while PR negativity has been associated with early disease recurrence and worse survival (55, 56).

Since all patients receiving levothyroxin for primary hypothyroidism were excluded from this study, only a small percentage of patients with BC (5.1%) had TSH levels above the upper normal limit for the euthyroid reference range. It can be speculated that the influence of TSH on disease course is related not to thyroid hypofunction, but to the biology of the hormone itself, for example its influence on the expression of PR. A recent large population-based cohort study did not find any association between thyroid hypofunction at BC diagnosis and disease recurrence (57).

In other types of cancer, various effects of TSH levels have been reported. Patients with non-small cell lung cancer and normal TSH levels were found to have a better median survival compared to patients with reduced TSH levels (58), whereas in patients with endometrial cancer, elevated pre-therapeutic serum TSH has been independently associated with poor DFS (59). This inconsistency may reflect differences in tumorigenesis between BC and other types of cancer, and the multifunctional nature of TSH (58).

Conclusion

We report a high incidence of elevated serum FT4 levels in patients with BC compared to BBD. Elevated FT4 significantly correlated with FT3 in patients with BBD, but not in BC. We found that the FT3/FT4 ratio was lower in BC compared with BBD. Taken together, these data suggest differences in the control of TH homeostasis in BBD and BC.

In BC tissue, the expression of SMRT was higher than in BBD, with over-expression detected only in BC, suggesting the involvement of SMRT in malignant transformation. The significant differences in FT4 levels, FT3/FT4 ratio and expression of SMRT between BBD and BC warrant further investigation to determine the usefulness of these parameters in the diagnostic work-up of BC.

Our study found a significant reverse association between serum FT3 levels and tumor expression of SMRT in the total cohort including both patients with BBD and BC. Most patients with abnormally low FT3 levels had tumours over-expressing SMRT, while in most cases with abnormally elevated FT3, the expression of SMRT was down-regulated. The same pattern was observed in patients with BC. This is the first time a relationship between thyroid function in terms of serum FT3 levels and tumor SMRT expression has been reported, specifically in patients with BBD and BC.

Supporting the data on the involvement of SMRT in proliferation is our finding of a positive association with tumor grade and Ki67 proliferation index. In addition, we found that TSH is a prognostic factor, positively associated with PR expression and survival. In multivariate analysis, SMRT and serum TSH were both associated with DFS, implying their independent prognostic significance for BC patients, specifically in patients with ER positive disease receiving adjuvant hormone therapy. Thus, the results of this study showed that in BC, the expression of SMRT is associated with thyroid function in terms of serum FT3 levels, tumor proliferative activity and a more aggressive clinical course.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conception and design: BN, TA, TP. Acquisition of data: TA, BN, EC, TP, LK, OM. Analysis and interpretation of data: BN, TA, TP, LK, AM. Histological examination of the breast: BM Writing, review, and/or revision of the manuscript: BN, TA, TP, LK.

Acknowledgements

The Authors thank Dr. Mario Baras of the Hebrew University, Hadassah School of Public Health, for advice on statistical analysis.

References

1 Søgaard M, Farkas DK, Ehrenstein V, Jørgensen JO, Dekkers OM and Sørensen HT: Hypothyroidism and hyperthyroidism and breast cancer risk: a nationwide cohort study. Eur J Endocrinol *174*: 409-414, 2016. PMID: 26863886. DOI: 10.1530/EJE-15-0989

2 Kim EY, Chang Y, Lee KH, Yun JS, Park YL, Park CH, Ahn J, Shin H and Ryu S: Serum concentration of thyroid hormones in abnormal and euthyroid ranges and breast cancer risk: A cohort study. Int J Cancer *145*: 3257-3266, 2019. PMID: 19179309. DOI: 10.1002/ijc.32283

3 Smyth PPA: The thyroid and breast cancer: a significant association? Ann Med *29*: 1989-1991, 1997. PMID: 9240623. DOI: 10.3109/07853899708999335

4 Kuijpens JL, Nyklictek I, Louwman MW, Weetman TA, Pop VJ and Coebergh JW: Hypothyroidism might be related to breast cancer in post-menopausal women. Thyroid *15*: 1253-1259, 2005. PMID: 16356089. DOI: 10.1089/thy.2005.15.1253

