Predictive Value of Syndecan-1 Expression for the Response to Neoadjuvant Chemotherapy of Primary Breast Cancer

MARTIN GÖTTE[1], CHRISTIAN KERSTING[2], MARIA RUGGIERO[1,4], JOKE TIO[1], AUGUSTINUS H. TULUSAN[3], LUDWIG KIESEL[1] and PIA WÜLFING[1]

Departments of [1]Obstetrics and Gynecology and [2]Pathology, Münster University Hospital, Münster; [3]Department of Obstetrics and Gynecology, Klinikum Bayreuth, Bayreuth, Germany; [4]Department of Reproductive Medicine and Child Development, University of Pisa, Italy

Abstract. *Background: The overexpression of syndecan-1 in breast carcinomas correlates with poorer prognosis and an aggressive phenotype. The effect of syndecan-1 expression on tumor response to neoadjuvant chemotherapy was determined in locally advanced breast cancer. Patients and Methods: Semi-quantitative syndecan-1 immunohistochemistry was performed in pre-chemotherapy breast cancer biopsies of 37 patients undergoing high-dose neoadjuvant treatment with cyclophosphamide and epirubicin. Results: 43.2% of breast carcinomas stained positive for syndecan-1. Syndecan-1 expression was more frequent in ductal invasive carcinomas than in other histological types (p=0.062). The pathological response to chemotherapy was decreased in syndecan-1-positive patients: 37.5% of syndecan-1-positive vs. 19% of syndecan-1-negative patients attained pathologically "no change". No syndecan-1-positive patient showed complete remission. Also, a correlation between syndecan-1 immunostaining intensity and response to chemotherapy was observed. Of the responding tumors, none showed strong syndecan-1 expression (Score 3+), whereas 20% of the non-responding tumors were strongly syndecan-1-positive. Conclusion: Syndecan-1-expressing breast carcinomas show a trend towards a decreased response to chemotherapy.*

The cell surface heparan sulfate proteoglycan syndecan-1 (SDC-1) is predominantly expressed by different epithelia and plays multiple roles in the regulation of cell migration, cell-cell and cell-matrix interactions, growth factor and chemokine activity, and in the modulation of protease activity (1, 2). These functions are reflected by changes in SDC-1 expression under several pathological conditions, including wound repair (3, 4), pathological angiogenesis (4, 5) and malignant diseases (6). Recently, SDC-1 has also emerged as a molecular marker for breast cancer (7-12). In the study by Barbareschi *et al.* (9), which included 254 breast carcinoma cases with long-term follow-up, a strong epithelial expression of SDC-1 was observed in 42% of the carcinomas. High expression levels of SDC-1 were associated with high histological grade, high mitotic count, large tumor size, c-erbB-2 overexpression and estrogen receptor (ER)- and progesterone receptor (PR)-negative status. The study by Leivonen *et al.* (10) involved 200 patients with a median follow-up of 17 years. Epithelial SDC-1 expression was associated with negative ER status, whereas stromal SDC-1 expression was associated with positive ER status. In contrast to Barbareschi *et al.*, no statistically significant association was found between SDC-1 expression and tumor size or PR status, respectively. Ten-year breast cancer-specific overall survival was significantly reduced both for patients displaying epithelial or stromal SDC-1 expression, respectively. Since the 10-year overall survival was reduced to an even greater extent in patients showing both epithelial and stromal SDC-1 expression, the authors proposed that this constellation may be a predictor of unfavourable prognosis in breast cancer (10). While several studies have assigned a prognostic value to altered SDC-1 expression in breast carcinomas (9, 10, 12), there is no study to date investigating the predictive value of SDC-1 expression in breast carcinomas on response to neoadjuvant chemotherapy. The neoadjuvant setting allows the evaluation of medical therapies prior to surgery and the destruction of residual or disseminated tumor cells, thus improving breast-conserving operability in locally advanced breast cancer (13, 14). Consequently, the clinical response to neoadjuvant chemotherapy has been shown to predict both disease-free and overall survival (15, 16). The identification of biological markers, which are predictors of a good response to neoadjuvant therapy, the assessment of not only

Correspondence to: Dr. Martin Götte, Department of Obstetrics and Gynecology, Münster University Hospital, Albert-Schweitzer-Str. 33, D-48129 Münster, Germany. Fax: ++49-251-8348267, e-mail: mgotte@uni-muenster.de

Key Words: Syndecan-1, heparan sulfate proteoglycan, chemoresistance, breast cancer.

allows survival benefits from a given treatment, but also facilitates risk-adapted individual therapy concepts and the development of pharmacological compounds targeting these markers. Thus, the objective of our study was to investigate the predictive value of SDC-1 expression for response to neoadjuvant chemotherapy in patients treated for locally advanced breast cancer.

