DC-SCRIPT: Nuclear Receptor Modulation and Prognostic Significance in Primary Breast Cancer


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Background

Nuclear receptors, including estrogen receptor (ER), progesterone receptor (PR)-B, peroxisome proliferator–activated receptor gamma, and retinoic acid receptor alpha, have been implicated in breast cancer etiology and progression. We investigated the role of dendritic cell–specific transcript (DC-SCRIPT) as coregulator of these nuclear receptors and as a prognostic factor in breast cancer.

Methods

The effect of DC-SCRIPT on the transcriptional activity of nuclear receptors was assessed by luciferase reporter assays. DC-SCRIPT expression in normal and tumor tissue from breast cancer patients was analyzed by polymerase chain reaction and immunohistochemistry. The prognostic value of tumor DC-SCRIPT mRNA expression was assessed in three independent cohorts of breast cancer patients: a discovery group (n = 47) and a validation group (n = 97) (neither of which had received systemic adjuvant therapy) and in a tamoxifen-treated validation group (n = 68) by using a DC-SCRIPT to porphobilinogen deaminase transcript ratio cutoff of 0.15 determined in the discovery group. Univariate and multivariable Cox proportional hazards model analyses were performed. All statistical tests were two-sided.

Results

DC-SCRIPT suppressed ER- and PR-mediated transcription in a ligand-dependent fashion, whereas it enhanced the retinoic acid receptor alpha– and peroxisome proliferator–activated receptor gamma–mediated transcription. In breast tissue samples from nine patients, DC-SCRIPT mRNA was expressed at lower levels in the tumor than in the corresponding normal tissue (P = .010). Patients in the discovery group with high tumor DC-SCRIPT mRNA levels (66%) had a longer disease-free interval than those with a low DC-SCRIPT mRNA level (34%) (hazard ratio [HR] of recurrence for high vs low DC-SCRIPT level = 0.23, 95% confidence interval [CI] = 0.06 to 0.93, P = .039), which was confirmed in the validation group (HR of recurrence = 0.50, 95% CI = 0.26 to 0.95, P = .034). This prognostic value was confined to patients with ER- and/or PR-positive tumors ( discovery group: HR of recurrence = 0.16, 95% CI = 0.03 to 0.89, P = .030; validation group: HR of recurrence = 0.42, 95% CI = 0.19 to 0.91, P = .028) and was also observed in the second validation group (HR = 0.46, 95% CI = 0.22 to 0.97, P = .040). DC-SCRIPT was an independent prognostic factor after correction for tumor size, lymph node status, and adjuvant therapy (n = 145; HR = 0.50, 95% CI = 0.29 to 0.85, P = .010).

Conclusion

DC-SCRIPT is a key regulator of nuclear receptor activity that has prognostic value in breast cancer.

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Nuclear receptors form a unique class of phylogenetically conserved transcriptional regulators (1). They execute a transcriptional program upon binding to a ligand. Ligands for the nuclear receptor family of proteins vary from hormones to vitamins and metabolic products. In accordance with the wide variety of ligands, nuclear receptors are key regulators in a diversity of physiological functions, including development, metabolism, cell differentiation, and immune responses (2). Malfunction of nuclear receptors has been associated with diseases, such as diabetes, chronic inflammatory diseases, and cancer (2–4).

Clinically, a connection between hormone-dependent nuclear receptor function and breast cancer development has long been recognized. Most research has focused on the expression and function of two nuclear receptors, estrogen receptor (ER)–α and progesterone receptor (PR), which are preserved or increased in approximately 70% of breast tumors (5). Estrogens, the ligands for the ER, are often associated with the initiation and progression of breast cancer (6) and are well known for their proliferative effect on breast cancer cells (7–9). Antiestrogen therapy with tamoxifen has been applied successfully in the treatment of breast cancer patients (5). In line with the importance of the ER and PR in breast cancer, the expression of transcriptional coregulators of ER and PR is also of prognostic significance in breast cancer (10,11). For example, the genes encoding nuclear receptor coactivator 3 (NCOA3; also
known as AIB1) and nuclear receptor corepressor 2 (NCOR2; also known as SMRT) have been shown to serve as a tumor suppressor gene and an oncogene for breast cancer, respectively (12–17).

Another class of nuclear receptors, the retinoid X receptor alpha (RXRα) heterodimers (ie, RXRα–retinoic acid receptor alpha [RARα] and RXRα–peroxisome proliferator–activated receptor gamma [PPARγ]), has recently been implicated in breast cancer. For example, whereas the presence of ER and PR is associated with breast tumor development and breast tumor cell proliferation, RARα and PPARγ play a predominantly antitumorigenic role in human breast cancer by inhibiting cell growth and inducing apoptosis (7,18). These properties imply that an imbalance in the activity of nuclear receptors may contribute to the development and progression of breast cancer. How the activity of the nuclear receptor repertoire in cells is regulated and which factors determine the response of the nuclear receptor repertoire to environments in which multiple nuclear receptor ligands are present are still open questions.

We previously identified and characterized a novel protein, dendritic cell–specific transcript (DC-SCRIPT; also known as ZNF366), that is preferentially expressed by dendritic cells in the immune system (19,20). DC-SCRIPT contains an amino-terminal proline-rich domain, 11 Cys-His-type zinc fingers, and a carboxyl-terminal acidic region. The acidic region of DC-SCRIPT contains a functional binding motif for the corepressor protein CtBP (19,21) and an LXXLL nuclear receptor interaction motif, which is thought to be involved in ER function (22). Herein, we investigate the effect of DC-SCRIPT on the function of multiple members of the nuclear receptor family as well as the prognostic relevance of DC-SCRIPT expression in breast cancer patients.

Materials and Methods

Cells

Human embryonic kidney 293 (HEK293) cells were cultured in Dulbecco’s modified Eagle medium containing GlutaMAX (Invitrogen, Breda, the Netherlands) supplemented with 10% heat-inactivated fetal calf serum (FCS; Greiner Bio-one, Alphen a/d Rijn, the Netherlands), 1% nonessential amino acids (Invitrogen), and 0.5% antibiotic–antimycotic (Invitrogen). Human hepatocellular carcinoma Hep3B cells were cultured in Iscove’s modified Dulbecco’s medium (Invitrogen), 10% heat-inactivated FCS, and 0.5% antibiotic–antimycotic. Human breast adenocarcinoma MCF-7 cells were cultured in RPMI-1640 medium (Invitrogen), 10% heat-inactivated FCS, and 0.5% antibiotic–antimycotic. Human breast adenocarcinoma Drosophila Line 2 (SL2) cells were grown in Schneider drosophila medium (Invitrogen) and 10% heat-inactivated FCS. All cell lines were originally obtained from American Type Culture Collection (Manassas, VA). Cells were regularly checked morphologically to verify the identity of each cell line.

