Significance of Syndecan-1 Expression in Ductal Carcinoma *In Situ* of the Breast

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Abstract. *Background: Fibroblast growth factor-2 (FGF-2) supports tumor progression in breast cancer. FGF-2 signaling is modulated by heparan sulfate proteoglycans, such as syndecan-1 (CD138). The exact role of CD138 in ductal carcinoma in situ of the breast (DCIS) is still uncertain. Differential expression depending on grading could suggest a role for syndecan-1 during growth and tumor progression. Materials and Methods: Samples of 127 cases of breast DCIS associated with follow-up data were included. CD138 staining intensity, number of positive cells, intracellular and tissue localization were examined. Results: Median follow-up was 45.4 months and median recurrence-free survival (RFS) 86 months. Age, menopausal status and previous hormone replacement therapy had no significant influence on RFS. Smoking significantly influenced RFS (p=0.008). Endocrine therapy or radiotherapy did not improve RFS. Grading was not correlated with CD138 staining intensity, but was significantly associated with the percentage of CD138-positive cells (low-vs. high-grade, p=0.043). Estrogen receptor (ER) expression did not influence staining intensity of CD138 (p=0.247), but negatively correlated with the proportion of CD138-positive cells (p=0.032). Progesterone receptor (PR) expression significantly influenced the intensity of staining (p=0.010) and the percentage of CD138-positive cells (p=0.004); both were increased in PR-negative cases. CD138 staining intensity and percentage of positive cells did not correlate with RFS. Nuclear grade and syndecan-1 staining localization were significantly associated (p=0.001). ER-positive, and PR-positive DCIS more often exhibited membrane-bound syndecan-1 than ER- or PR-negative cases (p=0.001). Nuclear grade and tissue localization of CD138 correlated significantly (p=0.005). PR influenced CD138 tissue distribution, while ER did not. Syndecan-1 localization did not statistically impact RFS. Conclusion: In DCIS of different nuclear grades, tissue localization of syndecan-1 is significantly divergent, suggesting a specific effect on biology and progression of DCIS*

Ductal carcinoma *in situ* (DCIS) of the breast is a non-invasive lesion. DCIS may give rise to invasive breast cancer if it remains without appropriate therapy. Within 20 years, 20-50% of cases of DCIS progress to invasive tumors (1, 2). Good differentiation is commonly correlated with hormone receptor (HR) positivity. Fewer than 30% of poorly-differentiated DCIS are HR-positive (3, 4). Most cases of DCIS are currently treated by lumpectomy and postoperative radiotherapy. The local recurrence rate is 10%; half of these recurrences are invasive. A tumor-free margin after surgery is essential for prevention of relapse (5-7). To individualize treatment of DCIS, biomarkers are required to better identify DCIS with aggressive biology (8, 9).

Fibroblast growth factor-2 (FGF-2) is pro-angiogenic and also influences breast cancer cells directly. These effects may influence DCIS biology towards invasive growth. For effective signaling, FGF-2 activity is modulated by the heparan sulfate proteoglycan (HSPG) family as co-factors (10, 11). Syndecan-1 is a member of this family (12, 13). Syndecan-1 is important not only for normal breast physiology, but also for breast cancer growth. In breast

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cancer, syndecan-1 expression is found in epithelial tumor cells, in stroma or in both components (14, 15). High syndecan-1 expression is often correlated with aggressive tumor behavior. Syndecan-1 expression in DCIS is enhanced in high-grade tumors (4) and is associated with enhanced angiogenesis and lymphangiogenesis. The significance of differential syndecan-1 expression in DCIS of different nuclear grades is unclear.

The objectives of this investigation were to measure the impact of individual and epidemiological factors on recurrence-free survival (RFS) after DCIS. In particular, the role of syndecan-1 expression in DCIS was examined. The syndecan-1 staining intensity, percentage of positively-stained cells, cellular and tissue localization in DCIS are important variables. The study aimed to clarify whether there is differential expression between the DCIS sub-groups. Differences could suggest an active role of syndecan-1 during tumor progression.

Materials and Methods

Representative paraffin-embedded tissue samples of DCIS and associated clinical data were available for 127 patients. Patients underwent surgery between 2000 and 2006 at the University Hospital Kiel, Kiel, Germany, and had given informed consent. This study was approved by the local Review Board (# AZ: D 452/13). Clinical and pathological data were collected retrospectively.

De-paraffinization, rehydration, epitope retrieval and immunohistochemical staining with EnVision™ Detection Systems, peroxidase/DAB (Dako, Glostrup, Denmark), counterstainig, dehydration and mounting were performed according to the manufacturers protocol (Dako). Syndecan-1 was labeled by a primary antibody (monoclonal CD138 mouse antibody (BC-03 405, 1:1000; Biozol, Eching, Germany). Pathological evaluation was performed independently by two pathologists. HR status was determined by immunohistochemistry and described by immune reactive score (IRS) according to Remmele and Stegner (17). The proportion of stained (*i.e.* positive) tumor cells was scored semi-quantitatively. The staining intensity was rated from 0-3 (0 no staining, 1 low, 2 moderate, 3 high staining intensity). Additionally, localization of cellular staining (membrane bound, cytoplasmic or nuclear) was precisely assessed, as was how syndecan-1 was distributed within the DCIS tissue (central, peripheral and diffuse).

Quantitative variables are presented using the mean and standard deviation. Potential dependencies were examined by Chi-square test. In case of inadequately small expected frequencies, the exact test according to Fisher was used. Kaplan–Meier analysis and log-rank test or Cox regression were examined with regard to the influence of investigated parameters on RFS. Tests were two-sided and a significance level of 5% was set. For statistical calculations, PASW Statistics 20 software (SPSS Inc., an IBM Company, Chicago, IL, USA) was used.