Patients and Methods

Patients and treatment. The study population included 37 women with locally advanced breast cancer (T2-4, N0-2, M0), treated with epirubicin and cyclophosphamide neoadjuvant chemotherapy (17). The patients were enrolled at the Department of Obstetrics and Gynecology, Klinikum Bayreuth, Germany, between August 1997 and March 2002, and they were staged according to the International Union Against Cancer - Tumour Node Metastasis (UICC-TNM). The median age of the patients was 51 years (range 29-66). Table I lists their main clinicopathological features. In all women, the diagnosis was histologically confirmed by tru-cut biopsy before chemotherapy. Clinical examination, bilateral mammography, sonography of the breast and axillary region, chest X-ray, liver sonography and bone scintigraphy were performed to rule out metastatic disease at the time of diagnosis. The study was approved by the Ethics Committees of the University of Erlangen-Nürnberg, Germany, prior to patient recruitment and each woman gave written informed consent.

Eligible patients, having a histologically-proven breast cancer with advanced disease and no evidence of metastases or inflammatory breast cancer, were randomly assigned to one of 2 groups (arm A and arm B) to receive different neoadjuvant chemotherapy schedules.

Eighteen out of 37 patients were included in treatment arm A, which consisted of 3 cycles of epirubicin (120 mg/m^2, Pharmacia, Erlangen, Germany) and cyclophosphamide (600 mg/m^2, Baxter, Frankfurt, Germany) followed by 5 μg/kg·day GM-CSF (Amgen, München, Germany) *s.c.* or *i.v.* between days 2-12. The cycles were repeated every 2 weeks. The remaining 19 patients were included in treatment arm B, which consisted of 3 cycles of epirubicin (120 mg/m^2) and cyclophosphamide (600 mg/m^2) given every 3 weeks. In this arm, supportive GM-CSF was only given if leukocytes were ≤2000/μl. Two to 3 weeks after completion of the third chemotherapy cycle, all patients underwent surgical therapy; patients showing a clinical response to chemotherapy (n=33) received breast-conserving surgery and irradiation of the residual breast at a dose of 60 Gy in 6 weeks. In 8 of these patients, reconstruction of the breast was performed. Patients showing no regression of the tumor (n=4) underwent a modified radical mastectomy.

Assessment of response. To monitor clinical response, the tumor and axillary lymph node size were assessed after each treatment cycle by palpation using a calliper. Changes in the calculated product of the bi-dimensional tumor measurements at diagnosis and on completion of chemotherapy were recorded. Standard UICC criteria (18) were used to classify the clinical response to chemotherapy: complete response (CR) was defined as the absence of any residual clinical signs of disease, partial response (PR) as a greater than 50% tumor shrinkage, no change (NC) as <50% decrease or <25% increase in tumor size, and progressive disease

Table I. *Patient and tumor characteristics at the time of diagnosis (prior to neoadjuvant chemotherapy).*

No. of patients	37
Age (years)	
Median (range)	51 (29 – 66)
Menopausal status	
Premenopausal	11 (29.7%)
Postmenopausal	26 (70.3%)
Tumor size	
T2	32 (88.9%)
T3	3 (8.3%)
T4	1 (2.8%)
Unknown	1
Lymph node status	
N0	20 (55.6%)
N1	16 (44.4%)
Unknown	1
Grading*	
I	0
II	10 (33.3%)
III	20 (66.7%)
Unknown	7
Histology	
Ductal	25 (67.6%)
Lobular	9 (24.3%)
Other	3 (8.1%)
Estrogen receptor	
Negative	7 (20%)
Positive	28 (80%)
Unknown	2
Progesterone receptor	
Negative	10 (28.6%)
Positive	25 (71.4%)
Unknown	2
Her-2/neu	
Negative	24 (70.6%)
Positive	10 (29.4%)
Unknown	3
Ki-67	
≤18%	13 (38.2%)
>18%	21 (61.8%)
Unknown	3
p53	
Normal	23 (67.6%)
Mutation	11 (32.4%)
Unknown	3

*Grading of surgical resection specimens postchemotherapy; not applicable in seven patients.