Coimmunoprecipitation Assay

The nuclear receptors (National Center for Biotechnology Information accession number) ERα (NM_000125), PR-B (NM_000926), RARα (NM_000964), RXRα (NM_002957), and PPARγ (NM_138712) were each cloned into the expression vector pHA-n1 to enable expression of fusion proteins that contain a carboxyl-terminal hemagglutinin (HA) epitope tag. pHA-n1 was generated by replacing the green fluorescent protein (GFP) DNA sequence from the plasmid pEGFP-n3 (Clontech, Mountain View, CA) with the HA DNA sequence. The DNA for DC-SCRIPT (NM_152625) was cloned into the mammalian expression vector pEYFP (Clontech) to enable expression of a DC-SCRIPT fusion protein tagged with enhanced yellow fluorescent protein (YFP). HEK293 cells were plated in 10-cm dishes (6 x 10⁶ cells per dish) and incubated for 24 hours. The cells were cotransfected with 5 µg pDC-SCRIPT-EYFP or pEYFP (control) and 5 µg of a vector expressing HA-tagged ERα, PR-B, RARα, or PPARγ by using Metafectene transfection reagent (Biontex, Martinsried/Planegg, Germany) according to the manufacturer’s protocol. The cells were stimulated 8 hours after transfection with the following ligands (all from Sigma, Zwijndrecht, the Netherlands): 10 nM β-estradiol for cells expressing HA-tagged ER, 1 µM all-trans-retinoic acid (AtRA) for cells expressing HA-tagged RAR, 1 µM GW1929 (a PPARγ ligand) for cells expressing HA-tagged PPARγ, or the PR ligand 10 nM R5020 (PerkinElmer, Groningen, the Netherlands) for cells expressing HA-tagged PR-B. For all ligands, a 1000-times

Context and Caveats

Prior knowledge

An imbalance in the transcriptional activities of estrogen receptor, progesterone receptor-B, peroxisome proliferator–activated receptor gamma, and retinoic acid receptor alpha may contribute to the development and progression of breast cancer, but how the activities of these nuclear receptors are regulated is unclear.

Study design

The role of the novel transcriptional repressor dendritic cell–specific transcript (DC-SCRIPT) as a coregulator of estrogen receptor, progesterone receptor-B, peroxisome proliferator–activated receptor gamma, and retinoic acid receptor alpha in human cancer cells was assessed by luciferase reporter assays. DC-SCRIPT expression in normal and tumor tissue from breast cancer patients was analyzed by polymerase chain reaction assays and immunohistochemistry, and the prognostic value of tumor DC-SCRIPT mRNA expression was assessed in three independent cohorts of breast cancer patients.

Contribution

In vitro, DC-SCRIPT suppressed estrogen receptor– and progesterone receptor–mediated transcription in a ligand-dependent fashion, whereas it enhanced retinoic acid receptor alpha– and peroxisome proliferator–activated receptor gamma–mediated transcription. Breast tumors expressed lower levels of DC-SCRIPT than normal breast tissue from the same patient. Quantification of DC-SCRIPT mRNA expression in three cohorts of breast cancer patients revealed that DC-SCRIPT mRNA expression is an independent prognostic factor for breast cancer patients with estrogen receptor– and/or progesterone receptor–positive tumors.

Implications

DC-SCRIPT is a key regulator of nuclear receptor activity that has prognostic value in breast cancer.

Limitations

The clinical conclusions about DC-SCRIPT mRNA expression as a prognostic marker in breast cancer were based on nonrandomized retrospective analyses and could not be independently validated.

From the Editors
stock dilution in ethanol was used. Twenty-four hours after transfection, the cells were lysed in radioimmunoprecipitation assay buffer (50 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 50 mM Tris–HCl [pH 7.5], 5 mM EDTA, and 0.5% sodium-deoxycholate) containing the protease inhibitors 2 μg/mL leupeptin (Sigma, Zwijndrecht, the Netherlands), 2 μg/mL aprotonin (Roche, Woerden, the Netherlands), and 1 mM phenylmethylsulfonyl fluoride (Sigma, Zwijndrecht, the Netherlands). The cell lysates were used for immunoprecipitation of YFP-tagged proteins with anti-GFP antibody–coupled protein G beads (GE Healthcare, Zeist, the Netherlands) (this antibody recognizes YFP-tagged proteins). Cells transfected with YFP and HA-tagged ERα, PR-B, RARα, or PPARγ were used as controls for nonspecific binding. Immunoprecipitated proteins were mixed with sample buffer containing 5% glycerol, 6% sodium dodecyl sulfate, 125 mM Tris–HCl (pH 6.8), 0.1 mg/mL bromophenol blue (Gebr. Schmid GmbH + Co, Freudenstadt, Germany), and 10% β-mercaptoethanol (Sigma); heated at 95°C for 5 minutes; and then cooled on ice. The proteins were resolved by electrophoresis on an 8% polyacrylamide gel (ratio of acrylamide to bisacrylamide, 37.5:1) and transferred overnight to Protran nitrocellulose transfer membranes (Schleicher and Schuell, ‘s-Hertogenbosch, the Netherlands) at 30 mA and 4°C. To block nonspecific protein binding, the membranes were incubated in 2% skimmed milk powder (Campina, Eindhoven, the Netherlands) and 1% bovine serum albumin (Roche) in phosphate-buffered saline with 0.01% Tween (PBST) for detecting HA-tagged proteins or in 1% skimmed milk powder and 3% bovine serum albumin in PBST for detecting YFP-tagged proteins. The membranes were then incubated for 2 hours with a mouse anti-GFP antibody (1:1000 dilution; Roche Applied Science, Almere, the Netherlands), washed three times in PBST, and subsequently incubated for 1 hour with the secondary antibody IRDye 800CW goat anti-mouse IgG (1:5000 dilution; Li-cor Biosciences, Bad Homburg, Germany) to detect YFP-tagged proteins. To detect HA-tagged proteins, the membranes were incubated with a rat monoclonal anti-HA antibody (1:1000 dilution, clone 3F10; Roche), washed three times in PBST, and incubated for 1 hour with the secondary antibody Alexa Fluor 680–conjugated goat anti-rat IgG (1:5000 dilution; Invitrogen). All membranes were then washed three times in PBST. After staining the YFP- or HA-tagged proteins, the membranes were scanned by using an Odyssey Infrared Imaging System (Li-cor Biosciences) to visualize the YFP- or HA-tagged proteins.