5 Weng CH, Chen YH, Lin CH, Luo X and Lin TH: Thyroid disorders and breast cancer risk in Asian population: a nationwide population-based case-control study in Taiwan. BMJ Open *30*: e020194, 2018. PMID: 29602850. DOI: 10.1136/bmjopen-2017-020194

6 Giustarini E, Pinchera A, Fierabracci P, Roncella M, Fustaino L, Mammoli C and Giani C: Thyroid autoimmunity in patients with malignant and benign breast diseases before surgery. Eur J Endocrinol *154*: 645-649, 2006. PMID: 16645010. DOI: 10.1530/eje.1.02108

7 Ditsch N, Liebhardt S, Von Koch F, Lenhard M, Vogeser M, Spitzweg C, Gallwas J and Toth B: Thyroid function in breast cancer patients. Anticancer Res *30*: 1713-1717, 2010. PMID: 20592366.

8 Angelousi A, Diamanti-Kandarakis E, Zapanti E, Nonni A, Ktenas E, Mantzou A, Kontzoglou K and Kouraklis G: Is there an association between thyroid function abnormalities and breast cancer? Arch Endocrinol Metab *61*: 54-61, 2017. PMID: 28273204. DOI:10.1590/2359-3997000000191

9 Shi Y, Li X, Ran L, Arshad B, Li H, Xu Z, Zhao C, Wu Y, Wu H, Chen H, Li HY, Wu KN and Kong LQ: Study on the status of thyroid function and thyroid nodules in chinese breast cancer patients. Oncotarget *8*: 80820-80825, 2017. PMID: 29113346. DOI: 10.18632/oncotarget.20542

10 Szychta P, Szychta W, Gesing A, Lewiński A and Karbownik-Lewińska M: TSH receptor antibodies have predictive value for breast cancer – retrospective analysis. Thyroid Res *6(1)*: 8, 2013; PMID: 23680448. DOI: 10.1186/1756-6614-6-8

11 Cheng SY, Leonard JL and Davis PJ: Molecular aspects of thyroid hormone actions. Endocr Rev *31*: 139-170, 2010. PMID: 20051527. DOI: 10.1210/er.2009-0007

12 Onate SA, Tsai SY, Tsai MJ and O'Malley BW: Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science *270*: 1354-1357, 1995. PMID: 7481822. DOI: 10.1126/science.270.5240.1354

13 Chen JD and Evans RM: A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature *377*: 454-71995. PMID: 7566127. DOI: 10.1038/377454a0

14 Hörlein AJ, Näär AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Söderström M and Glass CK: Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature *377*: 397-404, 1995. PMID: 7566114. DOI:10.1038/377397a0

15 Alland L, Muhle R, Hou H Jr, Potes J, Chin L, Schreiber-Agus N and DePinho RA: Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature *387*: 49-55, 1997. PMID: 9139821. DOI:10.1038/387049a0

16 Shimizu H, Astapova I, Ye F, Bilban M, Cohen RN and Hollenberg AN: NCoR1 and SMRT play unique roles in thyroid hormone action *in vivo*. Mol Cell Biol *35*: 555-565, 2015. PMID: 9139821. DOI: 10.1128/MCB.01208-14

17 Shimizu H, Lu Y, Vella KR, Damilano F, Astapova I, Amano I, Ritter M, Gallop MR, Rosenzweig AN, Cohen RN and Hollenberg AN: Nuclear corepressor SMRT is a strong regulator of body weight independently of its ability to regulate thyroid hormone action. PLoS One *14(8)*: e0220717, 2019. PMID: 31404087. DOI: 10.1371/journal.pone.0220717

18 Zhang L, Gong C, Lau SL, Yang N, Wong OG, Cheung AN, Tsang JW, Chan KY and Khoo US: SpliceArray profiling of breast cancer reveals a novel variant of NCOR2/SMRT that is associated with tamoxifen resistance and control of ERα transcriptional activity. Cancer Res *73*: 246-255, 2013. PMID: 23117886. DOI: 10.1158/0008-5472.CAN-12-2241

19 Ryo A, Wulf G, Lee TH and Lu KP: Pinning down HER2-ER crosstalk in SMRT regulation. Trends Biochem Sci *34*: 162-165, 2009. PMID: 19269830. DOI: 10.1016/j.tibs.2008.12.004

20 Heldring N, Pawson T, McDonnell D, Treuter E, Gustafsson JA and Pike AC: Structural insights into corepressor recognition by antagonist-bound estrogen receptors. J Biol Chem *282*: 10449-10455, 2007. PMID: 17283072. DOI: 10.1074/jbc.M611424200