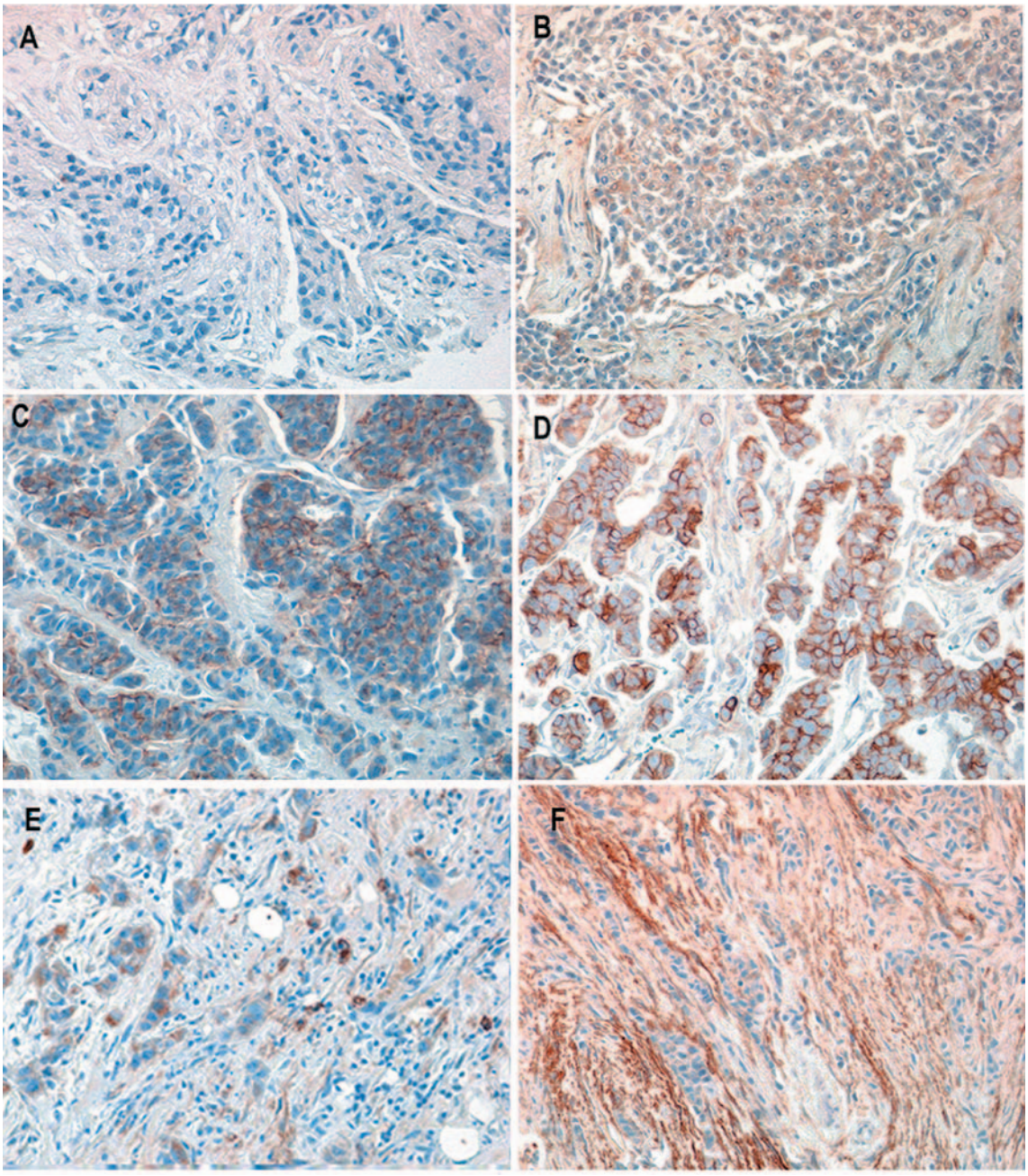

Figure 1. *Representative immunohistochemical staining patterns for syndecan-1. A) 0, B) 1+, C) 2+, D) 3+, E) strong cytoplasmic staining, F) strong stromal staining.*

(PD) as a 25% increase in tumor size or as the appearance of new lesions, respectively. Additionally, the course of tumor size was evaluated by sonography and mammography.