Transcription Assays
The transcription reporter plasmids pTk-RARE3-luc, which contains three RAR response elements upstream of a firefly luciferase reporter, and pTk-luc were described previously (23). pAc5.1 (Invitrogen), which contains the Drosophila actin 5C (Ac5) promoter for high-level expression of the gene of interest in SL2 cells (24), was used to generate the following plasmids for expression in insect cells: pAc-RAR containing RARα, pAc-RXR containing RXRα, and pAc-DCSCRIPT containing DC-SCRIPT. The mammalian expression plasmids pCATCH and pCATCH-DCSCRIPT were described previously (19) and were used in the transcription assays in Hep3B and MCF-7 cells. MMTV-luc, a transcription reporter plasmid containing the mouse mammary tumor virus (MMTV) promoter, which is positively regulated by several classes of steroid hormones including ligands for the PR (25,26), was kindly provided by Prof Dr Stunnenberg (Department of Molecular Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, the Netherlands). MMTV-luc was used to generate MMTV-RLuc, a reporter plasmid in which Renilla luciferase expression is under the control of the MMTV promoter, which was used in the MCF-7 transcription assay. The transcription reporter PPRE-luc (Addgene plasmid number 1015) contains the PPAR response elements upstream of a firefly luciferase reporter and has been described previously (27). The PR expression plasmid pSG5-PR-B (28) was kindly provided by Prof Dr Horwitz (Department of Medicine/Endocrinology, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colorado) and was used in the transcription assays in Hep3B and MCF-7 cells. The transcription reporter ERE3-TATA-luc (Addgene plasmid number 11354), which contains three copies of the vitellogenin estrogen response element, has been described previously (29) and was used in the transcription assays in Hep3B cells.

Hep3B cells were plated in 24-well plates (6 × 104 cells per well) 8 hours before transfection, and all plasmids were transfected into these cells by using a calcium phosphate precipitation kit (Invitrogen) according to the manufacturer’s protocol. SL2 cells were plated in medium without FCS in 24-well plates (1 × 106 cells per well) just before transfection by using a calcium phosphate precipitation kit (Invitrogen). MCF-7 cells were plated in 24-well plates (5 × 104 cells per well) 8 hours before transfection, and all plasmids were transfected into these cells by the use of Metafectene reagent (Biontex). Twenty-four hours after transfection, Hep3B, MCF-7, and SL2 cells were stimulated 16 hours after transfection with the following ligands or vehicle (ethanol) for 16 hours. Transfected Hep3B and MCF-7 cells were stimulated 16 hours after transfection with the following ligands or vehicle (ethanol) for 24 hours: the ER ligand β-estradiol (10 nM; Sigma, Zwijndrecht, the Netherlands), the PR ligands progesterone (100 nM; Sigma, Zwijndrecht, the Netherlands) or R5020 (10 nM; PerkinElmer), or the PPARγ ligands GW1929 (1 μM; Sigma, Zwijndrecht, the Netherlands) or troglitazone (10 μM; Sigma, Zwijndrecht, the Netherlands). Forty hours after transfection, the cells were lysed in 100 μL passive lysis buffer (Promega, Leiden, the Netherlands), and the lysates were analyzed for luminescence with the use of the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol and a Victor 3 luminometer (PerkinElmer). Relative light units were calculated after correction for transfection efficiency based on the activity of a cotransfected reporter plasmid encoding Renilla luciferase under the control of the SV40 promoter (pRL–SV40; Promega). The data are expressed as the mean relative luciferase activity of at least three independent experiments with 95% confidence intervals (CIs).

Dissociation and Purification of Epithelial Cells From Breast Biopsy Samples
Normal breast tissue located distally from the tumor was obtained during surgical resection of the primary tumor at the Radboud University Nijmegen Medical Centre (RUNMC) from six anonymous breast tumor patients and was macrodissected within 12 hours of surgical removal and dissociated as previously described (30); tissue procurement was approved by the institutional ethics committee of the RUNMC. In short, the tissue was minced with a
scalpel and dissociated in 0.1% DNase (Roche) and 0.14% collagenase A (Roche) in RPMI-1640 medium by incubating the minced tissue three times for 45 minutes each time in fresh medium containing DNase and collagenase. The cell suspension was passed through a 30-µM MACS preseparation filter (Miltenyi Biotec, Utrecht, the Netherlands), washed to remove tissue debris, and stored overnight at 4°C. The cell suspension was incubated with allopheocyanin-conjugated anti-human CD326 antibody (Miltenyi Biotec), which recognizes the epithelial cell surface marker epithelial cell adhesion molecule followed by incubation with anti-allopheocyanin MACS beads (Miltenyi Biotec), and the CD326-positive and CD326-negative cells were fractionated by magnetic separation. The CD326-positive and CD326-negative cell fractions were lysed for RNA isolation and analyzed for the expression of the leukocyte marker CD45, the dendritic cell marker CD11c, the epithelial cell marker CD326, and DC-SCRIPT by means of quantitative polymerase chain reaction (PCR) analysis.

**cDNA Synthesis and Quantitative PCR Analysis**

For quantitative PCR analysis of DC-SCRIPT, PBGD, CD45, CD11c, and CD326 mRNA levels, total RNA was isolated from fresh normal breast tissue or cell lines with the use of Trizol reagent (Gibco BRL, Gaithersburg, MD) according to the manufacturer’s protocol. RNA was treated with DNase I (amplification grade; Invitrogen) and reverse-transcribed into cDNA by using random hexamers and Moloney murine leukemia virus reverse transcriptase (Invitrogen). mRNA levels for the genes of interest were determined with a PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) with SYBR Green (Applied Biosystems). We used reaction mixtures and program conditions that were recommended by the manufacturer (Applied Biosystems) as the fluorophore and the following oligonucleotide primers (forward, reverse): DC-SCRIPT (5′-AAGCATGGAGTCTATGGAG-3′, 5′-TTCTGAGAGGTTGAGAAGG-3′), PBGD (5′-GGCAATGCGGCTGCAA-3′, 5′-GGTTACCCCAGGAAATTCAC-3′), CD326 (5′-GTGTCTGTAAGTAAACTACAGTCTG-3′, 5′-AGCCATTCATTCTGCTCCTCATC-3′), CD45 (5′-ACCACAGTTTTTACTAGCAGTTGT-3′, 5′-TTGTTGAAGGGGATTTCAGGTAA-3′), and CD11c (5′-ATCCACTTCTTGGCTACCT-3′, 5′-TGAGGTATTGGTGATCCATT-3′). We used reaction mixtures and program conditions that were recommended by the manufacturer (Applied Biosystems).

Quantitative PCR data were analyzed with 7000 Systems SDS Software v1.2.3 (Applied Biosystems) and checked for correct amplification and dissociation of the products. mRNA levels of the genes of interest were normalized to mRNA levels of the housekeeping gene porphobilinogen deaminase (PBGD) (31) and were calculated according to the cycle threshold method (32).