Results

The median follow-up was 45.4 months. For 88 patients with DCIS, sufficient follow-up was available to calculate RFS. Eleven patients experienced relapse (DCIS or invasive breast cancer). Four patients developed secondary malignancies or have died (non breast cancer-related). The median RFS was 86 months. The sub-groups with different nuclear gradings are represented by 16 low-grade, 44 intermediate-grade, 21 high-grade and 7 high-grade cases with microinvasion (Figure 1a-f). No patient with a low-grade or a high-grade DCIS with microinvasion experienced recurrence; 6 intermediate-grade and 4 high-grade DCIS relapsed. The median duration of RFS is 85 months in the intermediate and 77 months in the high-grade DCIS group. No significant difference in RFS in the two groups were revealed (log-rank test, $p=0.208$) (Figure 2a). Neither estrogen receptor (ER) ($p=0.657$), nor progesterone receptor (PR) ($p=0.674$) expression had any impact on RFS.

General epidemiological factors influencing the recurrence-free survival. Patient age at primary diagnosis had no significant effect on RFS (Cox regression analysis, $p=0.111$). Patients' age was on average 58 years (median=59 years, range=36-82 years). The menopausal status of 81 patients was known; out of these, 19 were pre-menopausal and 62 post-menopausal. There was a statistical trend towards an improved RFS after menopause ($p=0.057$) (Figure 2b). Information on previous hormone replacement therapy (HRT) was available for 69 patients; 38 patients had never used HRT and 31 were on HRT. Previous HRT was not related to an adverse outcome after DCIS ($p=0.128$).

We also examined the influence of previous and current nicotine consumption on RFS after DCIS. Smoking history was available for 69 patients: 46 never smoked, 17 had smoked prior to DCIS diagnosis, and 6 patients were current smokers when diagnosed with DCIS. In the never-smoking group, three breast cancer-related recurrences occurred and in the group of previous smokers, two events occurred, whereas in the group of current smokers, three events occurred (50% of all currently smoking patients). A significant negative influence of smoking on RFS (never smokers *vs*. previous and current smokers) was found (log-rank test, $p=0.008$) (Figure 2c).

Influence of different treatment modalities. Follow-up data regarding endocrine therapy were available for 75 patients. Of these, 28 had not received endocrine therapy; 39 women had received tamoxifen, seven had received aromatase inhibitors and one a switch therapy with aromatase inhibitor and tamoxifen. The log-rank test shows no advantage for any single regimen. The principal use of endocrine therapy (n=47) compared to no-drug therapy (n=28) did not improve RFS ($p=0.815$).

From a total of 127 patients, 124 had sufficient surgical reports available. A total of 92 were treated with breast-conserving surgery (BCS) and 32 with mastectomy; 29 patients after ablation and 84 after BCS remained free of recurrence. Of 77 patients, 42 had not and 35 had received

postoperative radiation therapy; four low-, 18 intermediate-, 11 high-grade, and two high-grade with microinvasion were irradiated. No advantage for RFS by radiotherapy (log-rank test $p=0.941$) was seen in this cohort.

Influence of general pathological factors on RFS. No significant effect of tumor size on risk of recurrence was found (Cox regression analysis, $p=0.206$). The tumor-free resection margin was also not correlated with disease relapse ($p=0.381$). Data of ER and PR expression and subsequent follow-up were available for 54 cases. Seven cases of DCIS did not express ER, whereas 47 were ER-positive; 22 were PR-negative and 32 PR-positive. ER ($p=0.657$) and PR ($p=0.674$) expression were not correlated with RFS.

Effect of syndecan-1 (CD138) staining intensity and percentage of positive cells. Patient age and menopausal status were not correlated with the percentage of syndecan-1-positive cells or CD138 staining intensity. Data regarding previous HRT were available for 77 women; 33 had received HRT previously, 44 had not. Previous HRT had no statistical impact on cellular staining intensity and the proportion of positively stained cells.

A total of 51 out of 77 patients never smoked, 19 smoked previously and seven were current smokers. No differences among the different groups in relation to staining intensity ($p=0.88$) or percentage of CD138-positive cells ($p>0.999$) were revealed (Kruskal–Wallis test).

ER and PR status were known for 75 patients; 14 were ER-negative and 61 ER-positive. ER expression did not correlate with staining intensity of syndecan-1 ($p=0.247$) (Figure 3a) but of note, ER correlated inversely with the proportion of CD138-positive cells ($p=0.032$) (Figure 3b).

Thirty-three cases were PR negative and 42 PR positive. A statistically significant inverse correlation of PR status with CD138 staining intensity ($p=0.010$) was found (Figure 3c), as well as with the percentage of syndecan-1-positive cells ($p=0.004$) (Mann–Whitney *U*-test) (Figure 3d). The CD138 staining intensity was significantly greater and the proportion of CD138-positive cells higher in PR-negative DCIS cases.

Thirty low-grade, 58 intermediate-grade, 29 high-grade and 8 high-grade with microinvasion were included in this analysis. Staining intensity was not correlated with nuclear grading (Figure 4a). This is opposed by results which show significant differences between low- and high-grade DCIS (Kruskal–Wallis test, $p=0.043$) and between intermediate- and high-grade DCIS ($p=0.049$) with respect to the number of CD138-positive cells. A higher nuclear grade was significantly correlated with a higher proportion of CD138-positive cells (Figure 4b). Syndecan-1 staining intensity ($p=0.629$) and the percentage of syndecan-1 positively- stained cells did not influence RFS (Cox regression analysis, $p=0.169$).

Cellular localization of syndecan-1. One hundred and eighteen cases of DCIS were included in this analysis. Out of 28 low-grade DCIS, 25 exhibited membrane-bound, one cytoplasmic and two diffuse (membranous and cytoplasmic) syndecan-1 staining. Forty-three out of 54 intermediate-grade DCIS exhibited membrane-bound, four cytoplasmic and seven diffuse cytoplasmic CD138 staining. Sixteen out of 28 high-grade DCIS cases exhibited membrane-bound, six cytoplasmic and six diffuse syndecan-1 expression. Three out of eight high-grade DCIS with microinvasion exhibited membrane-bound, two cytoplasmic and three diffuse CD138 staining. A significant association between histological grade and cellular staining localization of syndecan-1 was shown (Chi-square test, $p=0.001$).