The pathological tumor response was graded according to Sinn *et al.* (19): grade 4, no microscopic evidence of residual tumor cells in all resected specimens of the breast; grade 3, only residual, non-invasive tumor; grade 2, focal invasive tumor ≤5 mm; grade 1, resorption and tumor sclerosis; grade 0, no effect. The final pathological response was designated as follows: grade 4: CR; grade 1-3: PR; grade 0: NC. Pathological non-responders were postoperatively treated with 12 cycles of 5-FU (2 g/m^2, weekly) and 4 cycles of Taxol (Bristol-Meyers-Squibb, München, Germany) (125 mg/m^2, every 3 weeks) (20). Patients with complete or partial response to neoadjuvant chemotherapy were subjected to 1 additional cycle of high-dose epirubicin and cyclophosphamide followed by 2 cycles of cyclophosphamide-, methotrexate-, 5-fluorouracil (CMF) (methotrexate: Medac, Hamburg, Germany; 5-fluorouracil: GRY-Pharma, Kirchzarten, Germany).

Table II. *Immunohistochemical analysis of SDC-1 expression in human breast carcinomas (n=37); epithelial staining.*

Score	No. (%)
0	21 (56.8)
1+	6 (16.2)
2+	8 (21.6)
3+	2 (5.4)
negative	21 (56.8)
positive	16 (43.2)
stromal+*	12 (33.3)

*additional stromal staining was scored independently of epithelial staining.

Table III. *SDC-1 expression stratified for clinical response to neoadjuvant chemotherapy.*

	CR	PR	NC	No.
SDC-1 *				
0	4 (19.0%)	14 (66.7%)	3 (14.3%)	21
1	2 (13.3%)	13 (86.7%)	0	15

*$p=0.249$ (CR *vs.* PR *vs.* NC).

All tumor specimens were obtained at the time of mastectomy or breast-conserving therapy. The tumor tissue was fixed in formalin and embedded in paraffin; 3-μm hematoxylin-eosin-stained slides were finally obtained and reviewed by a pathologist. Tumor size, classified according to the pT system, was measured macroscopically on stained sections containing the largest tumor and, in some samples, the size was estimated on the basis of a sequential slide series. The TNM classification (21) was applied, and tumor grade was classified based on UICC criteria as follows: grade I: well-differentiated, grade II: moderately-differentiated, and grade III: poorly-differentiated. Each sample was independently assessed by 2 experienced pathologists unaware of the clinical outcome of the patients.

Immunohistochemical analysis. The tru-cut/core biopsy taken at the first clinic attendance for diagnostic purposes was used as pre-random assignment tumor sample. All tissue samples were fixed in formalin (standard formaldehyde fixation) immediately after removal, followed by embedding in paraffin for sectioning and subsequent analysis of biological markers.

Estrogen receptor (ER) and progesterone receptor (PgR) status, Her-2/neu, p53 and Ki-67 were assessed by immunohistochemistry, as described previously (22). ER, PgR and the p53 mutation were assessed to be positive when the percentage of reacting cells was higher than 10%. Her-2/neu was scored according to the Herceptest (DakoCytomation, Glostrup, Denmark) system. A high proliferative activity was assigned to samples containing more than 18% of tumor cell nuclei immunoreactive for the Ki-67 antigen.

Immunostaining of pre-chemotherapy biopsies for SDC-1 was performed as follows: 3-μm sections of paraffin-embedded breast cancer samples were mounted on slides coated with poly-L-lysine, followed by dewaxing and rehydrating through a graded ethanol series. For antigen retrieval, the sections were boiled in Reveal buffer (BioCarta, Hamburg, Germany) in a pressure cooker (103 kPa/15 psi, 5 min). After blocking of non-specific binding sites, the sections were incubated with the mouse anti-human SDC-1 primary antibody BB4 (Serotec, Düsseldorf, Germany) diluted 1:100 in PBS/1% BSA, overnight at 4˚C. Endogenous peroxidase was quenched with methanol / 0.6% H_2O_2 and the slides were washed 3 times with Tris-buffered saline. Bound primary antibody was visualised using DAKO Mouse-EnVision-HRP and the NovaRed substrate (Vector Laboratories, Burlingame, CA, USA). Positive controls were sections of epithelial (membranous and cytoplasmic) SDC-1 staining of non-malignant tissue. Negative controls were obtained by omitting the primary antibodies. Counterstaining was performed using hematoxylin. Positive staining for SDC-1 presented as predominantly membraneous or cytoplasmic staining, respectively (Figure 1). The immunostaining intensity was categorized semi-quantitatively into 4 groups, as described previously (23): negative (score 0): no staining; weakly-positive (score 1+): faint/barely perceptible staining in the majority of tumor cells; moderately-positive (score 2+): moderate staining in the majority of tumor cells; and strongly-positive (score 3+): strong staining of the majority of tumor cells. A score of 0 was deemed as a negative absolute score, while a score of 1-3 was considered to be a positive absolute score. Strong stromal immunostaining was scored independently (Figure 1, Table II).

