Clinical significance of the nuclear receptor co-regulator DC-SCRIPT in breast cancer: an independent retrospective validation study

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Abstract

Introduction: In this study we aimed to validate the prognostic value of DC-SCRIPT mRNA expression in a large independent breast cancer cohort. In addition, since DC-SCRIPT is a transcriptional co-regulator of nuclear receptors, we explored its prognostic value in relation to estrogen-receptor-α (ESR1) and -β (ESR2) and evaluated its predictive value for response to tamoxifen treatment.

Methods: DC-SCRIPT mRNA levels were measured by real-time PCR in 1,505 primary invasive breast cancers and associated with outcome (disease-free survival (DFS), metastasis-free survival (MFS) and overall survival (OS)) using univariate and multivariable Cox regression analysis. Logistic and Cox regressions were used to associate DC-SCRIPT levels with clinical benefit and progression-free survival (PFS) for 296 patients treated with first-line systemic tamoxifen for advanced disease.

Results: In univariate and multivariable analysis higher DC-SCRIPT levels were associated with a favorable outcome for both the entire cohort and patients with lymph node-negative (LNN) disease that did not receive adjuvant therapy (DFS, MFS and OS; all, P < 0.001). This association was most pronounced in small (pT1) tumors, in ESR1-positive tumors and in tumors with low ESR2 expression. For first-line endocrine therapy for advanced disease no predictive association was seen with clinical benefit or PFS.

Conclusions: This study provides a higher level of evidence that DC-SCRIPT is indeed an independent, pure prognostic factor for primary breast cancer and shows that DC-SCRIPT mRNA expression is most informative for either ESR1-positive and/or ESR2-low pT1 tumors.

Introduction

Estrogens influence the aggressiveness of breast cancer through their cognate nuclear receptors. In particular, the estrogen receptor-alpha (ERα) (ESR1) - present in tumor cells of about 70% to 75% of all breast tumors - is considered crucial because of its proliferation-inducing actions and for that reason is an important target for therapy. Next to ESR1, a second ER exists, ERβ (ESR2). ESR2 counteracts the activity of ESR1 in many systems [1,2] and is also expressed in the majority of breast cancers. Apart from breast epithelial tumor cells, ESR2 is also expressed in adjacent infiltrating lymphocytes, fibroblasts, and endothelial cells, all of which are known to influence tumor growth [3]. However, its precise role in breast cancer progression is less well defined.

DC-SCRIPT (zinc finger protein 366 [ZNF366]) is a recently identified nuclear receptor co-regulator first identified in immune cells [4-6]. Nuclear receptor co-regulators are proteins that can activate or repress the transcriptional activity of nuclear receptors. DC-SCRIPT is in this respect a unique co-regulator as we have shown that it enhances the activities of the nuclear retinoic acid receptor (RAR) and peroxisome proliferator-activated receptor (PPAR) heterodimers, RARα/RXRα.
and PPARγ/RXRo, but represses the activities of ESR1 and progesterone receptor (PGR) [7]. We also showed that DC-SCRIPT was an independent prognostic factor, particularly for hormone receptor-positive breast cancer. This led us to postulate that the anti-proliferative effect of DC-SCRIPT in breast cancer cells could be mediated by simultaneous modulation of the activity of multiple nuclear receptors.

To provide a higher level of evidence for DC-SCRIPT mRNA expression as a prognostic marker, we now report on DC-SCRIPT expression and its significance in a retrospective validation study of 1,505 breast cancer patients with known ESR1, ESR2, and PGR expression levels. The primary objective of this study was to confirm the relationship between DC-SCRIPT mRNA levels measured in primary breast cancers and tumor aggressiveness in a much larger, independent, breast cancer cohort. The main clinical endpoints for assessing the prognostic value of DC-SCRIPT expression were disease-free survival (DFS), metastasis-free survival (MFS), and overall survival (OS) in lymph node-negative (LNN) patients who had not received adjuvant systemic therapy; this approach allowed us to determine tumor aggressiveness during the natural course of the disease. As DC-SCRIPT modulates ER activity, we also analyzed the prognostic value of DC-SCRIPT separately in tumors stratified by ESR1 and ESR2 expression. Since several co-regulators of nuclear receptors also modulate response to therapy [8,9], we also assessed, as a secondary aim of this study, the predictive value of DC-SCRIPT by using clinical benefit and progression-free survival (PFS) after first-line tamoxifen for advanced disease as the main endpoints.

