Research Article

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Stanniocalcin Expression as a Predictor of Late Breast Cancer Recurrence

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Abstract

Background: Expression of human paracrine hormones stanniocalcin 1 (STC1) and stanniocalcin 2 (STC2) may potentiate late breast cancer recurrence. We tested the hypothesis that expression of STC1 and STC2 in primary breast tumors is more strongly associated with late versus early recurrences.

Methods: A total of 541 estrogen receptor–positive, tamoxifen-treated (ER^+/TAM^+) and 300 ER-negative, tamoxifen-untreated (ER^-/TAM^-) breast cancer patients who experienced recurrence within 10 years of primary diagnosis and matched recurrence-free controls were selected from a cohort of 11,251 Danish breast cancer patients diagnosed with stage I, II, or III breast cancer during 1985 to 2001. The association between IHC expression of STC1 and STC2 in primary breast tumor tissue microarrays and breast cancer recurrence was evaluated within median time to recurrence quintiles.

Results: The association between STC1 expression, dichotomized as positive or negative, and recurrence was strongly positive for the final time quintile (6–10 years postdiagnosis) in the ER^+/TAM^+ group [aOR = 2.70; 95% confidence interval (CI): 1.22–5.98]. Regression of the log ORs relating dichotomous STC1 and STC2 expression to recurrence by median time to recurrence (year) resulted in a relatively large positive effect estimate for STC1 ($\beta = 0.16$; 95% CI, −0.03–0.36) and a near-null positive effect estimate for STC2 ($\beta = 0.04$; 95% CI, −0.14–0.21).

Conclusions: Our results suggest a stronger association between primary tumor STC1 expression and late recurrence, as opposed to early recurrence, although no clear trend was apparent.

Impact: STC1 expression in the primary tumor may potentiate late recurrences, suggesting dormancy pathways that merit further investigation. *Cancer Epidemiol Biomarkers Prev; 27(6); 653–9. ©2018 AACR.*

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Introduction

Median disease-free survival time for breast cancer patients has improved considerably in recent years due to advances in screening and surgical, adjuvant, and radiation treatments (1). However, risk of recurrence persists even after years of disease-free survival. In breast cancer patients treated with curative intent, more than half of recurrences occur 3 or more years after diagnosis (2). In a study of U.S. women ≥65 years old at breast cancer diagnosis, approximately 5% of 5-year survivors developed a breast cancer recurrence 6 to 10 years after diagnosis (3). In a cohort of younger Danish breast cancer patients (87% <70 years old at diagnosis; ref. 4) who received guideline treatment (5), approximately 7% of 5-year survivors developed a recurrence 6 to 15 years after diagnosis. Cases of breast cancer recurrence have been reported as late as 39 years after primary diagnosis and treatment (6, 7).

Treatment stratification of breast cancer patients by recurrence risk can improve outcomes (8), although current risk prediction methods (9–13) are not robust for evaluating later recurrence risk (14–16). To improve upon these models, a molecular marker that predicts late recurrence specifically, without also predicting early recurrence, is needed. Such a marker would likely enable long-term cellular survival in the stressed tumor microenvironment, with the potential to eventually release the micrometastasis from dormancy (17). On the basis of these criteria, expression of human paracrine hormones stanniocalcin 1 (STC1) and 2 (STC2) has been hypothesized as a potentially predictive molecular marker in the primary tumor specific to late recurrence risk.

Stanniocalcins stabilize cells under stressed conditions, including neural cells and cardiomyocytes (18, 19), by decreasing reactive oxygen species and inhibiting apoptosis (20, 21). This stabilization assists tumor cells in long-term survival. Expression of both STC1 and STC2 has been associated with increased cancer risk (22–25), and with poor prognosis following cancer diagnosis, including breast cancer (26–29). Endopredict (EP), an RNA-based multigene risk score including primary tumor expression of STC2, showed improved discrimination between patients with estrogen receptor–positive (ER^+)/$HER2^-$ breast cancer at high versus low risk of recurrence 10 years following primary breast cancer diagnosis, compared with use of clinicopathologic features alone (30). Small studies of breast cancer patients have revealed higher proportion of STC1- and STC2-expressing cells in primary tumors among women who experienced recurrences 5 or more years after primary diagnosis, compared with those who experienced a recurrence within 2 years of primary diagnosis (31). Expression

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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of STC2 in primary tumor cells has also been associated with longer disease-free survival (32). Studies thus far have been limited by small patient populations, a focus on survival instead of recurrence as the primary outcome, and insufficient control for covariate information.