Statistical analysis. After evaluation of immunohistological staining in a blinded fashion, statistical analyses were carried out using the SPSS 10.0 statistical software. The χ-square test was used to test associations between SDC-1 expression in tumors and response to neoadjuvant chemotherapy. To analyze the predictive value of SDC-1 expression for clinical and pathological response, datasets were divided into the following categories: "response" [complete response (CR) + partial response (PR)] and "non-response" [no change (NC)]. The significance level was $p \leq 0.05$.

Results

Immunohistochemical SDC-1 expression. Immunolabeling for SDC-1 presented as cytoplasmic or membranous staining, respectively. In accordance with previous observations (9, 10), the intensity of SDC-1 staining among different tumors varied from complete absence of staining to strong diffuse staining. Moderate or strong staining intensity, defined as "positive" immunoreaction, was present for SDC-1 in 43.2% in evaluable breast carcinomas (Figure 1). Additional strong stromal immunostaining was frequently detected in 33.3% of all cases (Table II).

Response to neoadjuvant chemotherapy – treatment activity. All 37 investigated patients completed the treatment according to the study protocol. The clinical response could not be determined in 1 patient. At the end of the chemotherapy administration, 6 patients (16.7%) attained

Table IV. *SDC-1 expression stratified for pathological response to neoadjuvant chemotherapy.*

	Pathological response				
	No.	CR*	PR	NC	*p*
SDC-1					
negative (score 0)	21	1 (100%)	16/26 (61.5%)	4/10 (40%)	
positive (score 1-3)	16		10/26 (38.5%)	6/10 (60%)	0.211

*CR = complete response; PR= partial response; NC= no change. *p* = response (CR+PR) *vs.* non-response (NC) (χ^2 test).

clinical CR (cCR) and 27 patients (75.0%) attained clinical PR (cPR), for an overall clinical response rate of 91.7% (Table III). Three patients (8.3%) showed clinically NC (cNC), and no patient progressed. Information on the pathological response was available for all patients (Table IV). One patient (2.7%) was found to have a pathological CR (pCR) and 26 patients (70.3%) had a pathological PR (pPR). Thus, the overall pathological response rate was 73%. Ten patients (27%) showed pathological NC (pNC).

Relationship between expression of SDC-1 and response to chemotherapy. The pathological response to chemotherapy was decreased in SDC-1-positive patients (Table IV). Pathological NC was obtained in 37.5% of SDC-1-positive patients as compared to 19% of SDC1-negative patients. None of the SDC-1-positive patients had a pCR. Also, with respect to staining intensity, a correlation between the SDC-1 immunoreaction and response to chemotherapy was observed. None of the responding tumors showed strong SDC-1 expression (Score 3+), whereas 20% of the non-responding tumors were strongly SDC-1-positive (Table V). However, regarding the stromal staining pattern, a different relationship was observed: those patients with stromal SDC-1 expression tended to respond better to chemotherapy than those without stromal staining (n.s.; $p=0.414$; Table VI). No correlation was observed between SDC-1 expression and the clinical response.

Correlation between expression of SDC-1 and tumor biological factors. Besides an association of SDC-1 expression with ductal carcinomas ($p=0.062$), no further correlations with tumor characteristics, such as tumor size, lymph node involvement, histological grading or hormone receptor status, were observed (Table VII). Both steroid hormone receptors correlated positively with each other ($p<0.001$), and the ER- and PgR-statuses were (inversely) correlated with the proliferation rate as assessed by Ki-67.

Table V. *Relationship between the degree of SDC-1 expression and pathological response.*

	Response	
	0 n=10	1 n=27
SDC-1 grading		
0	4 (40%)	17 (63%)
1	3 (30%)	4 (14.8%)
2	1 (10%)	6 (22.2%)
3	2 (20%)	–

$p=0.056$

Table VI. *Relationship between stromal syndecan-1 expression and pathological response.*

	Stromal SDC-1 expression	
	negative (n=24)	positive (n=12)
no response	7/24 (29.2%)	2/12 (16.7%)
response	17/24 (70.8%)	10/12 (83.3%)

Table VII. *Relationship between syndecan-1 expression, histology, ER* and PgR* status.*