21 Ciriello G, Sinha R, Hoadley KA, Jacobsen AS, Reva B, Perou CM, Sander C and Schultz N: The molecular diversity of Luminal A breast tu¬mors. Breast Cancer Res Treat *141*: 409-420, 2013. PMID: 24096568. DOI: 10.1007/s10549-013-2699-3

22 van Agthoven T, Sieuwerts AM, Veldscholte J, Meijer-van Gelder ME, Smid M, Brinkman A, den Dekker AT, Leroy IM, van Ijcken WF, Sleijfer S, Foekens JA and Dorssers LC: CITED2 and NCOR2 in anti-oestrogen resistance and progression of breast cancer. Br J Cancer *101*: 1824-1832, 2009. PMID: 19904269. DOI: 10.1038/sj.bjc.6605423

23 Green AR, Burney C, Granger CJ, Paish EC, El-Sheikh S, Rakha EA, Powe DG, Macmillan RD, Ellis IO and Stylianou E: The prognostic significance of steroid receptor co-regulators in breast cancer: co-repressor NCOR2/SMRT is an independent indicator of poor outcome. Breast Cancer Res Treat *110*: 427-437, 2008. PMID: 19904269. DOI: 10.1038/sj.bjc.6605423

24 Smith CL, Migliaccio I, Chaubal V, Wu MF, Pace MC, Hartmaier R, Jiang S, Edwards DP, Gutiérrez MC, Hilsenbeck SG and Oesterreich S: Elevated nuclear expression of the SMRT corepressor in breast cancer is associated with earlier tumor recurrence. Breast Cancer Res Treat *136*: 253-265, 2012. PMID: 23015261. DOI: 10.1007/s10549-012-2262-7

25 Shi Y, Li X, Ran L, Arshad B, Li H, Xu Z, Zhao C, Wu Y, Wu H, Chen H, Li HY, Wu KN and Kong LQ: Study on the status of thyroid function and thyroid nodules in chinese breast cancer patients. Oncotarget *24*: 80820-80825, 2017. PMID: 29113346. DOI: 10.18632/oncotarget.20542

26 Takatani O, Okumoto T, Kosano H, Nishida M, Hiraide H and Tamakuma S: Relationship between the levels of serum thyroid hormones or estrogen status and the risk of breast cancer genesis in Japanese women. Cancer Res *49*: 3109-3112, 1989. PMID: 2720668.

27 Kuijpens JL, Nyklíctek I, Louwman MW, Weetman TA, Pop VJ and Coebergh JW: Hypothyroidism might be related to breast cancer in post-menopausal women. Thyroid *15*: 1253-1259, 2005. PMID: 16356089. DOI: 10.1089/thy.2005.15.1253

28 Karpaghavalli VG, Sumathy S and Dolia PB: Thyroid profile in patients with breast tumors. Int J Pharm Bio Sci *7*: 249-253, 2016. DOI: 10.22376/ijpbs.2016.7.4.b249-253

29 Ortega-Olvera C, Ulloa-Aguirre A, Ángeles-Llerenas A, Mainero-Ratchelous FE, González-Acevedo CE, Hernández-Blanco ML, Ziv E, Avilés-Santa L, Pérez-Rodríguez E and Torres-Mejía G: Thyroid hormones and breast cancer association according to menopausal status and body mass index. Breast Cancer Res *20*: 94, 2018. PMID: 30092822. DOI: 10.1186/s13058-018-1017-8

30 Tosovic A, Becker C, Bondeson AG, Bondeson L, Ericsson UB, Malm J and Manjer J: Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to breast cancer risk. Int J Cancer *131*: 2126-2133, 2012. PMID: 22323002. DOI: 10.1002/ijc.27470

31 Brandt J, Borgquist S and Manjer J: Prospectively measured thyroid hormones and thyroid peroxidase antibodies in relation to risk of different breast cancer subgroups: a Malmö Diet and Cancer Study. Cancer Causes Control *26*: 1093-1104, 2015. PMID: 26033776. DOI: 10.1007/s10552-015-0602-8

32 Tang HY, Lin HY, Zhang S, Davis FB and Davis PJ: Thyroid hormone causes mitogen-activated protein kinase-dependent phosphorylation of the nuclear estrogen receptor. Endocrinology *145*: 3265-3272, 2004. PMID: 15059947. DOI: 10.1210/en.2004-0308