**Immunohistochemistry**

Snap-frozen breast cancer specimens from eight anonymous breast cancer patients were obtained from the Rijnstate Hospital (Arnhem, the Netherlands; approved by the institutional ethics committee of the RUNMC) and embedded in OCT embedding matrix (CellPath, Newtown, UK) and sectioned (5-µm-thick tissue sections). The sections were placed on Superfrost slides (Thermo Scientific, Etten-Leur, the Netherlands), fixed with acetone, and incubated with 4 µg/mL goat anti-human DC-SCRIPT antibody (R&D Systems, Abingdon, UK), followed by incubation with a biotinylated horse anti-goat IgG (Vector Laboratories), and signal development was performed using a Vectorstain ABC-AP Kit (Vector Laboratories, Burlingame, CA) and fast red (Sigma). The epithelial cell surface marker epithelial cell adhesion molecule was detected with a mouse monoclonal anti-CD326 antibody (Miltenyi Biotec) and a biotinylated horse anti-mouse IgG (Vector Laboratories). Isotype-matched goat IgG (R&D Systems) and mouse IgG1 (BD Bioscience) were used as controls. Sections were counterstained with hematoxylin to visualize the cell nuclei and analyzed by using a Leica DM LB microscope (Leica Microsystems B.V., Rijswijk, the Netherlands).

**Patients**

Our use of coded tumor tissues in this study was performed in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands (http://www.federa.org/; a link to the English version is available at this site) and was approved by the institutional ethics committee of the RUNMC. All steps in tissue processing and marker assaying were performed by individuals who blinded to the clinical outcomes, and, as much as possible, in accordance with the guidelines for biomarker characterization described by Pepe et al. (33).

From nine anonymous patients who underwent surgery in the early 1990s, both breast tumor and distally located normal breast tissue were macrodissected and stored in liquid nitrogen until RNA isolation as described below. No other information on these patients or tissues is available.

Other tumor tissues were from patients who had operable unilateral breast cancer and had undergone resection of their primary tumor between November 1987 and December 1997. We included patients who had no previous carcinoma diagnosis, no distant metastases at diagnosis, and no evidence of disease within 1 month after primary surgery. We excluded patients who had received neoadjuvant systemic therapy or who were diagnosed with carcinoma in situ only.

The discovery group (n = 47) was selected from among a previously described cohort of patients (34) based on the availability of tumor RNA and consisted of stage pT1 or pT2 (n = 42) or lobular (n = 5) node-negative breast tumors that had received no adjuvant systemic therapy. The tumors were collected, snap-frozen, and embedded in OCT compound (Tissue-Tek) as soon as possible after surgical resection (breast-conserving lumpectomy: n = 34; modified radical mastectomy: n = 13) at the Rijnstate Hospital. Tumors in the validation (n = 97) and tamoxifen-treated (n = 68) groups were also selected based on tumor RNA availability and receipt of adjuvant treatment (no adjuvant systemic treatment [validation group] or adjuvant systemic treatment with tamoxifen) from among a different cohort of patients that was also described earlier (36). The tumors from this cohort of patients were obtained from a tumor bank in the Department of Chemical Endocrinology (RUNMC) that contains frozen tumor tissue collected from breast cancer patients who were treated at the nine hospitals that form the Comprehensive Cancer Center East in the Netherlands. The tumor tissues were collected by these hospitals for the central measurement of ER and PR levels by ligand-binding assay. Patients were defined as ER and/or PR positive (level of either or both
receptors $\geq$10 fmol/mg protein) or as ER and PR negative (levels of both receptors $<10$ fmol/mg protein).

All patients were reviewed once every 3 months during the first 2 years after surgery once every 6 months for the next 5 years, and once a year thereafter by means of a medical history, physical examination, and routine laboratory investigations. Each patient received once yearly x-ray mammography, and for those with suspicious results, magnetic resonance imaging of the breast.

Tumor Tissue RNA Isolation

Aliquots of frozen tissue were pulverized using a microdismembrator (Braun, Melsungen, Germany), and the resulting tissue powder was stored in liquid nitrogen. Total RNA was isolated from 20 mg of tissue powder with the use of an RNeasy Mini Kit (Qiagen, Hilden, Germany) with on-column DNase I treatment. Aliquots of RNA were stored at $-80^\circ$C. RNA quality was verified by examining the ribosomal RNA bands after agarose gel electrophoresis of each RNA sample and by PCR amplifying PBGD mRNA. RNA concentrations were determined from the spectrophotometric absorption at 260 nm by using a Genequant spectrophotometer (Amersham, Eindhoven, the Netherlands). We observed no effect of storage time in liquid nitrogen on the quality of the RNA.

Statistical Analyses

Statistical analyses were carried out using SPSS software (version 16.0; SPSS, Chicago, IL). The normality of distributions was tested by the method of Kolmogorov–Smirnov (37). A normal distribution for DC-SCRIPT mRNA expression values was achieved after taking the $\log$ of each value. Differences in the proportions of clinicopathological characteristics among the three patient groups were assessed with Pearson $\chi^2$ tests. Differences in PBGD-normalized DC-SCRIPT mRNA expression levels between normal breast tissue and tumor tissue from the same patient were assessed using paired $t$ tests, and between tumor tissues and cell lines by unpaired Student $t$ tests. Differences in DC-SCRIPT mRNA levels for variables with more than two groups were assessed by analysis of variance. Because data on histological grading were missing for a substantial number of patients, patients with missing data were included in all analyses as a separate group. The disease-free interval (defined as the time from surgery until the diagnosis of recurrent disease) was used as follow-up endpoint. Contralateral breast cancer or second malignancies were not considered to be recurrent disease. Overall survival was not evaluated because data on breast cancer-specific causes of death were difficult to retrieve from the patient records, and thus, the number of events was too small for reliable statistical analysis. Survival curves were generated by using the Kaplan–Meier method. Cox proportional hazards modeling was used to assess the prognostic value of DC-SCRIPT mRNA expression after dichotomization of the patient group. The proportional hazard assumption was confirmed by examination of the Schoenfeld residuals. An optimal cut point was selected in a discovery group at the lowest possible $P$ value at such cut point, thus representing the best dichotomization of the patient group. The cut point was selected by multiple testing and validated in the two other independent patient cohorts. Multivariable Cox proportional hazards modeling was then used to assess the independent prognostic value of DC-SCRIPT mRNA expression in the combined validation and tamoxifen-treated patient cohorts. All clinicopathological variables were entered into the model. Subsequently, variables that did not contribute to the model ($P > .1$) according to the likelihood ratio statistic were removed in a stepwise fashion until only statistically significant variables remained in the model. Interactions between DC-SCRIPT mRNA expression and ER and PR status, and between DC-SCRIPT mRNA expression and tamoxifen treatment were assessed by entering an interaction variable into the model. Two-sided $P$ values less than .05 were a priori considered to be statistically significant.