	SDC-1-negative	SDC-1-positive	*p*
Histology	n=21	n=16	0.062
ductal	11 (52.4%)	14 (87.5%)	
lobular	7 (33.3%)	2 (12.5%)	
other	3 (14.3%)	–	
Estrogen receptor	n=21	n=14	0.863
negative	4 (19%)	3 (21.4%)	
positive	17 (81%)	11 (78.6%)	
Progesterone receptor	n=21	n=14	0.445
negative	5 (23.8%)	5 (35.7%)	
positive	16 (76.2%)	9 (64.3%)	

* preoperative determination of ER and PgR receptor status

Treatment arms. Statistical analysis, stratified for both treatment arms, showed no differences between arm A and arm B concerning the distribution of histopathological characteristics, expression of SDC-1 and response rates to chemotherapy.

Discussion

SDC-1 expression is frequently altered in human carcinomas, but the role of SDC-1 in tumor growth is still controversial.

Some carcinomas down-regulate this receptor whereas others display variable or up-regulated expression profiles (6, 11, 24). Concerning human breast carcinomas, recent reports show SDC-1 overexpression in more than one-third of the evaluated tissues, with high expression correlating to the most invasive tumors (9, 12, 24). In the present study, the question of whether SDC-1 expression in advanced breast carcinomas is correlated with clinicopathological markers or response to neoadjuvant chemotherapy was analyzed.

In a recently published study, Leivonen *et al.* (10) evaluated clinicopathological markers in a large series of human breast carcinomas, searching for a correlation with SDC-1 expression. No statistically significant association between stromal SDC-1 expression and age, tumor size, axillary nodal status, histological grade, ploidy, S-phase fraction or PR status was found. Our analysis of the breast carcinomas prior to chemotherapy confirm these findings, showing no significant correlation between the expression patterns of SDC-1 with tumor size, lymph node involvement or histological grading. Of note, our analysis confirmed a relationship between the histological type and SDC-1 expression: invasive ductal carcinoma, which is the most frequent histotype, was SDC-1-positive in 87.5% of samples and SDC-1-negative in 52.4%.

The main objective of this study was to evaluate whether the expression of SDC-1 in locally advanced breast cancer is predictive for the response to neoadjuvant chemotherapy. Analysis of the pathological response to neoadjuvant chemotherapy with respect to SDC-1 expression revealed a reduced effect in SDC-1-positive patients as compared to SDC-1-negative patients. In this study, 73% overall patients showed a pathological response, with a pCR in 2.7% patients and pNC in 27%. With respect to SDC-1 expression, pathological "no change" was obtained in 37.5% of SDC1-positive patients as compared to 19% of SDC-1-negative patients. None of the SDC-1-positive patients had a pCR. Furthermore, none of the responding tumors was strongly SDC-1-positive, whereas 20% of the non-responding tumors showed high SDC-1 expression. To determine whether our results were merely a reflection of differences between distinct groups, we evaluated whether tumor biological factors with well-established prognostic relevance were related to SDC-1 expression. No significant correlations between SDC-1 expression and histopathological factors were found. Concerning clinical response to chemotherapy, no relationship with SDC-1 expression was observed. In a recently published study, Leivonen *et al.* observed that epithelial SDC-1 expression was associated with negative ER status, whereas stromal SDC-1 expression was associated with positive ER status (10). Ten-year survival was significantly reduced for patients displaying both epithelial and stromal SDC-1 expression, leading to the conclusion that this concomitant expression may be a predictor of unfavorable prognosis in breast cancer. These findings indicate different functional roles for stromal and epithelial syndecan-1, respectively. In a study by Stanley *et al.* (7), SDC-1 staining was greatly reduced in malignant cells within infiltrating ductal carcinomas (n=20) as compared to the ductal epithelium of normal breast and stromal-epithelial neoplasms, which exhibited extensive basolateral epithelial staining. Strong SDC-1 staining was detected within the connective tissue and on stromal cell surfaces. In contrast, it was absent in control tissues and on neoplasms (7). In contrast to Stanley *et al.* (7), we observed an increased SDC-1 staining in malignant cells within invasive ductal carcinomas. Thus, our findings are more in line with the studies of Leivonen *et al.* (10) and Barbareschi *et al.* (9), who observed an increased epithelial staining for SDC-1, investigating much larger patient collectives than Stanley *et al.* (7). Importantly, increased SDC-1 staining within the group of ductal carcinomas correlated with a decreased breast cancer-specific survival ($p=0.02$) in the study by Leivonen *et al.* (10), and both Leivonen *et al.* and Barbareschi *et al.* assigned a negative prognostic value for increased SDC-1 expression in breast carcinomas (9, 10).