Menopausal status had no effect on cellular syndecan-1 expression (Fisher's exact test, $p=0.332$). Neither previous HRT nor cigarette smoking (former or current) had a significant effect on the cellular localization of syndecan-1.

ER expression affected the cellular distribution of syndecan-1. Three out of 13 ER-negative DCIS exhibited membrane-bound, five cytoplasmic and five diffuse (membranous and cytoplasmic) cellular staining. A total 49 of 58 ER-positive cases exhibited membrane-bound syndecan-1, three cytoplasmic and six diffuse distributions. Differences in cellular staining were statistically significant ($p<0.001$, Fisher's exact test). ER-positive DCIS significantly more frequently exhibited membrane-bound syndecan-1 staining. Fourteen out of 32 PR-negative DCIS exhibited membrane-bound, eight cytoplasmic and 10 diffuse cellular syndecan-1 distribution. Syndecan-1 localization in PR-positive cases was significantly different; 39 cases had membrane-bound and none cytoplasmic syndecan-1 distribution, while one case did not show any syndecan-1 expression. The influence of PR status on cellular CD138 staining was statistically highly significant (Fisher's exact test, $p<0.001$). PR-positive cases almost exclusively exhibited membranous syndecan-1. Fifty percent of PR-negative cases exhibited no membrane-bound but a diffuse or cytoplasmic staining pattern.

Syndecan-1 localization in DCIS tissues. Syndecan-1 tissue localization differed depending on nuclear grade (Figure 1a-f). Out of 28 low-grade cases 11 had diffuse tissue expression of syndecan-1, 11 had peripheral and six a centrally located tissue expression of syndecan-1. Out of 54 intermediate-grade DCIS, CD138 tissue expression was diffuse in 26, peripheral in 19 and central nine. Of 28 high-grade DCIS, 22 exhibited diffuse syndecan-1 expression, two peripheral and four central tissue expression patterns. In six out of seven high-grade DCIS with microinvasion, one with diffuse and one with peripheral distribution pattern were detectable. There was a significant correlation between grading and localization of syndecan-1 expression in DCIS

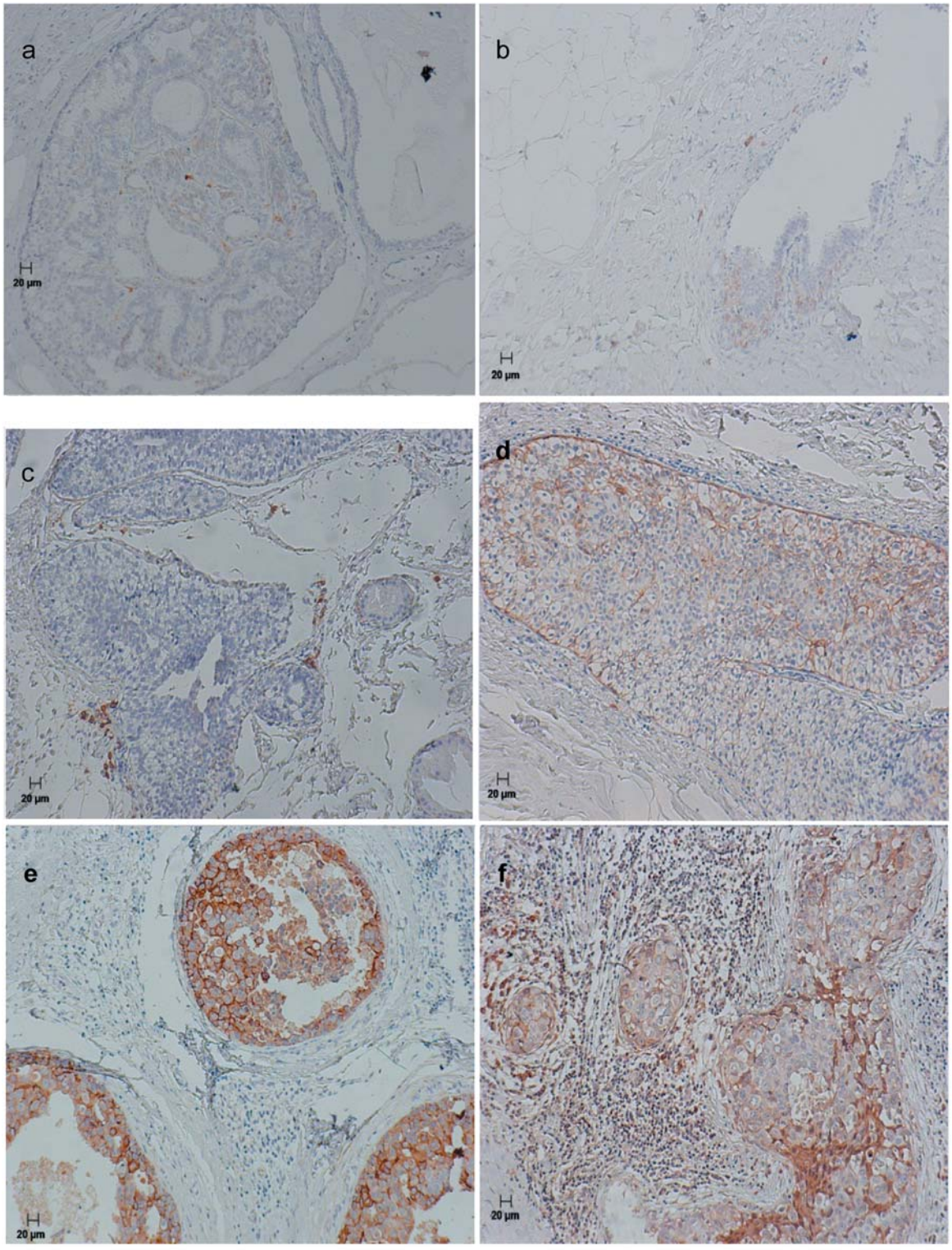

Figure 1. *Examples of distinct immunohistochemical staining patterns of syndecan-1 in ductal carcinoma in situ (DCIS) (×100 magnification). a: Low-grade with weak membranous cellular syndecan-1 staining in <5% of tumor cells and diffuse tissue expression. b: Low-grade with weak membranous cellular staining in 10% of tumor cells centrally in DCIS tissue. c: Intermediate-grade with strong membranous staining in 80% of tumor cells and diffuse tissue expression. d: Intermediate-grade with weak membranous staining in <5% of tumor cells and peripheral tissue expression. e: High-grade with strong cytoplasmatic staining in 90% of tumor cells and diffuse tissue expression. f: High-grade with microinvasion, with strong membranous and cytoplasmatic cellular staining in 70% of tumor cells and diffuse tissue expression.*