Materials and methods
Patients
The protocol to study biological markers associated with disease outcome was approved by the medical ethics committee of the Erasmus Medical Center (Rotterdam, The Netherlands) (MEC 02.953). This retrospective study used 1,505 M0 (no metastasis) and 32 M1 (with metastasis) blind-coded freshly frozen primary tumor tissues of female patients with primary operable breast cancer from 1978 through 2000. The study was performed in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in The Netherlands [10], and consent was not required. Wherever possible, the study has been reported in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines [11]. The primary breast tumors were from patients with detailed clinical follow-up as previously described [12-14]. ER protein status was determined by routine ligand-binding assays or enzyme immunoassays [15], and ESR1, ESR2, and PGR mRNA status was determined by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) [14,16,17]. Follow-up, tumor staging, and response to therapy were defined by standard International Union Against Cancer (Geneva, Switzerland) classification criteria [18] and applied previously by Foekens and colleagues [19]. All 1,537 patients underwent breast-conserving lumpectomy (44%) or modified mastectomy (56%). Of the 1,505 patients included for the evaluation of tumor aggressiveness, 462 lymph node-positive patients (31%) were treated with adjuvant systemic therapy, 207 patients received hormonal therapy, 233 chemotherapy, and 22 combination therapy. Disease recurrence occurred in 836 patients, and 703 developed a distant metastasis. The median follow-up time of patients alive was 90 months (range of 4 to 260 months).

Eight hundred thirty-seven patients had no involved nodes and did not receive systemic adjuvant therapy. Of these 837 LNN patients, 383 had a disease relapse, 300 developed a distant metastasis, and 273 died during follow-up. Of the 703 patients who developed a distant metastasis, 296 ER-positive patients, including the 32 M1 patients, received hormonal therapy as first-line therapy for advanced disease. Clinical benefit of first-line tamoxifen treatment was observed in 185 patients. Median follow-up time for treatment of advanced disease was 38 (4 to 120) months. Two hundred nineteen patients had died at the end of the follow-up. None of these patients had received prior adjuvant hormonal therapy, whereas 19% received prior adjuvant chemotherapy. A more detailed description of the patients and their therapy is given in the Supplementary materials and methods (Additional file 1). Patient and tumor characteristics combined with DC-SCRIPT mRNA expression and clinical outcome are listed in Table 1.

RNA isolation and quantitative RT-PCR
Tissue processing, RNA isolation, cDNA synthesis, and quantitative RT-PCR were performed as previously described [16]. Real-time quantitative PCRs were performed in a 25-µL reaction volume in an Mx3000P™ Real-Time PCR System (Agilent, Amsterdam, The Netherlands). In addition to an SYBR-based assay to detect a 129-base pair (bp) DC-SCRIPT transcript covering exon 4 to 5 (forward primer: 5′-AAAGTCAAGCATGGAGTG-CATG-3′; reverse primer: 5′-GCTTCTGAGAGAGGT-CAAAAG-3′), a commercially available Taqman Gene Expression Assay from Applied Biosystems (Nieuwkerk aan de IJssel, The Netherlands) covering exon 3 to 4 and generating a 62-bp product was used (Hs00403536_m1, RefSeq NM_152625.1). DC-SCRIPT levels were readily detected with both assays, and data generated with these assays correlated significantly.
### Table 1 Associations of DC-SCRIPT with clinicopathological and biological factors