We used a large study of Danish breast cancer patients followed up to 10 years for recurrence to address the hypothesis that expression of STC1 and STC2 in primary tumors is associated with risk for late recurrence, defined as recurrence occurring more than 5 years after primary diagnosis, and is weakly associated with risk of earlier recurrence.

Materials and Methods

Study population

Cases and controls were selected from a cohort of 11,251 female breast cancer patients living on Denmark's Jutland Peninsula who were registered with the Danish Breast Cancer Cooperative Group (DBCG). Women were eligible for inclusion in the cohort if they were diagnosed with stage I, II, or III breast cancer between 1985 and 2001 and were between 35 and 69 years old at diagnosis. We restricted to 1,826 ER^+, tamoxifen-treated (ER^+/TAM^+) patients and 1,808 ER^-, tamoxifen-untreated patients (ER^-/TAM^-) who survived at least one year without recurrence; all other patients were excluded ($n = 7{,}617$) due to unknown treatment, unknown ER status, or tamoxifen protocol or survival period of under one year.

For all female breast cancer patients registered in DBCG, follow-up for recurrence occurs every 3 to 6 months for 5 years and annually in years 5 to 10, from Danish Departments of Surgery, Histopathology, Radiotherapy, and Medical Oncology. Women who experienced a recurrence within 10 years of primary diagnosis or by December 31, 2006, were selected from ER^+ and ER^- patient groups as cases. A recurrence was defined as any type of breast cancer or distant metastases diagnosed after initial course of therapy, following the DBCG definition (33). Completeness of recurrent case identification was high (34), and a DBCG validation study found a positive predictive value of 99.4%, indicating few false positives (35). Controls were women who did not experience a recurrence by the same duration of follow-up of their matched case, selected by risk-set sampling from the same cohort of breast cancer patients. Vital status was ascertained by linkage to the Danish Central Personal Registry, which assured that controls were alive at the same duration of follow-up as the matched case. Controls were matched to recurrent cases on ER/TAM status and menopausal status, date of breast cancer surgery (within one year), county, and tumor stage defined by the Union for International Cancer Control (UICC). Before exclusions, there were 541 ER^+ and 300 ER^- recurrent cases and their matched controls.

TMA construction and IHC staining

For all laboratory assays performed, personnel were blinded to clinical information. Using each patient's unique Danish Civil Personal Registration number to link datasets, the locations of the original diagnostic specimens were identified. Formalin-fixed, paraffin-embedded primary breast tumor specimens were collected from the pathology archives of the participating hospitals by a medical research technician. New tissue sections were cut and were used by an experienced pathologist to identify blocks with invasive carcinoma. Tissue microarrays (TMAs) were constructed using a TMA Master (3DHISTECH), with 1-mm tissue cores sampled from each tumor specimen. Cores from the individual tumors were included in duplicate TMAs. If possible, up to three representative tumor cores, and one nonneoplastic core from marginal or normal tissues, were sampled.