Results

Interaction Between DC-SCRIPT and Multiple Nuclear Receptors

We performed coimmunoprecipitation experiments to examine whether DC-SCRIPT interacts with the steroid nuclear receptors ER$\alpha$ and PR-B or with the RXR heterodimers RAR$\alpha$ and PPAR$\gamma$. For this experiment, whole-cell lysates were prepared from HEK293 cells that had been cotransfected with expression vectors encoding YFP-tagged DC-SCRIPT or YFP- (control) and HA-tagged nuclear receptors ER$\alpha$, PR-B, RAR$\alpha$, or PPAR$\gamma$. YFP–DC-SCRIPT was immunoprecipitated with anti-GFP antibody–coupled beads, and the immunoprecipitated fraction was subjected to immunoblotting with an anti-GFP antibody. Equal expression of the HA-tagged nuclear receptors was demonstrated by immunoblotting of total lysates of the transfected cells with an anti-HA antibody (Figure 1, top panels). YFP–DC-SCRIPT and the control protein YFP were both effectively immunoprecipitated by the anti-GFP antibody–coupled beads (Figure 1). In
addition to the intact YFP–DC-SCRIPT protein, some additional protein bands were also observed that correspond to YFP–DC-SCRIPT breakdown products (Figure 1 and data not shown). Interestingly, in cells transfected with YFP–DC-SCRIPT, the HA-tagged nuclear receptor ERα was efficiently coimmunoprecipitated by the anti-GFP antibody–coupled beads (Figure 1, lane 2). HA-tagged PR-B (lane 4), RARα (lane 6), and PPARγ (lane 8) were also specifically coimmunoprecipitated with YFP–DC-SCRIPT but not with the control protein YFP. We repeated these coimmunoprecipitation experiments using the same stringent lysis conditions in the presence or absence of the ligand and observed no substantial effect on the efficiency of the coimmunoprecipitation experiments (data not shown). Additional yeast two-hybrid experiments showed no direct interaction between DC-SCRIPT and RAR, RXR, or ER (Supplementary Figure 1, available online). These coimmunoprecipitation data combined with the yeast two-hybrid results demonstrate that DC-SCRIPT is present in a protein complex with either of these nuclear receptors and imply that these interactions are indirect and ligand-independent.

**Effect of DC-SCRIPT on ER- and PR-B–Mediated Transcription**

Next, we used luciferase reporter assays to assess the effect of DC-SCRIPT on the transcriptional activity of the steroid-induced nuclear receptors ER and PR. Hep3B cells were transfected with a reporter construct containing response elements for ER (ERE3-TATA-luc) (29) and stimulated with the ER ligand estradiol. In the absence of exogenous ER, very little luciferase was produced by transfected cells that were exposed to estradiol (Figure 2, A, left). By contrast, Hep3B cells that were cotransfected with a mammalian expression vector encoding ER displayed luciferase activity in an estradiol-dependent manner (Figure 2, A, right). Introduction of increasing amounts of an expression vector encoding DC-SCRIPT into cells expressing exogenous ER revealed a dose-dependent repression of estradiol-dependent ER-mediated luciferase activity (Figure 2, A, right). These data are in agreement with previous findings that demonstrated that DC-SCRIPT can repress expression levels of endogenous targets of ER (ie, cathepsin D and pS2) (22). Similarly, DC-SCRIPT strongly repressed the hormone-induced (ie, progesterone and R5020) transcription mediated by PR-B on the MMTV promoter in a dose-dependent manner (Figure 2, B). These data indicate that DC-SCRIPT specifically represses transcription mediated by the steroid receptors ER and PR-B in a dose- and hormone ligand–dependent manner.

**Effect of DC-SCRIPT on RARα/RXRα- and PPARγ/RXRα- Mediated Transcription**

To examine the effect of DC-SCRIPT on transcription mediated by two members of the RXR subclass of nuclear receptors, the RXR heterodimers with RARα and PPARγ, we performed additional luciferase reporter assays in Hep3B cells. Hep3B cells transfected with the luciferase reporter construct ptk-RARE3-luc (23), which contains three response elements for the heterodimer RARα/RXRα, exhibited luciferase activity after stimulation with the RARα/RXRα ligand AtRA (Figure 3, A). Strikingly, coexpression of increasing amounts of DC-SCRIPT resulted in a dose-dependent increase in luciferase activity upon addition of AtRA (Figure 3, A), which is in contrast to the repressive effect of DC-SCRIPT on the transcriptional activity of the steroid receptors. To unequivocally demonstrate that the observed increase in luciferase activity was mediated by RARα/RXRα, we repeated the experiment in insect SL2 cells, which do not express RARα or RXRα (38) (Figure 3, B). DC-SCRIPT activated AtRA-dependent transcription in a dose-dependent fashion in SL2 cells only when RAR/RXR was coexpressed. In addition, removal of the RARα/ RXRα response elements in the reporter construct completely
abolished luciferase production in both Hep3B and SL2 cells (data not shown). We further demonstrated that DC-SCRIPT enhanced the transcriptional activity of PPARγ/RXRα in Hep3B cells. Hep3B cells transfected with a reporter construct containing the response elements for PPARγ/RXRα (PPRE-luc (27)) failed to display luciferase activity when exposed to the PPARγ ligands GW1929 and troglitazone. However, coexpression of PPARγ/RXRα in these cells resulted in ligand-dependent induction of luciferase activity that was enhanced by DC-SCRIPT in a dose-dependent manner (Figure 3, C). These data indicate that in contrast to the repressive effect of DC-SCRIPT on the transcriptional activity of the steroid receptors ER and PR-B, DC-SCRIPT enhances transcription mediated by the RXR heterodimers RARα and PPARγ.

**Effect of DC-SCRIPT on Nuclear Receptor–Induced Transcription in Breast Carcinoma MCF-7 Cells**

Our finding that DC-SCRIPT can repress transcription mediated by the steroid receptors ER and PR-B, whereas it can enhance the transcriptional activity of the RXR heterodimers RARα and PPARγ, implies that DC-SCRIPT may be a key regulator that balances the cell’s response to multiple nuclear receptor ligands, such as vitamins, fatty acids, and hormones. To corroborate these findings, we examined the effect of DC-SCRIPT on nuclear receptor–induced transcription in human breast carcinoma MCF-7 cells, which endogenously express both PR and RARα. Therefore, MCF-7 cells were cotransfected with MMTV-Rluc, a progesterone-inducible reporter construct that drives the expression of Renilla luciferase, and ptk-RARE3-luc, an AtRA-inducible reporter construct that drives the expression of firefly luciferase. Control experiments showed that both types of luciferase are induced in cells that are exposed to either the corresponding specific nuclear receptor ligand (PR ligand: R5020 and RARα ligand: AtRA) or both nuclear receptor ligands compared with MCF-7 cells treated with vehicle (Figure 4, A and B). In addition, the effect of DC-SCRIPT on the activity of these endogenously expressed nuclear receptors was in agreement with our findings in transfected Hep3B cells (Figures 2, B and 3, A). Interestingly, coexpression of DC-SCRIPT together with both the AtRA-inducible reporter and the progesterone-inducible reporter enhanced RARα/RXRα-mediated transcription (white bars) and concurrently repressed the activity of PR (black bars) (Figure 4, C). We further found that DC-SCRIPT overexpression inhibited MCF-7 cell growth, as did treatment of MCF-7 cells with the known ER antagonist tamoxifen (Supplementary Figure 2, available online). These data demonstrate that the presence of DC-SCRIPT can simultaneously modulate the activity of endogenously expressed PR and RARα/RXRα in MCF-7 cells and affects MCF-7 cell growth.
DC-SCRIPT Expression in Breast Epithelial Cells