Apparently, SDC-1 overexpression by breast cancer cells appears to correlate with a decreased expression of (stromal) matrix proteins (12). This could indicate a possible role of SDC-1 in tumor matrix remodeling, which would, in turn, influence tumor cell signaling (25). Recently, a growth-promoting loop between breast cancer cells and stromal fibroblasts, which depended on the presence of SDC-1, has been shown (26). SDC-1 up-regulation was noted in cells of tumor stroma surrounding tumor connective tissue. These stromal cells, characterized as spindle cells with myofibroblastic differentiation, may contribute to the dedifferentiation of tumor cells and the development of metastasis (25). In our study, a strong stromal immunostaining for SDC-1 was observed in 33.3% of the evaluated tissues, with an overall SDC-1-positive immunoreaction in 43.2% of the cases. Importantly, 29.2% of the patients showing negative stromal expression of SDC-1 exhibited no pathological response, whereas only 16.7% of the SDC-1 stromal-positive patients fell in this category. Thus, our data would be compatible with a concept of different predictive functions of stromal and epithelial SDC-1 expression, respectively, in neoadjuvant chemotherapy of breast carcinomas.

Although previously published data suggest an important role for SDC-1 as a prognostic marker and as a molecule relevant for the etiology of human breast cancer, to date the predictive value of SDC-1 in breast cancer has been unclear. In this study, a decreased response to chemotherapy was observed in SDC-1-positive patients. Our data suggest a potential ability of SDC-1 expression to predict breast cancer susceptibility to chemotherapy.

In conclusion, the current study provides evidence for a negative predictive value of SDC-1 expression in breast carcinomas. SDC-1 expression in breast cancer may adversely

affect the response to chemotherapy treatment. However, due to the limited sample size in this study, our findings have to be confirmed in larger series. Nonetheless, this study represents a suitable background for further investigations in this field.

Acknowledgements

The authors thank Vera Samoilova for her excellent technical assistance. Funding was provided by Münster University Hospital "Innovative Medizinische Forschung" IMF grants WÜ 1 2 03 32 (P.W.) and GÖ 1 2 04 15 (M.G.), and Deutsche Forschungsgemeinschaft DFG GO 1392/1-1 (M.G.), Germany.