TMA 2.5-μm tissue sections were stained by the pathology laboratory at the Rollins School of Public Health at Emory University (Atlanta, GA) for STC1 and STC2. Briefly, slides were deparaffinized in xylene, hydrated in graded alcohols, and blocked for endogenous peroxidase for 5 minutes in UltraVision hydrogen peroxidase block (Thermo Fisher Scientific, ref. TA-125H202Q). Heat-induced epitope retrieval was performed in a decloaking chamber (PT Link, Agilent). Before staining, and in between each step, slides were washed with Tween 20 buffer (Cell Marque, ref. 935B-09). Automated staining was carried out at room temperature using the Dako Autostainer*Plus*. Following UltraV block (Thermo Fisher Scientific, ref. TA-125-UB), sections were incubated for 30 minutes with the primary antibodies [rabbit polyclonal anti-STC1 (Sigma Aldrich, cat. HPA023918) at 1:500 dilution and rabbit polyclonal anti-STC2 (Sigma Aldrich, cat. HPA045372) at 1:1,500 dilution, followed by UltraVision Goat Polyvalent Secondary (Thermo Fisher Scientific, ref. TL-125-BN) for 15 minutes, UltraVision Streptavidin Horseradish Peroxidase (Thermo Fisher Scientific, ref. TL-125-HR) for 15 minutes, and by diaminobenzidine (DAB; Thermo Fisher Scientific, ref. TA-125-HDX) for 5 minutes]. Slides were counterstained with hematoxylin (Thermo Fisher Scientific, ref. 7211), dried for at least 24 hours, and then digitalized using the Panoramic Scan 150 whole slide image scanner (3DHISTECH).

IHC expression was evaluated at Aarhus University (Aarhus, Denmark) on digitalized images. The semiquantitative protocol was developed by three observers (A.S. Nielsen, S. Hamilton-Dutoit, and K.L. Lauridsen) based on consensus diagnoses in pilot studies. Expression intensity was assigned on the ordinal scale 0 to 3, with 0 representing negative staining, and 3 representing strong staining. The proportion of cells in each core with each expression intensity was recorded. A combination of staining proportion and intensity was used to calculate the semiquantitative histologic score (H-score), with a possible range of 0 (0% of cells had expression) to 300 (100% of cells within the core staining with full intensity; refs. 36, 37).

Definition of analytic variables

Recurrences are defined by DBCG as the first event of local, contralateral, or distant recurrence. Given our biologic premise, we excluded five contralateral breast cancers and their matched controls from this analysis. Time to recurrence was categorized by approximate quintile distribution of cases: 1 to <2 years; 2 to <3years; 3 to <4 years; 4 to <6 years; and 6 to 10 years. Matched factors, obtained from DBCG, included diagnostic ER expression, receipt of tamoxifen therapy (yes/no), menopausal status at diagnosis, UICC stage at diagnosis (I, II, III), year of diagnosis, and county of residence. ER positivity was defined as $\geq$10% ERα staining based on previous DBCG recommendations applicable to the time period of diagnosis for this cohort (38). ER^+ tumors were identified with high validity (94% concordance with reassay from a validation substudy), although ER^- tumor identification was not as robust (74% concordance with reassay; ref. 34). Year of diagnosis was categorized in three groups: 1985–1993, 1994–1996, and 1997–2001. Other covariates, selected by *a priori* evidence as potential confounding factors, included age (continuous), primary treatment (radiation and chemotherapy),

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systemic chemotherapy receipt (yes/no), prescribed tamoxifen therapy duration, and Charlson comorbidity score. The Charlson comorbidity index (CCI) was categorized as 0, 1–2, or ≥3, based on health history information up to 10 years before primary breast cancer, as recorded in the Danish National Patient Registry, with scores defined by Charlson (39). Expression of STC1 and STC2 was defined in two ways: (i) by the calculated continuous H-scores (range, 0–300) and (ii) dichotomized as negative (0% staining) or positive (>0% staining).

Statistical analysis

All statistical analyses were conducted using SAS 9.4 (SAS Institute). The proportions of recurrent cases and controls were calculated within each ER/TAM group, along with covariate distributions. Crude associations between STC1 and STC2 expression, defined both continuously and dichotomously, and recurrence event and covariates were explored. Because of high number of tumors with 0% staining, a factor of +1 was added after logarithmic transformation of H-scores to better assess the influence of continuous expression. Because more than half of tumors in each ER strata had no STC1 or STC2 expression, dichotomous (positive/negative) expression was the focus of analyses of expression levels.