Among immune cells, DC-SCRIPT expression appears to be restricted to the dendritic cell lineage (19). Less is known about DC-SCRIPT expression in nonimmune cells. Because DC-SCRIPT regulates transcription mediated by multiple nuclear receptors that play an important role in breast cancer and affects growth of the breast carcinoma MCF-7 cells, we examined DC-SCRIPT mRNA levels in normal breast tissue and corresponding breast tumor tissue from nine patients. DC-SCRIPT mRNA expression was readily detected in normal breast tissue by quantitative PCR. Statistically significantly lower DC-SCRIPT mRNA levels were present in the corresponding breast tumor samples ($P = .010$ [paired $t$ test]; Figure 5, A). DC-SCRIPT mRNA was essentially undetectable in more than 50 different cell lines analyzed, including a panel of 16 breast tumor cell lines (data not shown).

To identify which cells in the breast tissue samples expressed DC-SCRIPT mRNA, total cells isolated from fresh normal breast tissue biopsy samples were separated into an epithelial cell–positive and an epithelial cell–depleted fraction by magnetic bead sorting using the epithelial cell marker epithelial cell adhesion molecule (CD326). Quantitative PCR analysis of these samples showed that, as expected, DC-SCRIPT mRNA was present in the CD326-depleted leukocyte-containing fraction, as were the mRNAs encoding the dendritic cell–specific marker CD11c and the leukocyte marker CD45 (Figure 5, B). Importantly, DC-SCRIPT mRNA was also detected in the CD326-positive epithelial cell fraction. The absence of CD11c mRNA in the CD326-positive fraction indicates that epithelial cells and not contaminating dendritic cells were the cells that expressed DC-SCRIPT mRNA.

Immunohistochemistry on frozen breast tumor sections confirmed that morphologically normal and malignant CD326-positive ductal epithelial cells express DC-SCRIPT protein (Figure 5, C and D). In concordance with the PCR data (Figure 5, A), a wide range of DC-SCRIPT protein expression levels was observed in the breast tumor biopsy samples (data not shown).

Prognostic Significance of DC-SCRIPT mRNA Expression in Breast Cancer

Next, we explored the prognostic value of DC-SCRIPT mRNA expression in three cohorts of breast cancer patients. Characteristics of these patient cohorts and tumor tissues used in this study are described in Table 1. Most patients (70%–80%) in the untreated patient (ie, the discovery and validation) cohorts were postmenopausal at the time of primary surgery, whereas all patients who were treated with tamoxifen were postmenopausal ($P < .001$). The discovery group consisted only of pT1 or pT2 tumors without axillary nodal involvement, whereas both the validation and the tamoxifen cohorts consisted of larger tumors (8%–16% were pT3; $P < .001$) and included patients with positive lymph nodes.
The discovery group mostly (72%) underwent breast-conserving lumpectomy, whereas most patients in both the nontreated and tamoxifen-treated cohorts underwent modified radical mastectomy (69%–70%, \( P < .001 \)). Patients treated with endocrine therapy received tamoxifen 40 mg twice daily for at least 2 years. The three study groups did not differ in the relative size of their subgroups as defined by DC-SCRIPT expression (\( \chi^2 P = .543 \)) (Table 1).

First, we measured the DC-SCRIPT mRNA levels in primary breast tumors from a cohort of patients who had not received systemic adjuvant treatment (the discovery group; \( n = 47 \)). The DC-SCRIPT mRNA level in this patient group was not associated with any of the clinicopathological parameters [ie, menopausal status, tumor type, grade (39), tumor size, lymph node status, hormone receptor status, or type of surgery or therapy] or with the percentage of tumor cells in the biopsy sample (data not shown). We then analyzed the prognostic value of DC-SCRIPT mRNA level in the discovery group after dichotomization of the patients according to an optimal DC-SCRIPT to PBGD transcript ratio cutoff of 0.15, which resulted in 16 (34%) patients with low levels of DC-SCRIPT mRNA and 31 (66%) patients with high levels of DC-SCRIPT mRNA in their primary tumors. In a Kaplan–Meier survival analysis, these two groups of patients differed statistically significantly with respect to disease-free interval: those with a high DC-SCRIPT mRNA level had a statistically significantly longer disease-free interval than those with a low DC-SCRIPT mRNA level (validation vs tamoxifen: 17% vs 74%, \( P < .001 \)).

**Figure 5.** Dendritic cell–specific transcript (DC-SCRIPT) expression in breast epithelial cells. A) DC-SCRIPT mRNA expression in paired tissue samples. DC-SCRIPT mRNA levels in healthy tissues (black bars) and corresponding breast tumor (white bars) tissue (\( n = 9 \) patients) relative to PBGD mRNA as determined by quantitative polymerase chain reaction. B) mRNA expression in CD326-positive and CD326-negative breast cells. Expression of CD326, CD45, CD11c, and DC-SCRIPT mRNA in CD326-positive (white bars) and CD326-negative (black bars) cells purified from breast tissue relative to PBGD mRNA as determined by quantitative polymerase chain reaction. Data from one of the two DC-SCRIPT–positive patients out of six are shown. Data are expressed as the mean of at least three independent quantitative PCR assays; error bars correspond to 95% confidence intervals. C and D) DC-SCRIPT and CD326 protein expression in breast tumor sections. Epithelial cell adhesion molecule was stained with an anti-CD326 (red), DC-SCRIPT with anti-DC-SCRIPT (red), or matched isotypes (red) as control staining on frozen breast tumor sections as detected by immunohistochemistry staining. Nuclei are counterstained with hematoxylin (blue). Magnification is indicated by size bars (C) 0.1 \( \mu \)m and (D) 1 \( \mu \)m in lower left corner. Representative sections are shown.
level (hazard ratio [HR] of recurrence = 0.23, 95% CI = 0.06 to 0.93, \( P = .039 \)) (Figure 6, A).

To validate this finding, we analyzed DC-SCRIPT expression in an independent cohort of patients who also had not received adjuvant systemic treatment (the validation group; \( n = 97 \)). Overall, the validation group included more patients with advanced disease than the discovery group (eg, pT3 tumors: 8% vs 0%; axillary nodal involvement: 17% vs 0%) (Table 1). Nevertheless, when we applied the same cutoff, the 64 patients (66%) with a high tumor DC-SCRIPT mRNA level had statistically significantly better prognosis than the 33 patients (34%) with low tumor DC-SCRIPT mRNA level (HR of recurrence = 0.50, 95% CI = 0.26 to 0.95, \( P = .034 \)) (Figure 6, B).