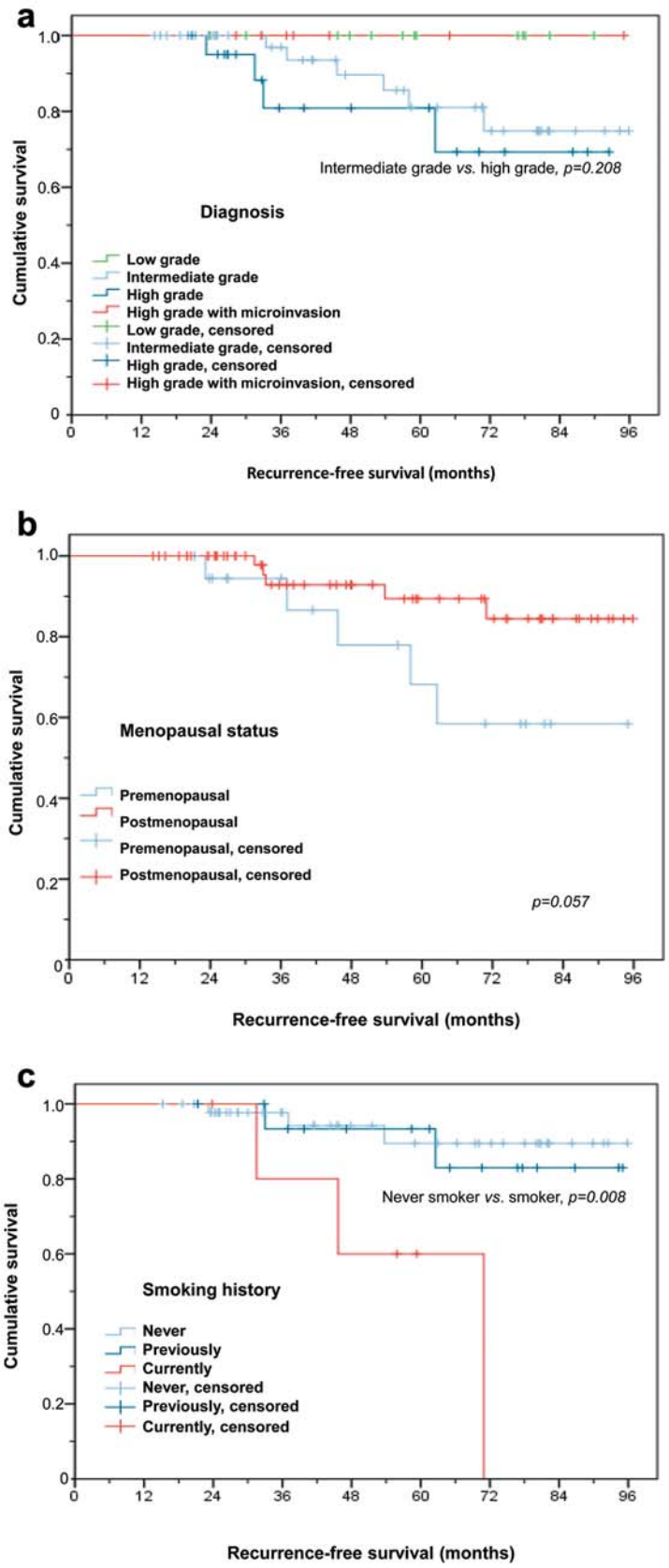

Figure 2. *Recurrence-free survival by: subtype (a), with no statistical differences among the groups of different nuclear grading (p=0.208); b: menopausal status, with a statistical trend for an improved survival for post-menopausal patients (p=0.057); c: previous or current smoking (never smoker vs. smoker, p=0.008).*