To evaluate the hypothesis that STC expression is higher in primary tumors of patients experiencing later recurrences compared with early recurrences, adjusted ORs associating expression with recurrence were calculated within each quintile of time to recurrence. Controls were pair matched to cases by design, and matched factors were controlled as categorical variables in unconditional logistic regression to optimize sample size. Matched factors can be categorized without loss of validity (40) in analyses of pair-matched case–control data, which dispenses with the need for conditional logistic regression and allows inclusion of cases whose pair-matched control has missing bioassay data, or vice versa. This analysis strategy does not compromise validity and optimizes precision. Adjusted models additionally controlled for selected covariates: chemotherapy, radiation treatment, CCI score, and duration of assigned tamoxifen therapy (ER^+ patients only). Because controls were selected by risk-set sampling, the case–control OR provides an estimate of the rate ratio, equivalent to the estimate that would be obtained from a full cohort analysis using proportional hazards regression (41). The log ORs calculated for each quintile of time to recurrence were regressed on median time to recurrence, summarized as five midpoints, with weighting by the inverse variance.

Results

Descriptive characteristics

Among recurrent cases in the cohort, 446 patients with ER^+ tumors and 253 patients with ER^- tumors had primary tumor samples in which STC1 or STC2 expression was adequate for inclusion in this analysis. The women included in the analysis were primarily postmenopausal at diagnosis (81%), although the distribution differed between ER^+ and ER^- groups (93% vs. 60% postmenopausal, respectively). This is explained by different age distributions; ER^+ patients were older (75% ≥55 years) than ER^- patients (38% ≥55 years; Table 1). Most women were diagnosed with stage II or III breast cancer (96%). Among the ER^+ group, tamoxifen was assigned for one (47%) or 5 years (37%), although a medical record review of a subsample of patients included in the cohort suggests that most patients received tamoxifen for a longer duration as guideline durations advanced during their follow-up period (34). Most patients (77%) had no comorbidity.

Expression of both STC1 and STC2 was higher on average among ER^+/TAM^+ patients, compared with ER^-/TAM^- patients. This difference remained when considering only patients with positive staining. Mean H-scores for STC1 and STC2 were similar between cases and controls within each ER stratum.

Association of STC1 and STC2 expression with recurrence, at any time during follow-up

Among the ER^+/TAM^+ patient group, there were 440 recurrent cases and 444 controls with available STC1-scored tumor samples, and 415 recurrent cases and 423 controls with available STC2-scored tumor samples (Table 2). STC1 expression, assessed as positive or negative, had a near-null association with odds of recurrence [aOR = 1.04; 95% confidence interval (CI), 0.79–1.38]. STC2 expression was associated with lower odds of recurrence (aOR = 0.73; 95% CI, 0.54–0.98). Results were near-null for both markers among ER^-/TAM^- patients.

Association of STC1 and STC2 expression with recurrence, by time of event

To assess whether the expression of STC1 and STC2 is differentially associated with recurrence based on time to recurrence, adjusted ORs were calculated within each quintile of time (1–<2 years; 2–<3years; 3–<4 years; 4–<6 years; and 6–10 years). Among ER^+/TAM^+ patients, STC1 expression was associated with breast cancer recurrence in years 6 to 10 following diagnosis (aOR = 2.70; 95% CI, 1.22–5.98). STC2 expression was associated with lower odds of breast cancer recurrence in years 3 to 4 following primary diagnosis (aOR = 0.37; 95% CI, 0.16–0.83), but showed no association with recurrence at other time quintiles assessed (Table 3). Within the ER^-/TAM^- group, smaller samples within later time quintiles resulted in imprecise estimates and in uninterpretable results (Supplementary Table S1).

Assessment of the primary study hypothesis resulted in a large positive effect estimate for STC1 among ER^+/TAM^+ patients ($\beta = 0.16$; 95% CI, −0.03–0.36), indicating a higher expression of STC1 in primary tumors appearing among women who experienced later recurrences. The effect estimate for STC2 was near-null ($\beta = 0.04$; 95% CI, −0.14–0.21; Table 4).

Discussion

In this study of Danish women diagnosed with stage I, II, or III breast cancer between 1985 and 2001 and followed up for 10 years for recurrence, there was a suggested trend in the association between STC1 and recurrence over time. No trend was revealed between STC2 and recurrence over time. STC1 expression was associated with later breast cancer recurrence (6–10 years after primary diagnosis), whereas STC2 was not associated with early or late breast cancer recurrence.