Because DC-SCRIPT represses the activity of both ER and PR, we explored the prognostic value of DC-SCRIPT in relation to the ER and PR status of the primary tumor. We found that the tumor DC-SCRIPT mRNA level had statistically significant prognostic value for patients in the discovery and validation groups with ER- and/or PR-positive tumors but not for those with ER- and PR-negative tumors (discovery group, patients with ER- and/or PR-positive tumors: HR of recurrence = 0.16, 95% CI = 0.03 to 0.89, \( P = .030 \); discovery group, patients with ER- and PR-negative tumors: HR of recurrence = 0.73, 95% CI = 0.07 to 8.07, \( P = .797 \); validation group, patients with ER- and PR-negative tumors: HR of recurrence = 0.42, 95% CI = 0.19 to 0.91, \( P = .028 \) [Figure 6, C]; validation group, patients with ER- and PR-positive tumors: HR of recurrence = 0.21, 95% CI = 2.22, \( P = .519 \) [Figure 6, D]).

We next assessed the prognostic value of DC-SCRIPT expression and tumor ER and PR status in a third cohort of patients who received antiestrogen therapy with tamoxifen (\( n = 68 \)). Strikingly, when we applied the same cutoff that was established in the

<table>
<thead>
<tr>
<th>Table 1. Patient and tumor characteristics by study group*</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>Postmenopausal</td>
</tr>
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</tr>
<tr>
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<tr>
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<tr>
<td>Tumor stage§ (size in mm)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>DC-SCRIPT mRNA level</td>
</tr>
<tr>
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<tr>
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</table>

* DC-SCRIPT = dendritic cell–specific transcript; ER = estrogen receptor; PR = progesterone receptor.
† \( \chi^2 \) test (two-sided).
‡ Bloom–Richardson grade (39).
§ American Joint Committee on Cancer staging (35).
‖ DC-SCRIPT/PBGD ratio higher or lower than 0.15, the optimal prognostic cut point in the discovery group.
Figure 6. Dendritic cell–specific transcript (DC-SCRIPT) expression as a prognostic marker in estrogen receptor (ER)– and progesterone receptor (PR)–positive breast cancer. A) Kaplan–Meier analysis of disease-free interval according to DC-SCRIPT mRNA expression in tumors from breast cancer patients who did not have axillary lymph node metastases and did not receive systemic adjuvant therapy (discovery group: }

(continued)
Table 2. Univariate and multivariable Cox proportional hazards modeling of factors associated with disease-free survival in the combined validation and tamoxifen-treated patient groups (n = 165)*

<table>
<thead>
<tr>
<th>Factor and comparison</th>
<th>Univariate analysis</th>
<th>Multivariable analysis</th>
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<td>HR of recurrence (95% CI)</td>
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<td>Postmenopausal vs premenopausal</td>
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<td>Lobular vs ductal</td>
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<tr>
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<tr>
<td>2 vs 1</td>
<td>0.94 (0.21 to 4.19)</td>
<td>.022</td>
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<tr>
<td>3 vs 1</td>
<td>2.29 (0.55 to 9.57)</td>
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<td>Tumor stage§</td>
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<td>pT2 vs pT1</td>
<td>1.73 (0.96 to 3.13)</td>
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<tr>
<td>pT3 vs pT1</td>
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<tr>
<td>No. of positive lymph nodes</td>
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<td>1–3 vs 0</td>
<td>1.63 (0.89 to 2.97)</td>
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<td>&gt;4 vs 0</td>
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<td>1.93 (1.09 to 3.43)</td>
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<td>Radiotherapy (yes vs no)</td>
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</tr>
<tr>
<td>Systemic adjuvant therapy (yes vs no)</td>
<td>1.14 (0.71 to 1.84)</td>
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<tr>
<td>DC-SCRIPT mRNA level (high vs low)</td>
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<tr>
<td>With systemic adjuvant therapy</td>
<td>0.83 (0.49 to 1.39)</td>
<td>.470</td>
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<tr>
<td>With ER and PR status</td>
<td>0.56 (0.34 to 0.91)</td>
<td>.017</td>
</tr>
</tbody>
</table>

*  DC-SCRIPT = dendritic cell–specific transcript; HR = hazard ratio; ER = estrogen receptor; PR = progesterone receptor; NA = not applicable.
†  Cox proportional hazards.
‡  Bloom–Richardson Grade (39).
§  American Joint Committee on Cancer staging (35).
||  DC-SCRIPT/PBGD ratio higher or lower than 0.15, the optimal prognostic cut point in the discovery group.

Table 2 summary: Disease-free survival analysis with univariate and multivariable Cox proportional hazards modeling. The factors evaluated include postmenopausal status, tumor type (lobular vs ductal, other vs unknown vs ductal), tumor grade (2 vs 1, 3 vs 1), tumor stage (pT2 vs pT1, pT3 vs pT1), number of positive lymph nodes, ER/PR positive vs ER/PR negative, mastectomy vs lumpectomy, radiotherapy (yes vs no), systemic adjuvant therapy (yes vs no), DC-SCRIPT mRNA level (high vs low), and systemic adjuvant therapy. The analysis reveals statistically significant associations for several factors, including positive lymph nodes, tumor stage, and DC-SCRIPT mRNA level, with disease-free survival.

Figure 6 (continued).

n = 47. B) Kaplan–Meier analysis of disease-free interval according to DC-SCRIPT mRNA expression in tumors from an independent validation group that included patients with larger tumors than those in the discovery group and with axillary lymph node metastases (n = 97). C) Kaplan–Meier analysis of disease-free interval according to DC-SCRIPT mRNA expression in tumors from patients in the validation group with ER- and PR-positive tumors (n = 65). D) Kaplan–Meier analysis of disease-free interval according to DC-SCRIPT mRNA expression in tumors from patients in the validation group with ER- and PR-negative tumors (n = 32). E) Kaplan–Meier analysis of disease-free interval according to DC-SCRIPT mRNA expression in tumors from patients treated with tamoxifen (n = 68). (A–E) Patients with high DC-SCRIPT expression were compared with patients with lower DC-SCRIPT expression. High DC-SCRIPT expression (dotted line) indicates expression above the optimal cutoff of DC-SCRIPT/PBGD transcript ratio of 0.15; low DC-SCRIPT expression (solid line) indicates expression below DC-SCRIPT/PBGD transcript ratio of 0.15. F) Kaplan–Meier analysis of disease-free interval according to tumor ER and PR status among patients treated with tamoxifen (n = 68). Dotted line corresponds to patients with ER- and PR-positive tumors; solid line corresponds to patients with ER- and PR-negative tumors. All P values are two-sided (Cox proportional hazards tests). Tick marks indicate censored events, and vertical lines indicate 95% confidence intervals. Numbers below graphs are the number of patients at risk at that time point.
tumor ER and PR status was \( (P = .017) \) (Table 2). These data indicate that the prognostic value of DC-SCRIPT mRNA level is independent of whether or not the patient received tamoxifen but does depend on whether or not the tumor expresses ER and PR.