| Characteristic                      | Number of patients | Percentage | DC-SCRIPT<sup>b</sup> (reference-normalized), × 10<sup>−2</sup> |
|-------------------------------------|--------------------|------------|---------------------------------------------------------------|
| All patients                        | 1,505              | 100%       | 0.69                                                          |
| Age, years                          |                    |            |                                                               |
| ≤ 40                                | 192                | 13%        | 0.69                                                          |
| 41-55                               | 561                | 37%        | 0.70                                                          |
| 56-70                               | 498                | 33%        | 0.70                                                          |
| >70                                 | 254                | 17%        | 0.64                                                          |
|                                    |                    |            | P = 0.15<sup>c</sup>                                         |
| Menopausal status                   |                    |            |                                                               |
| Premenopausal                       | 637                | 42%        | 0.72                                                          |
| Postmenopausal                      | 868                | 58%        | 0.66                                                          |
|                                    |                    |            | P = 0.06<sup>d</sup>                                         |
| Grade                               |                    |            |                                                               |
| Poor                                | 818                | 54%        | 0.64                                                          |
| Unknown                             | 452                | 30%        | 0.71                                                          |
| Moderate and good                   | 235                | 16%        | 0.80                                                          |
|                                    |                    |            | P = 0.001<sup>e</sup>                                         |
| Tumor size                          |                    |            |                                                               |
| pT1, ≤ 2 cm                         | 517                | 34%        | 0.81                                                          |
| >2 cm                               | 988                | 66%        | 0.63                                                          |
|                                    |                    |            | P < 0.001<sup>f</sup>                                         |
| Lymph nodes involved                |                    |            |                                                               |
| No                                  | 837                | 56%        | 0.69                                                          |
| Yes                                 | 668                | 44%        | 0.68                                                          |
|                                    |                    |            | P = 0.64<sup>d</sup>                                         |
| ESR1 mRNA status<sup>g</sup>        |                    |            |                                                               |
| Positive, ≥0.2                      | 1,176              | 78%        | 0.71                                                          |
| Negative, < 0.2                     | 329                | 22%        | 0.61                                                          |
|                                    |                    |            | P = 0.004<sup>d</sup>                                         |
| PGR mRNA status<sup>g</sup>         |                    |            |                                                               |
| Positive, ≥0.1                      | 949                | 63%        | 0.72                                                          |
| Negative, < 0.1                     | 556                | 37%        | 0.61                                                          |
|                                    |                    |            | P < 0.001<sup>c</sup>                                         |
| ESR2 mRNA status<sup>g</sup>        |                    |            |                                                               |
| Dichotomized high, ≥0.005           | 741                | 49%        | 0.89                                                          |
| Dichotomized low, < 0.005           | 742                | 49%        | 0.54                                                          |
|                                    |                    |            | P < 0.001<sup>c</sup>                                         |
| Invasive tumor cell content<sup>h</sup> |              |            |                                                               |
| ≥70%                                | 719                | 48%        | 0.57                                                          |
| < 70%                               | 786                | 52%        | 0.85                                                          |
|                                    |                    |            | P < 0.001<sup>d</sup>                                         |
| Histological type                   |                    |            |                                                               |
| DCIS + IDC                          | 194                | 13%        | 0.82                                                          |
| ILC                                 | 135                | 9%         | 0.81                                                          |
| IDC                                 | 810                | 54%        | 0.66                                                          |
| Mucinous                            | 40                 | 3%         | 0.56                                                          |
| Medullary                           | 31                 | 2%         | 0.69                                                          |
|                                    |                    |            | 1.18                                                          |
|                                    |                    |            | P = 0.012<sup>d</sup>                                         |
| Intrinsic breast cancer subtype<sup>h</sup> |      |            |                                                               |
| Normal-like                         | 22                 | 7%         | 1.43                                                          |
| ERBB2+                              | 63                 | 20%        | 0.75                                                          |
| Luminal A                           | 76                 | 25%        | 0.78                                                          |
|                                    |                    |            | 0.89                                                          |

<sup>a</sup> Percentage calculated as percentage of the total number of patients.

<sup>b</sup> DC-SCRIPT normalized to reference values.

<sup>c</sup> P-values for age and menopausal status.

<sup>d</sup> P-values for grade, tumor size, ESR1, PGR, ESR2, invasive tumor cell content, and intrinsic breast cancer subtype.

<sup>e</sup> P-value for lymph node involvement.

<sup>f</sup> P-values for ESR1 and PGR mRNA status.

<sup>g</sup> P-values for ESR2 mRNA status.

<sup>h</sup> P-value for histological type.
Table 1 Associations of DC-SCRIPT with clinicopathological and biological factors (Continued)

| Luminal B | Basal |
|----------|-------|
| 65       | 82    |
| 21%      | 27%   |
| 0.56     | 0.48  |
| 0.36     | 0.48  |

(Owing to missing cases, numbers do not always add up to 100%).