Our findings are consistent with our biologically based *a priori* hypothesis suggesting stanniocalcins as predictive markers for late recurrence (18, 19, 21, 30, 42, 43). Our result revealing an association of STC1 expression with recurrence in primary tumors of women who experienced recurrence 6 to 10 years following primary diagnosis, and not in tumors of women who experienced earlier recurrence, aligns with the results of a previous study by Joensuu and colleagues, which found a higher proportion of

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Table 1. Frequency and proportion of breast cancer recurrent case patients and matched control subjects with available STC1 or STC2 scores within group strata ($n = 1{,}407$)[a]

	ER^+/TAM^+, no. (%)[b] or mean (SD)		ER^-/TAM^-, no. (%)[b] or mean (SD)	
Patient characteristics	**Recurrent cases**	**Controls**	**Recurrent cases**	**Controls**
STC1 expression, dichotomous[c]				
Negative	222 (50)	228 (51)	165 (65)	168 (66)
Positive	218 (50)	216 (49)	87 (35)	85 (34)
Missing[d]	6	8	1	3
STC2 expression, dichotomous[c]				
Negative	156 (38)	128 (30)	143 (58)	133 (56)
Positive	259 (62)	295 (70)	102 (42)	104 (44)
Missing[d]	31	29	8	19
STC1 expression, continuous[e]	35.2 (49.8)	35.0 (51.7)	19.4 (39.6)	21.1 (40.4)
STC2 expression, continuous[e]	42.6 (61.9)	56.8 (73.9)	17.2 (33.6)	22.5 (40.7)
Diagnosis year				
1985–1993	187 (42)	188 (42)	89 (35)	82 (32)
1994–1996	90 (20)	91 (20)	67 (26)	73 (29)
1997–2001	169 (38)	173 (38)	97 (38)	101 (39)
Age category at diagnosis, years				
35–44	15 (3.4)	13 (2.9)	54 (21)	48 (19)
45–54	98 (22)	94 (21)	102 (40)	99 (39)
55–64	229 (51)	232 (51)	70 (28)	75 (29)
65–69	104 (23)	113 (25)	27 (11)	34 (13)
Menopausal status at diagnosis				
Premenopausal	30 (6.7)	31 (6.9)	100 (40)	105 (41)
Postmenopausal	416 (93)	421 (93)	153 (60)	151 (59)
UICC tumor stage at diagnosis				
I	9 (2.0)	6 (1.3)	17 (6.7)	22 (8.6)
II	201 (45)	204 (45)	136 (54)	134 (52)
III	236 (53)	242 (53.5	100 (39)	100 (39)
Histologic grade				
1	84 (19)	119 (26)	17 (6.7)	19 (7.4)
2	199 (45)	181 (40)	111 (44)	85 (33)
3	77 (17)	49 (11)	90 (36)	90 (35)
Missing	86 (19)	103 (23)	35 (14)	62 (24)
Surgery type				
Mastectomy	403 (90)	393 (87)	217 (86)	207 (81)
Breast conserving	43 (9.6)	59 (13)	35 (14)	49 (19)
Radiotherapy				
Yes	151 (34)	159 (35)	104 (42)	106 (48)
No	295 (66)	293 (65)	145 (58)	115 (52)
Missing	0	0	4	35
Tamoxifen protocol, years				
1	208 (47)	211 (47)		
2	75 (17)	74 (16)		
5	163 (36)	167 (37)		
Systemic adjuvant chemotherapy				
Yes	61 (14)	53 (12)	215 (85)	158 (62)
No	385 (86)	399 (88)	38 (15)	98 (38)
CCI score				
0	338 (76)	353 (78)	199 (79)	217 (85)
1	41 (9.2)	49 (11)	15 (5.9)	12 (4.7)
2	9 (2.0)	16 (3.5)	11 (4.3)	13 (5.1)
3+	58 (13)	34 (7.5)	28 (11)	14 (5.5)

NOTE: Percentage expression over available samples. Removed if staining was inadequate or scores were inconclusive.

[a]Initial cohort consisted of 11,251 women living on the Jutland Peninsula, Denmark, ages 35–69 years, who were diagnosed with stage I, II, or III breast cancer between 1995 and 2001.