References

1 Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J and Zako M: Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem *68*: 729-777, 1999.
2 Götte M: Syndecans in inflammation. FASEB J *17*: 575-591, 2003.
3 Gallo R, Kim C, Kokenyesi R, Adzick NS and Bernfield M: Syndecans-1 and -4 are induced during wound repair of neonatal but not fetal skin. J Invest Dermatol *107*: 676-683, 1996.
4 Elenius V, Götte M, Reizes O, Elenius K and Bernfield M: Inhibition by the soluble syndecan-1 ectodomain delays wound healing in syndecan-1 overexpressing mice. J Biol Chem *279*: 41928-41935, 2004.
5 Götte M, Joussen AM, Klein C, André P, Wagner DD, Kirchhof B, Hinkes MT, Adamis AP and Bernfield M: Role of Syndecan-1 in leukocyte-endothelial interactions in the ocular vasculature Invest Ophthalmol Vis Sci *43*: 1135-1141, 2002.
6 Beauvais DM and Rapraeger AC: Syndecans in tumor cell adhesion and signaling. Reprod Biol Endocrinol *2*: 3, 2004.
7 Stanley MJ, Stanley MW, Sanderson RD and Zera R: Syndecan-1 expression is induced in the stroma of infiltrating breast carcinoma. Am J Clin Pathol *112*: 377-383, 1999.
8 Matsuda K, Maruyama H, Guo F, Kleeff J, Itakura J, Matsumoto Y, Lander AD and Korc M: Glypican-1 is overexpressed in human breast cancer and modulates the mitogenic effects of multiple heparin-binding growth factors in breast cancer cells. Cancer Res *61*: 5562-5569, 2001.
9 Barbareschi M, Maisonneuve P, Aldovini D, Cangi MG, Pecciarini L, Angelo Mauri F, Veronese S, Caffo O, Lucenti A, Palma PD, Galligioni E and Doglioni C: High syndecan-1 expression in breast carcinoma is related to an aggressive phenotype and to poorer prognosis. Cancer *98*: 474-483, 2003.
10 Leivonen M, Lundin J, Nordling S, Von Boguslawski K and Haglund C: Prognostic value of syndecan-1 expression in breast cancer. Oncology *67*: 11-18, 2004.
11 Mennerich D, Vogel A, Klaman I, Dahl E, Lichtner RB, Rosenthal A, Pohlenz HD, Thierauch KH and Sommer A: Shift of syndecan-1 expression from epithelial to stromal cells during progression of solid tumors. Eur J Cancer *40*: 1373-1382, 2004.
12 Tsanou E, Ioachim E, Briasoulis E, Charchanti A, Damala K, Karavasilis V, Pavlidis N and Agnantis NJ: Clinicopathological study of the expression of syndecan-1 in invasive breast carcinomas. Correlation with extracellular matrix components. J Exp Clin Cancer Res *23*: 641-650, 2004.
13 Ayers M, Symmans WF, Stec J, Damokosh AI, Clark E, Hess K, Lecocke M, Metivier J, Booser D, Ibrahim N, Valero V, Royce M, Arun B, Whitman G, Ross J, Sneige N, Hortobagyi GN and Pusztai L: Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. J Clin Oncol *22*: 2284-2293, 2004.
14 Makris A, Powles TJ, Ashley SE, Chang J, Hickish T, Tidy VA, Nash AG and Ford HT: A reduction in the requirements for mastectomy in a randomized trial of neoadjuvant chemoendocrine therapy in primary breast cancer. Ann Oncol *9*: 1179-1184, 1998.
15 Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, Wickerham DL, Begovic M, DeCillis A, Robidoux A, Margolese RG, Cruz AB Jr, Hoehn JL, Lees AW, Dimitrov NV and Bear HD: Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol *16*: 2672-2685, 1998.
16 Cleator SJ, Makris A, Ashley SE, Lal R and Powles TJ: Good clinical response of breast cancers to neoadjuvant chemoendocrine therapy is associated with improved overall survival. Ann Oncol *16*: 267-272, 2005.
17 Euler U, Dresel V, Bühner M, Volkholz H and Tulusan AH: Dose and time intensified epirubicin/cyclophosphamide (EC) as preoperative treatment in locally advanced breast cancer. Breast Cancer Res Treat *76(Suppl.1)*: S51, 2002.
18 Hayward JL, Rubens RD, Carbone PP, Heuson JC, Kumaoka S and Segaloff A: Assessment of response to therapy in advanced breast cancer. Br J Cancer *35*: 292-298, 1977.
19 Sinn HP, Schmid H, Junkermann H, Huober J, Leppien G, Kaufmann M, Bastert G and Otto HF: Histologic regression of breast cancer after primary (neoadjuvant) chemotherapy. Geburtshilfe Frauenheilkd *54*: 552-558, 1994.
20 Klaassen U, Wilke H, Weyhofen R, Harstrick A, Eberhardt W, Muller C, Korn M, Hanske M, Diergarten K and Seeber S: Phase II study with cisplatin and paclitaxel in combination with weekly high-dose 24 h infusional 5-fluorouracil/leucovorin for first-line treatment of metastatic breast cancer. Anticancer Drugs *9*: 203-207, 1998.
21 Sobin LH WC: TNM Classification of Malignant Tumours. New York, Wiley-Liss, 1997.
22 Wülfing P, Tio J, Kersting C, Sonntag B, Buerger H, Wülfing C, Euler U, Boecker W, Tulusan AH and Kiesel L: Expression of Endothelin-A receptor predicts unfavourable response to neoadjuvant chemotherapy in locally advanced breast cancer. Br J Cancer *91*: 434-440, 2004.
23 Wülfing P, Diallo R, Kersting C, Wülfing C, Poremba C, Rody A, Greb RR, Böcker W and Kiesel L: Expression of Endothelin-1, Endothelin-A and Endothelin-B receptor in human breast cancer and correlation with long-term follow-up. Clin Cancer Res *9*: 4125-4131, 2003.
24 Burbach BJ, Friedl A, Mundhenke C and Rapraeger AC: Syndecan-1 accumulates in lysosomes of poorly differentiated breast carcinoma cells. Matrix Biol *22*: 163-177, 2003.
25 Burbach BJ, Ji Y and Rapraeger AC: Syndecan-1 ectodomain regulates matrix-dependent signaling in human breast carcinoma cells. Exp Cell Res *300*: 234-247, 2004.
26 Maeda T, Alexander CM and Friedl A: Induction of syndecan-1 expression in stromal fibroblasts promotes proliferation of human breast cancer cells. Cancer Res *64*: 612-621, 2004.

Received October 19, 2005
Accepted November 2, 2005