Results

Associations of DC-SCRIPT with clinicopathological factors and histological and intrinsic breast cancer subtypes

In analogy with our previous study, DC-SCRIPT mRNA expression was readily detected by quantitative RT-PCR in five normal breast tissues taken adjacent from tumor tissue and five prophylactic breast tissues (median [interquartile]: 0.063 [0.015] and 0.054 [0.035], respectively), whereas median levels were over 8-fold lower (P < 0.05) in 1,505 invasive breast tumors (0.0069 [0.0074]). Table 1 shows the median expression levels and interquartile ranges of DC-SCRIPT transcripts and relation with patient and tumor characteristics for these 1,505 patients who were evaluable for prognosis. DC-SCRIPT levels were positively associated with tumor grade and ESR1, PGR, and ESR2 steroid hormone receptor expression level and negatively associated with invasive epithelial tumor cell content and tumor size. In addition, ESR2 was more highly expressed in tumors with a higher percentage of stromal cells (786 tumors with 30% to 70% invasive epithelial cells), and ESR1 was...
more highly expressed in tumors with a high percentage of invasive epithelial cells (719 tumors with at least 70% invasive epithelial cells) \( (P < 0.001) \) (data not shown). High levels of DC-SCRIPT were found in breast tumors with a ductal carcinoma \textit{in situ} (DCIS) component or infiltrating lobular carcinoma compared with infiltrating ductal carcinomas \( (both \ P < 0.01) \). Of 308 LNN tumors, intrinsic subtyping data were available \[20\]. In these tumors, basal-like tumors had the lowest levels and normal-like breast tumors expressed significantly higher levels of DC-SCRIPT compared with the other intrinsic subtypes \( (P < 0.001; \text{Figure S1 in Additional file 2}) \).

Furthermore, luminal A tumors expressed higher levels of DC-SCRIPT and ESR2 but lower levels of ESR1 compared with luminal B tumors \( (median levels in luminal A versus luminal B: 0.0078 and 0.056 for DC-SCRIPT \[P = 0.003\], 0.0095 and 0.0023 for ESR2 \[P < 0.001\], and 6.1 and 13.6 for ESR1 \[P < 0.001\]) \). This may be explained at least partly by the fact that, in this cohort of 308 LNN tumors, the luminal B tumors contained a higher percentage of invasive epithelial cells \( (mean \pm \text{standard deviation [SD]}: 77\% \pm 9\% \text{for the } n = 64 \text{luminal B tumors versus 67\% \pm 12\% for the } n = 71 \text{luminal A tumors}) \).

**DC-SCRIPT and tumor aggressiveness in univariate and multivariable analyses**

In the analyses including all 1,505 M0 patients, increasing levels of DC-SCRIPT mRNA were significantly associated with favorable DFS, MFS, and OS \( (HR 0.78, 0.74, \text{and 0.77, respectively; all } P < 0.001) \). To test for a relation between DC-SCRIPT mRNA levels and tumor aggressiveness \( (that \ is, \ the \ natural \ course \ of \ the \ disease \ without \ the \ confounding \ effect \ of \ systemic \ adjuvant \ therapy) \), we restricted our next analyses of MFS to those 837 LNN disease patients who had not received \( \text{(neo)} \)adjuvant systemic therapy. The significant relationships of DC-SCRIPT as a continuous variable in these univariate analyses justified the use of the previously identified cut point that dichotomized the cohort in 33.3\% of the patients with low levels and 66.7\% of patients with high levels of DC-SCRIPT mRNA in their primary tumors \( [7]\). In univariate analysis, high levels of DC-SCRIPT were significantly associated with a favorable prognosis \( (HR 0.55; P < 0.001) \) \( (\text{Table 2}) \). When added to a multivariable base model for LNN disease - which included the traditional prognostic factors of age, menopausal status, grade, and PGR - stratified by ESR1 and tumor size to meet the proportional hazards assumption, the association of DC-SCRIPT with MFS remained highly significant \( (HR 0.60; P < 0.001) \) \( (\text{Table 2}) \). Adding ESR2 to the model did not significantly affect the prognostic value of DC-SCRIPT in these analyses \( (\text{Table 2}) \).