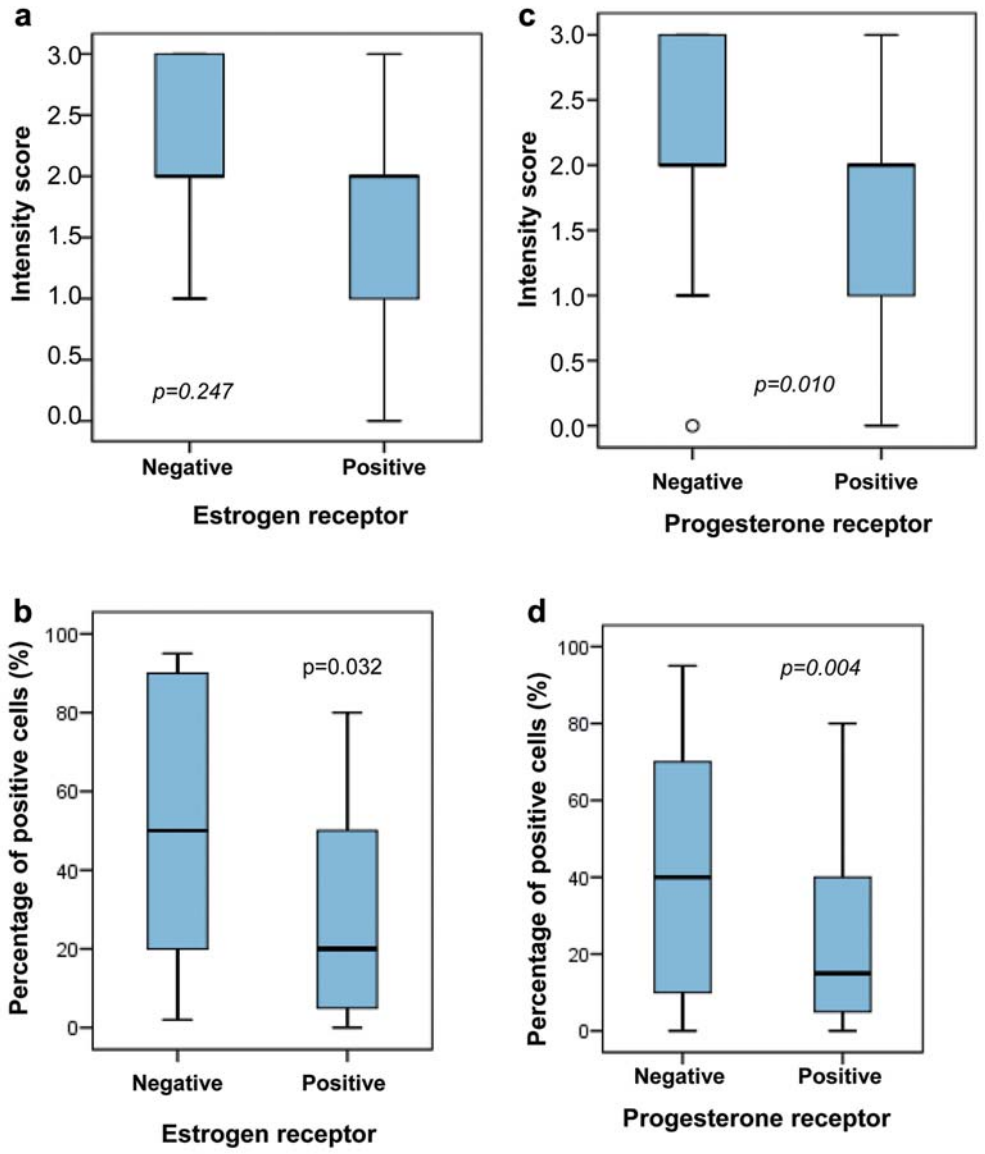

Figure 3. *Correlation of estrogen receptor (ER) status and syndecan-1 staining intensity (a) and number of syndecan-1-positive cells (b). Correlation of progesterone receptor (PR) status and syndecan-1 staining intensity (c) and number of syndecan-1-positive cells (d). In the box plots, the bottom and top of the box show the first and third quartiles of data. The band inside the box is the median. The whiskers represent the lowest and the highest value of 1.5× interquartile range. Data not included between the whiskers are plotted as outliers using a small circle.*

tissues (Chi-square test, p=0.005) (Figure 4a). A significant influence of ER expression on tissue distribution of syndecan-1 (located centrally, peripherally or diffuse) was not detected (Fisher exact test, p=0.250) (Figure 3a and b). Results for PR effects on tissue distribution differ significantly. Of 71 DCIS, 32 were PR-negative and 39 PR-positive. Of 32 PR-negative cases, 24 exhibited diffuse, five peripheral and three central syndecan-1 expression. In 39 PR-positive DCIS, 16 had diffuse, 14 peripheral and nine central syndecan-1 tissue expression. A significant correlation of PR expression and expression of syndecan-1 in DCIS tissue was found (Pearson's chi-square test, p=0.016) (Figure 3c and d).

Menopausal status did not affect syndecan-1 tissue localization (p=0.332). Moreover, previous HRT (p=0.067) and a history of smoking (p=0.691) were without statistical influence on syndecan-1 tissue distribution. Fifty DCIS cases with complete follow-up data exhibited diffuse tissue staining for syndecan-1. Eighteen cases exhibited a peripheral and 14 a central staining tissue pattern. No case