**Discussion**

Here, we have identified DC-SCRIPT as a regulator of the activity of several subclasses of nuclear receptors and as a prognostic marker for ER- and/or PR-positive breast cancer. We have shown that expression of DC-SCRIPT in Hep3B cells and MCF-7 cells represses the hormone-induced activity of ER and PR while it concurrently enhances RAR\( /RXR\alpha \) and PPAR\( \gamma /RXR\)-mediated transcription. In addition, we showed that ductal epithelial cells express DC-SCRIPT mRNA, that breast tumors express lower levels of DC-SCRIPT than normal breast tissue from the same patient, and that breast tumor cell lines do not express DC-SCRIPT mRNA. Moreover, quantification of DC-SCRIPT mRNA expression in three cohorts of breast cancer patients revealed that DC-SCRIPT mRNA expression is an independent prognostic factor for breast cancer patients with ER- and/or PR-positive tumors.

To our knowledge, we have provided the first evidence for the presence of DC-SCRIPT in multiple nuclear receptor protein complexes based on data from coimmunoprecipitation experiments. Results of yeast two-hybrid experiments imply that DC-SCRIPT, unlike its direct binding to CtBP1 (19), does not bind directly to nuclear receptors. This finding suggests that the interaction between DC-SCRIPT and nuclear receptors is likely to be mediated by other nuclear receptor coregulators that are present in these large multiprotein complexes (14). Because DC-SCRIPT was previously shown to interact with multiple proteins known to be present in these protein complexes (19,22), it will be interesting to investigate the molecular mechanism by which DC-SCRIPT differentially modulates the activity of multiple classes of nuclear receptors.

Because malfunction of nuclear receptors and their coregulators has been associated with breast cancer (2–4), we evaluated the expression of DC-SCRIPT in breast tissue. We found that DC-SCRIPT is expressed by normal breast tissue, whereas less DC-SCRIPT mRNA could be detected in the corresponding breast tumor tissue. Combined with our finding that DC-SCRIPT inhibits cell growth of the breast carcinoma cell line MCF-7, these data suggest that DC-SCRIPT may act as a tumor suppressor in breast cancer development. Such a function for DC-SCRIPT is also consistent with our finding that DC-SCRIPT overexpression in Hep3B and MCF-7 cells inhibits the activity of ER and PR, which were previously shown (6) to exhibit proliferative and anti-apoptotic activities in breast cancer cells. In contrast, RAR\( \alpha /RXR\alpha \) and PPAR\( \gamma /RXR\), the transcriptional activities of which are enhanced by DC-SCRIPT, are reported to have antiproliferative and proapoptotic effects in breast cancer cells (40–42). Moreover, a recent report showed that expression of RAR\( \alpha /RXR\alpha \) target genes identified in MCF-7 cells predicted a positive clinical outcome in breast cancer patients (43). Our findings in MCF-7 cells indicate that DC-SCRIPT affects the balance in the activities of endogenous PR and RAR\( \alpha /RXR\alpha \) in favor of RAR\( \alpha /RXR\alpha \) activity when the breast cancer cells are simultaneously stimulated with the respective ligands. To directly demonstrate a tumor suppressor function for DC-SCRIPT and to assess the impact of DC-SCRIPT expression on transcription at a genome-wide level are very difficult because prolonged overexpression of DC-SCRIPT in all of the cell lines tested thus far resulted in growth inhibition and death of the DC-SCRIPT-expressing cells (data not shown). Moreover, none of the cell lines analyzed endogenously expressed DC-SCRIPT, which prevented us from performing the obvious knockdown experiments of endogenous DC-SCRIPT to further investigate its antiproliferative effect. It will therefore be important to define the conditions and factors that regulate DC-SCRIPT expression, from both a physiological and a therapeutic perspective.

To our knowledge, this is the first time that DC-SCRIPT mRNA expression has been identified as a prognostic marker in breast cancer. Over the years, molecular profiling has yielded genetic signatures for many solid tumors (44–49), including breast cancer (50); however, DC-SCRIPT was not present in these signatures. The absence of DC-SCRIPT from these signatures can be explained by the fact that on older microarrays used to determine breast cancer signatures, the DC-SCRIPT gene was not yet present and the relatively low DC-SCRIPT mRNA expression may have prevented its detection in more recent studies. Our discovery of DC-SCRIPT as an independent prognostic marker for breast cancer patients with ER- and/or PR-positive tumors was possible because we used a unique cohort of patients who were not treated with systemic adjuvant therapy and followed up for 10 years (34,36). Prognostic significance can only be ascertained in such patient groups (51,52), which are becoming increasingly scarce because currently even patients with negative axillary lymph nodes almost all receive systemic adjuvant therapy. Our finding that DC-SCRIPT has prognostic value independent of a specific treatment suggests that DC-SCRIPT may contribute to tumor growth characteristics and is consistent with the proposed tumor suppressor function of DC-SCRIPT. The potential clinical significance of DC-SCRIPT extends beyond untreated patients, given that we showed that high DC-SCRIPT expression, like ER and PR status, is also prognostic in tamoxifen-treated patients. In line with this finding is our demonstration that DC-SCRIPT inhibited the growth MCF-7 cells treated without tamoxifen or with tamoxifen to block ER function. These data imply that DC-SCRIPT expression may be used to select ER- and/or PR-positive patients who might be candidates for more aggressive adjuvant therapy.

This study has several limitations. First, we have not found independent validation of DC-SCRIPT mRNA expression as a prognostic marker in breast cancer in publicly available databases; the absence of the DC-SCRIPT gene on older microarrays and the relatively low abundance of DC-SCRIPT mRNA may have prevented its detection. Second, our clinical conclusions are therefore based on nonrandomized retrospective analyses. To attain a higher level of evidence, independent multicenter and/or randomized prospective studies of DC-SCRIPT expression analyses are necessary.

It will be extremely interesting to assess in future validating studies whether the RAR\( \alpha /RXR\alpha \) and/or PPAR\( \gamma \) status of the tumors is of relevance regarding the prognostic value of DC-SCRIPT. The presence of RAR\( \alpha /RXR\alpha \) target genes in the genetic signature of breast tumor samples predicts a positive clinical outcome in breast
cancer patients (43). Stimulation of the nuclear receptors RAR/RXR and PPARγ/RXR has been explored as a novel therapy for breast cancer (53,54). So far, these therapies have shown only limited success because of retinoic acid resistance (18,55). Intriguingly, our data demonstrate that DC-SCRIPT is able to simultaneously enhance the activities of RARα/RXRα and PPARγ/RXRα and repress the activities of EREs and PR-B. On the basis of these findings, we hypothesize that the antiproliferative effect of DC-SCRIPT in breast cancer cells is mediated by modulating the activity of multiple nuclear receptors. It will be interesting therefore to also examine DC-SCRIPT expression levels in clinical trials that have explored the effect of stimulation of the RAR/RXR and PPARγ/RXR on the clinical outcome in breast cancer patients.

References


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