Because the proportional hazards assumptions were violated by ESR1 and tumor size and because DC-SCRIPT is a transcriptional co-regulator of nuclear receptors - including the, for breast cancer biologically relevant, steroid hormone receptors - we next explored its prognostic value as continuous variable in subgroups of tumors stratified by steroid hormone receptor status and tumor size \( (\text{Table 3 and Figure 1}) \). Subdividing the 837 primary LNN tumors into ESR1-positive and -negative \[14\] showed that increasing levels of DC-SCRIPT were, in univariate and multivariable analyses, associated with good prognosis only for the patients with ESR1-positive tumors. Subdividing these LNN tumors at the median level of ESR2 into high and low revealed that, in contrast to ESR1, increasing levels of DC-SCRIPT were, in both univariate and multivariable analyses, associated with good prognosis only for patients with primary tumors with low levels of ESR2. With respect to tumor size, in univariate and multivariable analyses, increasing levels of DC-SCRIPT were associated with good prognosis only for pT1 \( (\text{small tumor without lymphatic/vascular invasion}) \) tumors and not for larger tumors. These and additional exploratory Cox univariate analyses are summarized in Table 3. The prognostic value of DC-SCRIPT is visualized in Kaplan-Meier curves \( (\text{Figure 1}) \) as a dichotomized variable in these biologically relevant LNN ESR1-negative \( (\text{Figure 1a and -positive (Figure 1b}) \) and LNN ESR2-high \( (\text{Figure 1d and -low (Figure 1e}) \) subsets in combination with patients with pT1 primary tumors \( (\text{Figure 1c, f}) \).
breast cancers from Rotterdam. In addition, we confirm that DC-SCRIPT mRNA expression is a pure prognostic marker as it indicates - independently of current clinical prognostic markers such as age, menopausal status, grade, tumor size, and receptor status - the occurrence of distant metastasis in patients who did not receive any adjuvant systemic treatment. Because we used mRNA extracted from tumor tissue and a different mRNA isolation method (RNA-B versus column-based), an independent real-time PCR assay to detect DC-SCRIPT, a different type of machine to amplify the transcript, and personnel from another institute, we consider DC-SCRIPT a robust prognostic marker for patients with early breast cancer. The patients described in this

| Factor                                      | Univariate analysis | Multivariate analysisa |
|---------------------------------------------|---------------------|------------------------|
|                                            | Number | HR  | 95% CI | P value | HR  | 95% CI | P value |
| Age, years                                  | 837    |     |        |         |     |        |         |
| ≤ 40                                        | 114    | 1   |        | 1       | 1   |        | 1       |
| 41-55                                       | 295    | 0.88| 0.63  | 1.22    | 0.95| 0.67   | 1.35    |
| 56-70                                       | 270    | 0.72| 0.51  | 1.02    | 0.69| 0.40   | 1.20    |
| >70                                         | 158    | 0.53| 0.35  | 0.81    | < 0.01| 0.49 | 0.27   | 0.90    | 0.077  |
| Menopausal status                           |        |     |        |         |     |        |         |
| Premenopausal                               | 350    | 1   |        | 1       | 1   |        | 1       |
| Postmenopausal                              | 487    | 0.78| 0.62  | 0.97    | 0.028| 1.08 | 0.70   | 1.66    | 0.731  |
| Grade                                       |        |     |        |         |     |        |         |
| Poor                                        | 422    | 1   |        | 1       | 1   |        | 1       |
| Unknown                                     | 262    | 1.02| 0.79  | 1.30    | 1.12| 0.87   | 1.44    |
| Moderate and good                           | 153    | 0.49| 0.34  | 0.71    | < 0.001| 0.54 | 0.37   | 0.78    | < 0.001|
| PGR mRNA statusb                           |        |     |        |         |     |        |         |
| Negative, < 0.1                            | 312    | 1   |        | 1       | 1   |        | 1       |
| Positive, ≥0.1                              | 525    | 0.68| 0.54  | 0.85    | 0.001| 0.71 | 0.53   | 0.95    | 0.022  |
| Tumor size                                  |        |     |        |         |     |        |         |
| ≤ 2 cm                                      | 378    | 1   |        | 1       | 1   |        | 1       |
| >2 cm + unknown                             | 459    | 1.26| 1.00  | 1.59    | 0.047| Analyses stratified by tumor size to meet the proportional hazards assumption |
| ESR1 mRNA statusb                          |        |     |        |         |     |        |         |
| Negative, < 0.2                            | 199    | 1   |        | 1       | 1   |        | 1       |
| Positive, ≥0.2                              | 638    | 0.77| 0.59  | 0.99    | 0.040| 0.71 | 0.53   | 0.95    | 0.022  |
| Factor analyzed                             |        |     |        |         |     |        |         |
| DC-SCRIPT                                   |        |     |        |         |     |        |         |
| Continuous                                  | 837    | 0.77| 0.67  | 0.88    | < 0.001| 0.80 | 0.70   | 0.92    | 0.001  |
| 33.3% low                                   | 277    | 1   |        | 1       | 1   |        | 1       |
| 66.7% high                                  | 560    | 0.55| 0.43  | 0.69    | < 0.001| 0.60 | 0.47   | 0.76    | < 0.001|
| ESR2 mRNA statusb                          |        |     |        |         |     |        |         |
| Continuous                                  | 820    | 0.88| 0.79  | 0.99    | 0.034| 0.86 | 0.76   | 0.96    | 0.011  |
| Dichotomized low, < 0.005                   | 410    | 1   |        | 1       | 1.00| 0.73 | 0.59   | 0.94    | 0.014  |
| Dichotomized high, ≥0.005                   | 410    | 0.80| 0.63  | 1.00    | 0.052| 0.75 | 0.59   | 0.94    | 0.014  |
| DC-SCRIPT and ESR2 combined                 |        |     |        |         |     |        |         |
| Both low                                    | 183    | 1   |        | 1       | 1   |        | 1       |
| DC-SCRIPT low, ESR2 high                    | 91     | 0.74| 0.51  | 1.08    | 0.71| 0.49   | 1.04    |
| DC-SCRIPT high, ESR2 low                    | 227    | 0.49| 0.36  | 0.67    | 0.55| 0.40   | 0.76    |
| Both high                                   | 319    | 0.50| 0.38  | 0.67    | < 0.001| 0.52 | 0.39   | 0.69    | < 0.001|