[b]Missing not included in percent calculations.

[c]Dichotomous STC1 and STC2 expression defined as negative if 0% staining, positive if >0% staining.

[d]Missing if staining was inadequate or scores were inconclusive.

[e]Continuous H-score (0–300) determined by proportion and intensity of staining.

primary breast tumor cells expressing STC1 among women who had later versus earlier recurrence (31). We similarly observed a potential time-dependent trend between STC1 expression and recurrence. However, in years one to five following primary diagnosis, associations between STC1 expression and recurrence were irregular; the trend was instead driven by events after 6 years. This highlights a need for additional studies that continue past 10 years of follow-up. Also in contrast to the study of Joensuu and colleagues, our results do not indicate any association between STC2 expression and late recurrence. This difference may be attributed to the shorter period of follow-up in our study (10 years after primary diagnosis) compared with Joensuu and

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Table 2. Association of STC1 and STC2 expression with breast cancer recurrence

	ER^+/TAM^+			ER^-/TAM^-		
Expression of STC1 or STC2[a]	**Recurrent cases/controls**	**OR (95% CI)[b]**	**Adjusted OR (95% CI)[c]**	**Recurrent cases/controls**	**OR (95% CI)[b]**	**Adjusted OR (95% CI)[c,d]**
STC1	440/444	1.05 (0.80–1.38)	1.04 (0.79–1.38)	252/253	1.08 (0.72–1.60)	0.99 (0.65–1.51)
STC2	415/423	0.72 (0.54–0.96)	0.73 (0.54–0.98)	245/237	0.92 (0.63–1.34)	0.90 (0.60–1.36)
STC1[e]	409/415	1.11 (0.84–1.48)	1.11 (0.83–1.48)	244/234	1.11 (0.74–1.68)	1.05 (0.67–1.62)
STC2[e]	409/415	0.71 (0.53–0.96)	0.72 (0.53–0.98)	244/234	0.89 (0.61–1.30)	0.89 (0.58–1.34)

[a]Expression of STC1 and STC2 dichotomized as positive (>0%) or negative (0%).
[b]OR from unconditional logistic regression, controlling for matched factors: age group, year group of diagnosis, menopausal status, stage (I–III), and county of treatment.
[c]Adjusted OR includes adjustment for all matched factors and chemotherapy, radiation, CCI group, and tamoxifen duration (ER^+).
[d]Adjusted ORs resulted in a different number of cases/controls than matched ORs for ER^-/TAM^- group. For STC1 = 248/219, STC2 = 236/199, in combination = 240/203.
[e]STC1 and STC2 expression tested within same logistic model.

colleagues' study (up to 23 years after primary diagnosis). It is also possible that an attenuation of the association between STC2 expression and late recurrence resulted from our larger sample size of 446 ER^+ primary tumors, compared with previous studies, which analyzed only 30 (31) or 65 (44) ER^+ primary tumors.

It is conceivable that STC1 and STC2 may not have the same strength of association with regards to recurrence, as we observed here, based on the different mechanisms each hormone employs to promote cell stabilization. STC1 activates uncoupling protein 2 to decrease ATP synthesis and superoxide formation (20), whereas STC2 helps cells avoid apoptosis by inhibiting the store-operated calcium exchange, which depends on initial binding with hypoxia-inducible factor (43).

Although this is the largest study to date evaluating the association between stanniocalcins and breast cancer recurrence, sample size remained a limitation for assessment within time quintiles of interest. We were unable to adequately assess associations among the ER^-/TAM^- patient group. However, because STC1 and STC2 are estrogen-responsive genes (45), and because risk of recurrence in years 5 to 25 after primary diagnosis is much higher among ER^+ patients than ER^- patients (46), the inability to assess the ER^- group is less important. Moreover, previous studies that have observed an association between STC1 or STC2 expression and breast cancer recurrence in ER^+ tumors have not found an association for ER^- tumors (44). Defining expression as negative or positive avoided arbitrary categorization based on high versus low STC1 and STC2 expression, which would result in greater potential of misclassification; however, the dichotomized exposure simplifies expression and may not offer the most robust evaluation of these markers. We did perform all analyses using continuous expression, defined by H-scores, as the exposure, to examine how this exposure definition affected the results, although the high number of tumors showing zero expression for either marker precluded accurate evaluation of the continuously assessed ORs. Applying a factor of +1 prior to logarithmic transformation of the H-scores was consistent in demonstrating a positive association between increased transformed H-score for STC1 and breast cancer recurrence more than 6 years after primary diagnosis.