33 Davis PJ, Davis FB, Mousa SA, Luidens MK and Lin HY: Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. Annu Rev Pharmacol Toxicol *51*: 99-115, 2011. PMID: 20868274. DOI: 10.1146/annurev-pharmtox-010510-100512

34 Hammes, SR and Davis PJ: Overlapping nongenomic and genomic actions of thyroid hormone and steroids. Best Pract Res Clin Endocrinol Metab *29*: 581-593, 2015. PMID: 26303085. DOI: 10.1016/j.beem.2015.04.001

35 Tosovic A, Bondeson AG, Bondeson L, Ericsson UB, Malm J and Manjer J: Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in postmenopausal women. Breast Cancer Res *12*: R33, 2010. PMID: 20540734. DOI: 10.1186/bcr2587

36 Kumar SAC and Reshma S: A clinical study of free T3 in breast cancer and benign breast tumours. Int Surg J *5*: 197-199, 2018. DOI: 10.18203/2349-2902.isj20175894

37 Russell W, Harrison RF, Smith N, Darzy K, Shalet S, Weetman AP and Ross RJ: Free triiodothyronine has a distinct circadian rhythm that is delayed but parallels thyrotropin levels. J Clin Endocrinol Metab *93*: 2300-2306, 2008. PMID: 18364382. DOI: 10.1210/jc.2007-2674

38 Strich D, Karavani G, Edri S and Gillis D: TSH enhancement of FT4 to FT3 conversion is age dependent. Eur J Endocrinol *175*: 49-54, 2016. PMID: 27150496. DOI: 10.1530/EJE-16-0007

39 Brandt J, Borgquist S, Almgren P, Försti A, Huss L, Melander O and Manjer J: Thyroid-associated genetic polymorphisms in relation to breast cancer risk in the Malmö Diet and Cancer Study. Int J Cancer *142*: 1309-1321, 2018. PMID: 29134650. DOI: 10.1002/ijc.31156.

40 Goemann IM, Marczyk VR, Recamonde-Mendoza M, Graudenz MS, Wajner SM and Maia AL: Abstract 4926: Loss of deiodinase type 3 expression distinguishes patients with poor prognosis in breast cancer. Cancer Res *79(13)*, 2019. DOI: 10.1158/1538-7445.AM2019-4926

41 Kurebayashi J, Otsuki T, Kunisue H, Tanaka K, Yamamoto S and Sonoo H: Expression levels of estrogen receptor-alpha, estrogen receptor-beta, coactivators, and corepressors in breast cancer. Clin Cancer Res *6*: 512-518, 2000. PMID: 10690532.

42 Goodson ML, Jonas BA and Privalsky ML: 2005 Alternative mRNA splicing of SMRT creates functional diversity by generating corepressor isoforms with different affinities for different nuclear receptors. J Biol Chem *280*: 7493-7503, 2005. PMID: 15632172. DOI: 10.1074/jbc.M411514200.

43 Goodson M, Jonas BA and Privalsky MA: Corepressors: custom tailoring and alterations while you wait. Nucl Recept Signal *3*: e003, 2005. PMID: 16604171. DOI: 10.1621/nrs.03003

44 Stanya KJ and Kao H: New insights into the functions and regulation of the transcriptional corepressors SMRT and N-CoR. Cell Div *4*: 7, 2009. DOI: 10.1186/1747-1028-4-7

45 Park EJ, Schroen DJ, Yang M, Li H, Li L and Chen JD: SMRTe, a silencing mediator for retinoid and thyroid hormone receptors-extended isoform that is more related to the nuclear receptor corepressor. Proc Natl Acad Sci USA *96*: 3519-3524, 1999. PMID: 10097068. DOI: 10.1073/pnas.96.7.3519

46 Peterson TJ, Karmakar S, Pace MC, Gao T and Smith CL: The silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) corepressor is required for full estrogen receptor alpha transcriptional activity. Mol Cell Biol *27*: 5933-5948, 2007. PMID: 17591692. DOI: 10.1128/MCB.00237-07

47 Karmakar S, Gao T, Pace MC, Oesterreich S and Smith CL: Cooperative activation of cyclin D1 and progesterone receptor gene expression by the SRC-3 coactivator and SMRT corepressor. Mol Endocrinol *24*: 1187-1202, 2010. PMID: 20392877. DOI: 10.1210/me.2009-0480.