aMultivariable analyses were conducted in two blocks. First, a model including all established clinicopathological factors was fitted. The Cox proportional hazards assumptions were checked and the analyses were stratified by tumor size and ESR1 to meet the proportional hazards assumption. In a second block, the contributions of DC-SCRIPT and ESR2 (as continuous or dichotomized variables) were investigated. bWith quantitative polymerase chain reaction cut point for positive versus negative ESR1 and PGR, 0.2 and 0.1, respectively, and for ESR2 at the median level of 0.005 (mRNA levels relative to reference gene set). CI, confidence interval; DC-SCRIPT, dendritic cell-specific transcript gene; ESR, estrogen receptor gene; HR, hazard ratio; PGR, progesterone receptor gene; pT1, small tumor without lymphatic/vascular invasion.
retrospective study entered the clinic during 1978 to 2000. During this period, adjuvant therapy was not as widespread as it is nowadays; this circumstance was at the same time the strength of our cohort for the evaluation of a prognostic marker. The data that emerged from this study thus validate the hypothesis that DC-SCRIPT is associated with good prognosis in early disease and support the idea that DC-SCRIPT acts as a tumor suppressor in breast cancer progression [7].

Because of the size of this cohort and the biological function of DC-SCRIPT as a nuclear receptor co-regulator, we were able to include additional subgroup analyses to extend our insights into the clinical behavior and relevance of measuring DC-SCRIPT in primary breast cancers. High levels of DC-SCRIPT mRNA in primary tumors of breast cancer patients were significantly related with tumor characteristics that are associated with good prognosis, such as DCIS, infiltrating lobular carcinoma, breast tumors of the normal-like and luminal A subtype, and small (pT1), well-differentiated, steroid hormone receptor-positive tumors. While ESR1 is localized mainly in tumors with at least 70% invasive epithelial cells (P < 0.001), we showed for both ESR2 and DC-SCRIPT a positive correlation with tumors with less than 70% invasive epithelial cells (P < 0.001). As normal epithelial cells in tumors with less than 70% invasive epithelial cells express the highest levels of DC-SCRIPT, they could be responsible for this correlation. Furthermore, infiltrating leukocytes in the stroma might have contributed to the detected signal [4,5]. Alternatively, or additionally, stromal cells may have played a role in the induction of DC-SCRIPT in the epithelial tumor cells. In analogy, ESR2 is - apart from breast cancer epithelial tumor cells - also expressed in adjacent infiltrating lymphocytes, fibroblasts, and endothelial cells [3].