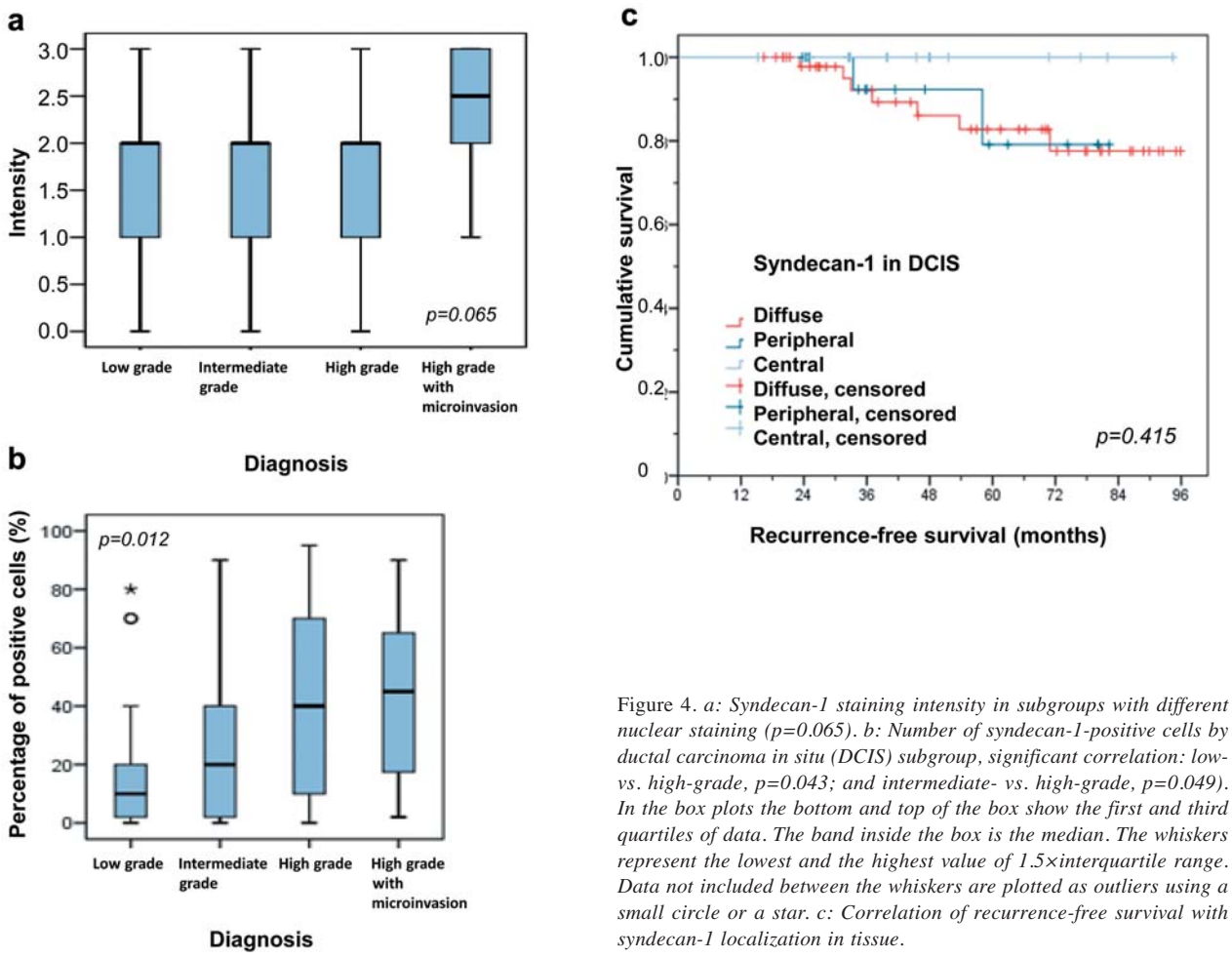

Figure 4. *a: Syndecan-1 staining intensity in subgroups with different nuclear staining (p=0.065). b: Number of syndecan-1-positive cells by ductal carcinoma in situ (DCIS) subgroup, significant correlation: low- vs. high-grade, p=0.043; and intermediate- vs. high-grade, p=0.049). In the box plots the bottom and top of the box show the first and third quartiles of data. The band inside the box is the median. The whiskers represent the lowest and the highest value of 1.5×interquartile range. Data not included between the whiskers are plotted as outliers using a small circle or a star. c: Correlation of recurrence-free survival with syndecan-1 localization in tissue.*

with central staining experienced relapse, while seven cases with diffuse and two with peripheral staining pattern did. No significant correlation between syndecan-1 tissue localization and RFS was found (log-rank test, p=0.415) (Figure 4c).

Discussion

There was a trend for a longer RFS in intermediate-grade (*vs*. high grade) DCIS (p=0.208) (Figure 2a). Numbers are small for sub-group analysis, therefore statistical conclusions are limited. ER/PR expression was not correlated with RFS after DCIS. Statistical results are limited as the HR status was only known in a minority of cases. A comparison of cases is difficult, as therapy standards have changed during the course of the study. This may have led to biological differences not being reflected in RFS data.

General epidemiological factors influencing RFS. Patient age had no significant influence on RFS (p=0.111). The age distribution is similar to that for other DCIS cohort studies (15, 16). There is a trend for improved RFS in post-menopausal women (p=0.057) (Figure 2b), although numbers of pre-menopausal cases were small. Previous HRT had no adverse effect on RFS. This is supported by observations of other groups, where discontinuation of HRT after diagnosis of ER-positive DCIS resulted in a rapid reduction of cell proliferation (18). In the present study, nicotine consumption was correlated with a worse RFS (Figure 2c) after DCIS. Women smokers should be advised about potential risks of continued smoking after DCIS. These data differ from those of larger epidemiological studies for invasive breast cancer. The Nurses Health Study revealed no smoking-related increased breast cancer mortality (19). The Women's Health Initiative revealed a trend for reduced risk for smokers (p=0.44). Other authors report no higher nuclear grade DCIS after smoking (20). Therefore the effects of smoking on prognosis after DCIS remain controversial.

Influence of different treatment modalities. No advantage for endocrine therapy at all was shown in this cohort. Endocrine therapy after HR-positive DCIS is not necessarily recommended (22). Radiotherapy after BCS showed no advantage for RFS, but the number of evaluated patients was small. Postoperative radiation is standard for DCIS after BCS (22). High-risk lesions were predominantly treated by mastectomy. Not surprisingly in this sub-group, there have been no recurrences.

Effect of syndecan-1 staining intensity and proportion of positively stained cells. Syndecan-1 staining intensity did not correlate with nuclear grading (Figure 4a). However, there were significant differences between low- and high-grade DCIS, and between intermediate- and high-grade DCIS in regard to the percentage of syndecan-1-positive cells. High nuclear grading was correlated with higher numbers of syndecan-1-positive cells (Figure 4b). It has previously been reported that in invasive carcinomas, expression of membrane-bound syndecan-1 is up-regulated compared to benign tissue (23-25). Thereby FGF-2 binding is altered in the tumor (10, 26, 27). Aggressive tumor biology correlates with strong syndecan-1 expression (15, 28-30). Well-differentiated tumors and benign epithelium often express syndecan-1 on the cell surface (30). Syndecan-1-positive cancers exhibit reduced chemosensitivity (25, 29, 31). It can be assumed that syndecan-1 directly affects DCIS progression. Other authors report an association of epithelial syndecan-1 pattern with an ER-negative phenotype and stromal syndecan-1 expression with an ER-positive tumor type (32-33). This study found no impact of ER expression on staining intensity of syndecan-1. But ER expression was inversely-correlated with the proportion of syndecan-1-positive cells. In ER-positive cases, significantly fewer syndecan-1 positive cells were present (Figure 3a and b). CD138 staining intensity was significantly stronger in PR-negative DCIS and the proportion of CD138-positive cells significantly increased in PR-negative cases (Figure 4c and d). Overall, there was no significant correlation between syndecan-1 staining intensity nor of percentage of syndecan-1 positively stained cells and RFS.