48 Adikesavan AK, Karmakar S, Pardo P, Wang L, Liu S, Li W and Smith CL: Activation of p53 transcriptional activity by SMRT: a histone deacetylase 3-independent function of a transcriptional corepressor. Mol Cell Biol *34*: 1246-1261, 2014. PMID: 24449765. DOI: 10.1128/MCB.01216-13

49 Blackmore JK, Karmakar S, Gu G, Chaubal V, Wang L, Li W and Smith CL: The SMRT coregulator enhances growth of estrogen receptor-α-positive breast cancer cells by promotion of cell cycle progression and inhibition of apoptosis. Endocrinology 155: 3251-3261, 2014. PMID: 24971610. DOI: 10.1210/en.2014-1002

50 Jonas BA, Varlakhanova N, Hayakawa F, Goodson M and Privalsky ML: Response of SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) and N-CoR (nuclear receptor corepressor) corepressors to mitogen-activated protein kinase kinase kinase cascades is determined by alternative mRNA splicing. Mol Endocrinol *21(8)*: 1924-1939, 2007. PMID: 17519355. DOI:10.1210/me.2007-0035

51 Perissi V, Aggarwal A, Glass CK, Rose DW and Rosenfeld MG: A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. Cell *116*: 511-526, 2004. PMID: 14980219. DOI: 10.1016/s0092-8674(04)00133-3

52 Tagami T, Madison LD, Nagaya T and Jameson JL: Nuclear receptor corepressors activate rather than suppress basal transcription of genes that are negatively regulated by thyroid hormone. Mol Cell Biol *17(5)*: 2642-2648, 1997. PMID: 9111334. DOI: 10.1128/mcb.17.5.2642

53 de Groot S, Janssen LG, Charehbili A, Dijkgraaf EM, Smit VT, Kessels LW, van Bochove A, van Laarhoven HW, Meershoek-Klein Kranenbarg E, van Leeuwen-Stok AE, van de Velde CJ, Putter H, Nortier JW, van der Hoeven JJ, Pijl H and Kroep JR: Thyroid function alters during neoadjuvant chemotherapy in breast cancer patients: results from the NEOZOTAC trial (BOOG 2010-01). Breast Cancer Res Treat *149(2)*: 461-466, 2015. PMID: 25556355. DOI: 10.1007/s10549-014-3256-4

54 Bardou VJ, Arpino G, Elledge RM, Osborne CK and Clark GM: Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol *21(10)*: 1973-1979, 2003. PMID: 12743151. DOI: 10.1200/JCO.2003.09.099

55 Lim E, Palmieri C and Tilley WD: Renewed interest in the progesterone receptor in breast cancer. Br J Cancer *115(8)*: 909-911, 2016. PMID: 27657336. DOI:10.1038/bjc.2016.303

56 Yao N, Song Z, Wang X, Yang S and Song H. prognostic impact of progesterone receptor status in Chinese estrogen receptor positive invasive breast cancer patients. J Breast Cancer *20(2)*: 160-169, 2017. PMID: 28690652. DOI: 10.4048/jbc.2017.20.2.160

57 Falstie-Jensen AM, Kjærsgaard A, Lorenzen EL, Jensen JD, Reinertsen KV, Dekkers OM, Ewertz M and Cronin-Fenton DP: Hypothyroidism and the risk of breast cancer recurrence and all-cause mortality – a Danish population-based study. Breast Cancer Res *21(1)*: 44, 2019. PMID: 30902106. DOI: 10.1186/s13058-019-1122-3.

58 Degirmencioglu S, Ugurlu E and Yaren A: Effects of serum thyroid stimulating hormone levels on prognosis in patients with advanced non-small cell lung cancer. J Carcinog Mutagen *7(4)*: 272, 2016. DOI: 10.4172/2157-2518.1000272

59 Seebacher V, Hofstetter G, Polterauer S, Reinthaller A, Grimm C, Schwameis R, Taucher S, Wagener A, Marth C and Concin N: Does thyroid-stimulating hormone influence the prognosis of patients with endometrial cancer? A multicentre trial. Br J Cancer *109*: 215-218, 2013. PMID: 23764750. DOI: 10.1038/bjc.2013.282

Received September 21, 2020
Revised October 1, 2020
Accepted October 5, 2020