Interestingly, in tumors that express relatively high ESR2 mRNA levels and that in general have a higher stromal content, DC-SCRIPT expression has little or no prognostic value. Thus, while in early ESR1-positive breast cancer DC-SCRIPT inhibits progression of breast cancer, this effect appears to be neutralized in tumors high in ESR2. Indeed, ESR2 has been reported to be dominant over ESR1 and able to counteract the proliferation-inducing activities of ESR1 [1,2]. Unraveling the precise role of DC-SCRIPT in the complex genomic and non-genomic interplay between ESR1, ESR2, and their isoforms [21-23] might turn out to be rewarding for elucidating the ‘yin-yang’ role of these factors in breast cancer.

As DC-SCRIPT can inhibit ERα and PR activity, a second aim of the study was to address whether DC-SCRIPT affects the response to endocrine therapy. In our previous study, we had already explored the value of DC-SCRIPT mRNA expression to indicate outcome in a cohort of breast cancer patients who received adjuvant tamoxifen [7]. However, in the adjuvant setting - that, for ethical reasons, nowadays includes only non-randomly assigned patients among treated and untreated

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Table 3 Disease-free survival, metastasis-free survival, and overall survival as a function of continuous DC-SCRIPT in lymph node-negative disease

| Association with continuous DC-SCRIPT | Cohort | Number | Disease-free survival | Metastasis-free survival | Overall survival |
|---------------------------------------|--------|--------|----------------------|-------------------------|-----------------|
|                                       |        |        | HR                   | 95% CI                  | P value         |
| Lymph node-negative                   | 837    | 0.82   | 0.73                 | 0.93                    | 0.001           |
| ESR1 mRNA-negativea                   | 199    | 0.94   | 0.76                 | 1.17                    | 0.59            |
| ESR1 mRNA-positivea                   | 638    | 0.79   | 0.68                 | 0.90                    | 0.001           |
| PGR mRNA-negativea                    | 312    | 0.88   | 0.74                 | 1.06                    | 0.19            |
| PGR mRNA-positivea                    | 525    | 0.81   | 0.69                 | 0.94                    | 0.007           |
| ESR2 mRNA-lowa                        | 410    | 0.76   | 0.64                 | 0.91                    | 0.003           |
| ESR2 mRNA-higha                       | 410    | 0.93   | 0.78                 | 1.11                    | 0.43            |
| Tumor size ≤ 2 cm (pT1)a               | 378    | 0.74   | 0.61                 | 0.89                    | 0.001           |
| Tumor size >2 cm                       | 459    | 0.92   | 0.79                 | 1.08                    | 0.31            |
| ESR1 mRNA-positive, tumor size ≤ 2 cm | 306    | 0.69   | 0.56                 | 0.85                    | 0.001           |
| ESR1 mRNA-positive, tumor size >2 cm  | 332    | 0.91   | 0.75                 | 1.10                    | 0.34            |
| ESR2 mRNA-low, tumor size ≤ 2 cm      | 175    | 0.57   | 0.43                 | 0.76                    | <0.001          |
| ESR2 mRNA-high, tumor size >2 cm      | 218    | 0.98   | 0.78                 | 1.23                    | 0.84            |
| ESR1-positive and ESR2-low, tumor size ≤ 2 cm | 147   | 0.63   | 0.45                 | 0.87                    | 0.005           |
| ESR1-positive and ESR2-low, tumor size >2 cm | 181   | 0.94   | 0.71                 | 1.24                    | 0.66            |
| ESR1-positive or ESR2-low or both, tumor size ≤ 2 cm | 334 | 0.65   | 0.53                 | 0.79                    | <0.001          |
| ESR1-positive or ESR2-low or both, tumor size >2 cm | 386 | 0.90   | 0.76                 | 1.08                    | 0.25            |