Cellular localization of syndecan-1. A majority of low- and intermediate-grade DCIS exhibited membrane-bound syndecan-1 staining. High-grade cases were slightly more often associated with membrane-bound staining than with cytoplasmic or diffuse cellular syndecan-1 pattern. The association between DCIS of different nuclear grade with cellular staining localization was statistically significant. ER and PR status were correlated with a specific cellular syndecan-1 pattern. ER-negative DCIS expressed syndecan-1 in the cytoplasm, while ER-positive DCIS expressed membrane bound syndecan-1. PR-negative DCIS revealed a mixed pattern of syndecan-1. PR-positive DCIS exclusively exhibited membrane-bound syndecan-1. The results suggest that the membranous expression of syndecan-1 is anti-proliferative, while cytoplasmic expression supports aggressive tumor biology. The finding that membrane-bound syndecan-1 may act in an anti-proliferative manner is surprising considering its role as a co-receptor for growth factors such as FGF-2. On the other hand, two recent articles showed that siRNA depletion of syndecan-1 in MDA-MB-231 breast cancer cells enhances invasiveness and proliferation (albeit moderately) *via* activation of integrin signaling (34, 35).

Syndecan-1 localization in DCIS. There was no significant influence of ER expression on syndecan-1 distribution in DCIS tissue. In invasive breast cancer, an association between ER negativity and syndecan-1 tissue localization has been described (36). The correlation between grading and localization of syndecan-1 expression in DCIS tissue was highly significant. Although no case with central syndecan-1 expression experienced relapse during follow-up, no significant effect of syndecan-1 tissue localization on RFS was shown herein (Figure 4c). This is surprising as expression patterns in sub-groups are strikingly different. Retrospectively, it cannot be determined whether this is due to small numbers within the subgroups, or perhaps a minor influence of syndecan-1 localization on RFS.

Conclusion

The essential results of this DCIS study are a significant correlation between nuclear grade and the proportion of syndecan-1-positive cells. This analysis shows a trend for a longer disease-free survival in low-grade DCIS. Tissue localization of syndecan-1 differed significantly in DCIS of different grades. HR expression was inversely correlated with the proportion of syndecan-1-positive cells. In HR-positive cases, significantly fewer syndecan-1-positive cells were present. Syndecan-1 staining intensity and percentage of syndecan-1-positive cells in DCIS were found to have no significant effect on RFS. The significantly diverse syndecan-1 staining in DCIS of different nuclear grades suggests an influential role of syndecan-1 in behavior and progression of DCIS.

Competing Interests

We declare we have no financial competing interests, regarding this study. We do not hold a patent and are not applying for a patent relating to the content of this article. We also have no non-financial competing interests.

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References

1 Purushotham AD: The diagnosis and management of pre-invasive breast disease Problems associated with management of pre-invasive lesions, Breast Cancer Res *5*: 309-312, 2003

2 Burstein HJ, Polyak K, Wong JS, Lester SC and Kaelin CM: Ductal carcinoma *in situ* of the breast. N Engl J Med *350*: 1430-1441, 2004.

3 Nelson NJ: DCIS prognostic markers: A few new candidates emerge, JNCI J Natl Cancer Inst *102(9)*: 588-590, 2010.

4 Porter D, Lahti-Domenici J, Keshaviah A, Bae YK, Argani P, Marks J, Richardson A, Cooper A, Strausberg R, Riggins GJ, Schnitt S, Gabrielson E, Gelman R and Polyak K: Molecular markers in ductal carcinoma *in situ* of the breast, Mol Cancer Res 1(5): 362-75, 2003.

5 Bijker N, Meijnen P, Peterse JL, Bogaerts J, Van Hoorebeeck I, Julien JP, Gennaro M, Rouanet P, Avril A, Fentiman IS, Bartelink H and Emiel JT and Rutgers EJT: Breast-Conserving Treatment With or Without Radiotherapy in Ductal Carcinoma-In-Situ: Ten-Year Results of European Organisation for Research and Treatment of Cancer randomized Phase III Trial 10853–A Study by the EORTC Breast Cancer Cooperative Group and EORTC Radiotherapy Group, J Clin Oncol *24*: 3381-3387, 2006.

6 Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Overview of the Randomized Trials of Radiotherapy in Ductal Carcinoma In Situ of the Breast, J Natl Cancer Inst Monogr *41*: 162-177, 2010.

7 Eng-Wong J, Costantino JP and Swain SM: The Impact of Systemic Therapy Following Ductal Carcinoma In Situ, J Natl Cancer Inst Monogr *41*: 200-203, 2010.

8 Schnitt SJ: Local outcomes in ductal carcinoma *in situ* based on patient and tumor characteristics. J Natl Cancer Inst Monogr *41*: 158-161, 2010.

9 Fisher ER, Dignam J, Tan-Chiu E, Costantino J, Fisher B, Paik S and Wolmark N: Pathologic findings from the National Surgical Adjuvant Breast Project (NSABP) eight-year update of Protocol B-17: intraductal carcinoma. Cancer *86*: 429-438, 1999.

10 Mundhenke C, Meyer K, Drew S and Friedl A: Heparan sulfate proteoglycans as regulators of fibroblast growth factor-2 receptor binding in breast carcinomas, Am J Pathol *160(1)*: 185-94, 2002.

11 Götte M, Kersting C, Radke I, Kiesel L and Wülfing P: An expression signature of syndecan-1 (CD138), E-cadherin and C-MET is associated with factors of angiogenesis and lymphangiogenesis in ductal breast carcinoma *in situ*. Breast Cancer Res *9(1)*: R8, 2007.

12 Ojeh N, Hiilesvuo K, Wärri A, Salmivirta M, Henttinen T and Määttä A: Ectopic expression of syndecan-1 in basal epidermis affects keratinocyte proliferation and wound re-epithelialization, J Invest Dermatol *128(1)*: 26-34, 2008.

13 Lai TH, King JA, Shih IeM, Vlahos NF and Zhao Y: Immunological localization of syndecan-1 in human endometrium throughout the menstrual cycle, Fertil Steril *87(1)*: 121-126, 2007.