Our study benefits from a standard follow-up protocol over 10 years after primary diagnosis, consistent across DBCG patients. Complete treatment, demographic, and potential covariate information is collected in DBCG, and use of these variables reduces potential for unmeasured confounding biasing our results. The DBCG has issued widely followed standardized treatment protocols for Danish breast cancer patients since 1977 (5), which assures baseline uniformity of quality of care in this single-payer health care system. Moreover, recurrence is well identified within the database, as demonstrated by a previous validation study (35), limiting the potential for disease misclassification. The ability to evaluate multiple tumor cores and adjacent normal sample enabled review of IHC expression levels representative of the whole tissue sample.

Overall, our results suggest that expression of STC1, but not STC2, may be differentially related to breast cancer recurrence based on time since primary diagnosis, in partial agreement with previous, smaller scale studies. On the basis of the lack of trend seen for STC2, and given that the time trend of STC1 association with breast cancer recurrence was not clear over the first 5 years of follow-up, our original hypothesis that STC1 and STC2 may be acting in a pathway to enable late breast cancer recurrence remains open. STC1 and STC2 expression profiling to determine treatment regimens for breast cancer stratified by risk of recurrence would not be clinically beneficial at this time. However, because we did observe an association between STC1 expression and breast

Table 3. Association between STC1 and STC2 expression and breast cancer recurrence, by median time to recurrence, ER^+/TAM^+ patient group

	STC1[a]			STC2[a]		
Median time to recurrence (y)[b]	**Recurrent cases/controls**	**OR (95% CI)[c]**	**aOR (95% CI)[d]**	**Recurrent cases/controls**	**OR (95% CI)[c]**	**aOR (95% CI)[d]**
1.5	106/109	0.75 (0.42–1.34)	0.68 (0.37–1.28)	101/103	0.76 (0.43–1.34)	0.76 (0.41–1.42)
2.4	77/81	1.23 (0.64–2.37)	1.27 (0.63–2.55)	75/76	0.95 (0.46–1.97)	0.88 (0.41–1.89)
3.4	83/75	1.11 (0.57–2.15)	1.03 (0.51–2.07)	76/70	0.35 (0.16–0.75)	0.37 (0.16–0.83)
4.8	111/107	0.75 (0.42–1.32)	0.77 (0.43–1.40)	103/104	0.72 (0.40–1.30)	0.75 (0.40–1.40)
7.3	63/72	2.80 (1.29–6.05)	2.70 (1.22–5.98)	60/70	0.88 (0.40–2.00)	0.99 (0.43–2.26)

[a]Expression of STC1 and STC2 dichotomized as positive (>0%) or negative (0%).
[b]Median time to recurrence based on recurrent cases.
[c]Based on unconditional logistic regression model, adjusting for matched factors: age group, year group of diagnosis, menopausal status, stage (I–III), and county of treatment.
[d]Adjusted OR includes matched factors and chemotherapy, radiation, CCI group, and tamoxifen duration (ER^+).

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Table 4. Association of STC1 or STC2 expression with time to recurrence, ER^+/TAM^+ patient group

Biomarker expression[a]	Intercept (SE)	Effect estimate (β), median time to recurrence (SE)[b]	95% CI around β	P[c]
STC1	−0.55 (0.41)	0.16 (0.10)	−0.03–0.36	0.20
STC2	−0.45 (0.36)	0.04 (0.09)	−0.14–0.21	0.71

[a]Expression of STC1 and STC2 dichotomized as positive (>0%) or negative (0%).
[b]Median time to recurrence assessed at $i = 5$ midpoints, by year.
[c]Testing $\beta = 0$.

cancer recurrence after 6 years from primary diagnosis, this biomarker and its biologic pathways merit further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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