*With quantitative polymerase chain reaction cut point for positive versus negative ESR1 and PGR, 0.2 and 0.1, respectively, and for ESR2 at the median level of 0.005 (mRNA levels relative to reference gene set). Interaction with continuous DC-SCRIPT (P < 0.05). CI, confidence interval; DC-SCRIPT, dendritic cell-specific transcript gene; ESR, estrogen receptor gene; HR, hazard ratio; PGR, progesterone receptor gene; pT1, small tumor without lymphatic/vascular invasion.
arms - one cannot discriminate between tumor aggressiveness and response to treatment [24]. The current retrospective study included hormone-naïve patients (that is, not having received any [neo]adjuvant endocrine treatment) who received first-line tamoxifen treatment for their advanced disease and therefore was better suited to study a putative relation of DC-SCRIPT and response to therapy. Despite the positive association of DC-SCRIPT with ESR1, DC-SCRIPT levels were unable to identify patients with ESR1-positive primary tumors at high or low risk to progress if treated with tamoxifen. Thus, although DC-SCRIPT can modulate the activity of ESR1, it does not affect the response to endocrine therapy with tamoxifen in advanced breast cancer. The early loss of DC-SCRIPT during cancer progression might explain this absence of a response in the metastatic disease setting.

Conclusions
This independent retrospective quantitative RT-PCR study validates that high levels of DC-SCRIPT are associated with reduced tumor aggressiveness. The association is particularly strong for small tumors with high ESR1 or low ESR2 mRNA levels or both. Finally, although DC-SCRIPT negatively regulates ESR1 and PGR activity, DC-SCRIPT levels measured in the
primary tumors are not associated with response to first-line endocrine treatment for advanced disease. This finding is in line with DC-SCRIPT as an early marker for disease.

Additional material

Additional file 1: Supplementary materials and methods. A word file containing additional Materials and methods [25-28].

Additional file 2: Figure S1 - DC-SCRIPT mRNA expression in breast cancer subtypes. The box-plot shows the five statistics (lower whisker is 9% minimum, lower box part is 25% percentile, solid line in box presents the median, upper box part is 75% percentile and upper whisker is 99% maximum). Figure depicts P for Mann-Whitney U test to identify significantly different expression of DC-SCRIPT in between subtypes.

Abbreviations

B2M: beta-2-microglobulin gene; bp: base product; Ct: cycle threshold; DCIS: ductal carcinoma in situ; DC-SCRIPT: dendritic cell-specific transcript; DFS: disease-free survival; ER: estrogen receptor; ESR: estrogen receptor gene; HMBS: hydroxymethylbilane synthase gene; HPR1: hypoxanthine guanine phosphoribosyltransferase 1 gene; HR: hazard ratio; LNN: lymph node-negative; M0: no metastasis; M1: with metastasis; MFS: metastasis-free survival; OS: overall survival; PCR: polymerase chain reaction; PFS: progression-free survival; PGR: progesterone receptor gene; PPAR: peroxisome proliferator-activated receptor; pT1: small tumor without lymphatic/vascular invasion; PR: progesterone receptor; RAR: retinoic acid receptor; RT-PCR: reverse transcriptase polymerase chain reaction; SYBR: N’N’-dihydro-N(4-(E)-3-methyl-1,3-benzothiazol-2-ylidene)methyl[1-phenyl]quinolin-1-um-2-yl]-N-propylpropane-1,3-diamine.

Acknowledgements

We especially thank the patients and surgeons, pathologists, and interns for their assistance in collecting tumor tissues and patients’ clinical follow-up data. We thank Joan Bolt, Marion Meijer, Mieke Timmermans, Anita Trapman, and Wendy van der Smissem for their excellent technical support. This work was financially supported by VCI grant 918-66-615 (awarded to GJA) from the Netherlands Organization for Scientific Research (NWO).

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Authors’ contributions

AMS participated in the study design, collected laboratory data on the patients, performed laboratory work and statistical analyses, and wrote the manuscript. MA participated in the study design, performed laboratory work, and provided critical revision of the manuscript. MPL collected laboratory data on the patients, performed the clinical statistical analyses, and provided critical revision of the manuscript. PNS provided critical revision of the manuscript and participated in the study design. VdW and AVG performed the laboratory work and provided critical revision of the manuscript. JAF and JVMM participated in the study design, provided the study material and clinical information, and provided critical revision of the manuscript. GJA participated in the study design and provided critical revision of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 31 August 2010 Revised: 12 November 2010 Accepted: 1 December 2010 Published: 1 December 2010

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doi:10.1186/bcr2786
Cite this article as: Siewuerts et al: Clinical significance of the nuclear receptor co-regulator DC-SCRIPT in breast cancer: an independent retrospective validation study. Breast Cancer Research 2010 12:R103.

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