14 Loussouarn D, Campion L, Sagan C, Frenel JS, Dravet F, Classe JM, Pioud-Martigny R, Berton-Rigaud D, Bourbouloux E, Mosnier JF, Bataille FR and Campone M: Prognostic impact of syndecan-1 expression in invasive ductal breast carcinomas. Br J Cancer *17;98(12)*: 1993-1998, 2008.

15 Lendorf ME, Manon-Jensen T, Kronqvist P, Multhaupt HA and Couchman JR: Syndecan-1 and syndecan-4 are independent indicators in breast carcinoma, J Histochem Cytochem *59(6)*: 615-629, 2011.

16 Silverstein MJ, Parker R, Grotting JC, Cote RJ and Russell CA: Ductal carcinoma *in situ* (DCIS) of the breast: diagnostic and therapeutic controversies. J Am Coll Surg *192(2)*: 196-214, 2001.

17 Remmele W and Stegner HE: Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue, Pathologe *8(3)*: 138-140, 1987.

18 Sprague BL, Trentham-Dietz A, Nichols HB, Hampton JM and Newcomb PA: Change in lifestyle behaviors and medication use after a diagnosis of ductal carcinoma *in situ*. Breast Cancer Res Treat *124(2)*: 487-495, 2010.

19 Boland GP, McKeown A, Chan KC, Prasad R, Knox WF and Bundred NJ: Biological response to hormonal manipulation in oestrogen receptor positive ductal carcinoma *in situ* of the breast, Br J Cancer *21;89(2)*: 277-283, 2003.

20 Kabat GC, Kim M, Kakani C, Tindle H, Wactawski-Wende J, Ockene JK, Luo J, Wassertheil-Smoller S and Rohan TE: Cigarette smoking in relation to risk of ductal carcinoma *in situ* of the breast in a cohort of postmenopausal women. Am J Epidemiol *172(5)*: 591-599, 2010.

21 Kerlikowske K: Epidemiology of ductal carcinoma *in situ*. J Natl Cancer Inst Monogr *2010(41)*: 139-141, 2010.

22 Arbeitsgemeinschaft für Gynäkologische Onkologie In: Therapieempfehlungen zur Diagnostik und Therapie primärer und metastasierter Mammakarzinome- State of the Art 2013, Ductales Carcinoma *in situ* (DCIS) *6*: 72-89, 2013.

23 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*: 478-483, 2003.

24 Sahlin L, Jiang S, Von Schoultz B, Fernstad R, Skoog L and Von Schoultz E: Expression of syndecan-1 in paired samples of normal and malignant breast tissue from postmenopausal women, Anticancer Res *27*: 3045-3050, 2007

25 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 *15;61(14)*: 5562-5569, 2001.

26 Nikolova V, Koo CY, Ibrahim SA, Wang Z, Spillmann D, Dreier R, Kelsch R, Fischgräbe J, Smollich M, Rossi LH, Sibrowski W, Wülfing P, Kiesel L, Yip GW and Götte M: Differential ro,les for membrane-bound and soluble syndecan-1 (CD138) in breast cancer progression, Carcinogenesis *30(3)*: 397-407, 2009.

27 Su G, Blaine SA, Qiao D and Friedl A: Shedding of syndecan-1 by stromal fibroblasts stimulates human breast cancer cell proliferation *via* FGF2 activation.J Biol Chem *18;282(20)*: 14906-14915, 2007.

28 Maeda T, Desouky J and Friedl A:, Syndecan-1 expression by stromal fibroblasts promotes breast carcinoma growth *in vivo* and stimulates tumor angiogenesis, Oncogene *2;25(9)*: 1408-1412, 2006.

29 Tokes AM, Szasz AM, Farkas A, Toth AI, Dank M, Harsanyi L, Molnar BA, Molnar IA, Laszlo Z, Rusz Z and Kulka J: Stromal matrix protein expression following preoperative systemic therapy in breast cancer, Clin Cancer Res *15(2)*: 2009.

30 Burbach BJ, Friedl A, Mundhenke C and Rapraeger AC: Syndecan-1 accumulates in lysosomes of poorly differentiated breast carcinoma cells, Matrix Biol *22(2)*: 163-177, 2003.
31 Götte M, Kersting C, Ruggiero M, Tio J, Tulusan AH, Kiesel L and Wülfing P: Predictive value of syndecan-1 expression for the response to neoadjuvant chemotherapy of primary breast cancer, Anticancer Res *26(1B)*: 621-627, 2006.
32 Lundström E, Sahlin L, Skoog L, Hägerström T, Svane G, Azavedo E, Sandelin K and von Schoultz B: Expression of syndecan-1 in histologically normal breast tissue from postmenopausal women with breast cancer according to mammographic density. Climacteric *9(4)*: 277-282, 2006.
33 Hallberg G, Andersson E, Naessén T and Ordeberg GE: The expression of syndecan-1, syndecan-4 and decorin in healthy human breast tissue during the menstrual cycle. Reprod Biol Endocrinol *16;8*: 35, 2010.
34 Ibrahim SA, Yip GW, Stock C, Pan JW, Neubauer C, Poeter M, Pupjalis D, Koo CY, Kelsch R, Schüle R, Rescher U, Kiesel L and Götte M: Targeting of syndecan-1 by microRNA miR-10b promotes breast cancer cell motility and invasiveness *via* a Rho-GTPase- and E-cadherin-dependent mechanism, Int J Cancer., *15;131(6)*: E884-896, 2012.
35 Hassan H, Greve B, Pavao MS Kiesel L, Ibrahim SA and Götte M: Syndecan-1 modulates β-integrin-dependent and interleukin-6-dependent functions in breast cancer cell adhesion, migration, and resistance to irradiation, FEBS J *280(10)*: 2216-2227, 2013.
36 Baba F, Swartz K, van Buren R, Eickhoff J, Zhang Y, Wolberg W and Friedl A: Syndecan-1 and syndecan-4 are overexpressed in an estrogen receptor-negative, highly proliferative breast carcinoma subtype. Breast Cancer Res Treat *98(1)*: 91-98, 2006.

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