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Syndecan-1 is a potential biomarker for triple-positive breast carcinomas in Asian women with correlation to survival

Geok-Hoon Lim[1], MBBS, FRCS, *Puay-Hoon Tan*[2], MBBS, FRCPA, *Ana Richelia Jara-Lazaro*[2], MD, *Aye Aye Thike*[2], MMed Sci, *Wey-Cheng Sim*[3], BSc, *Von-Bing Yap*[4], MSc, PhD, *George Wai-Cheong Yip*[3], MBBS, PhD

INTRODUCTION While overexpression of syndecan-1 has been associated with aggressive breast cancer in the Caucasian population, the expression pattern of syndecan-1 in Asian women remains unclear. Triple-positive breast carcinoma, in particular, is a unique subtype that has not been extensively studied. We aimed to evaluate the role of syndecan-1 as a potential biomarker and prognostic factor for triple-positive breast carcinoma in Asian women.
METHODS Using immunohistochemistry, staining scores of 61 triple-positive breast carcinoma specimens were correlated with patients' clinicopathological variables such as age, ethnicity, tumour size, histological grade, lymph node status, lymphovascular invasion, associated ductal carcinoma *in situ* grade, recurrence and overall survival.
RESULTS Syndecan-1 had intense staining scores in triple-positive invasive ductal breast carcinomas when compared to normal breast tissue. On multivariate analysis, syndecan-1 epithelial total percentage and immunoreactivity score showed statistical correlation with survival (p = 0.02).
CONCLUSION The intense staining scores of syndecan-1 and their correlation with overall survival in patients with triple-positive breast carcinoma suggest that syndecan-1 may have a role as a biological and prognostic marker in patients with this specific subtype of breast cancer.

Keywords: Asian women, biomarker, syndecan-1, triple-positive breast cancer

INTRODUCTION

Breast carcinoma is the commonest malignancy among Singaporean women, with an increasing annual incidence rate of about 3% since 1968.[1] Although there are similarities in breast cancers between Asian and Western countries, there also exists distinct differences. The peak age of breast cancer in Asian women is earlier, at 40–50 years, compared to 60–70 years in Western countries.[2] Also, the mortality rate of breast cancer in Asia is increased while that in Western countries is declining.[2] Even though this phenomenon could partly be explained by late detection and limited healthcare access among Asian women, there is some evidence that breast cancers in Asian women could have more aggressive tumour biology, such as higher tumour grade and hormone receptor negativity.[3]

Syndecan-1 is a transmembrane heparan sulphate proteoglycan. Previous studies have shown that heparan sulphate is overexpressed in breast carcinoma, regulating cancer proliferation and invasion. Overexpression of syndecan-1 is associated with aggressive breast cancer among Caucasian populations[4,5] and increased mortality risk.[6] However, the expression pattern of syndecan-1 in triple-positive breast carcinoma in Asian women is unknown.

In this study, we specifically evaluated the role of syndecan-1 as a biomarker and prognostic factor in a subgroup of patients with triple-positive breast cancer – defined as breast carcinoma with positive expression of oestrogen and progesterone receptors (ER/PR) and cerbB2 (HER2), which has been reported to account for 6.2%–15.5%[7,8] of all breast carcinomas.[9] While triple-negative breast cancer has been associated with poorer survival when compared to the other molecular subtypes,[10] data on triple-positive breast carcinoma is less widely published. Triple-positive breast cancer was selected in the present study, as it is a unique subtype with limited data in the literature.

METHODS

Archival specimens of triple-positive breast cancer and non-cancerous breast tissues from Asian women were obtained from the Department of Pathology at Singapore General Hospital, Singapore, between 2005 and 2007. ER (Neomarkers RM 9101-S, Clone: SP1, Thermo Fisher Scientific, CA, USA [dilution 1:50]) and PR (Neomarkers RM9102-S, Clone: PgR636, Thermo Fisher Scientific, CA, USA [dilution 1:200]) were then defined as positive if at least 10% of lesional cells displayed a minimal 2+ nuclear staining pattern. For cerbB2 (Neomarkers RM9103-S, Clone: SP3, Thermo Fisher Scientific, CA, USA [dilution 1:200]), a test was considered positive if at least 10% of the lesional cells exhibited 3+ cell membrane staining and a borderline/equivocal result was given when at least 10% of the lesional cells showed 2+ cytoplasmic membrane staining. Fluorescence *in situ* hybridisation was performed for the equivocal specimens to determine the final cerbB2 status.

Current updated guidelines from the College of American Pathologists/American Society of Clinical Oncology recommend that ER/PR be considered positive if at least 1% of the lesional cells show staining.[11] For cerbB2, at least 30% of the lesional cells have to demonstrate a 3+ cell membrane staining before it is to be deemed positive.[12] However, as management protocols in our group of women were based on our original definition

[1]Breast Department, KK Women's and Children's Hospital, [2]Department of Pathology, Singapore General Hospital, [3]Department of Anatomy, National University of Singapore, [4]Department of Statistics and Applied Probability, National University of Singapore, Singapore
Correspondence: Dr Lim Geok-Hoon, Consultant, Breast Department, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899. ghlimsg@yahoo.com.sg

of a 10% threshold, this cutoff was applied for the purpose of the current study. Results that failed to fulfil our criteria were regarded as negative.

Representative areas were selected for tissue microarray (TMA) construction by pathologists using the Beecher microarrayer (Beecher Instruments, Sun Prairie, WI, USA). Each TMA was constructed using 1-mm cores, with two cores per specimen. Immunohistochemical staining was carried out on tissue microarray blocks using a syndecan-1 antibody by the labeled streptavidin-biotin method. Briefly, tissue microarray sections were deparaffinised, rehydrated and endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide for 10 min. Antigen retrieval was performed using microwave at 100°C for 20 min in citrate buffer. Primary mouse syndecan-1 monoclonal immunoglobulin G1 antibody (Clone: DL-101; vCell Science, Santa Cruz, CA, USA) at 1:25 dilution was then added and incubated overnight at 4°C. Negative controls, which were normal breast tissue from the same patients, were obtained by omitting the primary antibody. After washing with Tris-buffered saline, a secondary antibody was added and incubated for 1 hr at room temperature. Visualisation was achieved by using diaminobenzidine (EnVision™+ Dual Link system-HRP [DAB+]; Dakocytomation, Carpinteria, CA, USA) as the substrate followed by counterstaining with haematoxylin.

The immunoreactivity of both epithelial (cytoplasmic) and stromal components was examined by two assessors who were blinded to the patient's clinical outcome. Staining intensity was scored as '0', '1+', '2+' and '3+', indicating nil, mild, moderate and marked intensity, respectively. The proportion of tumour cells stained was recorded as total percentage (TP), immunoreactivity score (IRS; the sum of each staining intensity × its respective TP) and weighted average intensity (WAI) score, which was calculated as the ratio of IRS:TP. Comparison was made between the two assessors' scores; when in conflict, the score was reviewed together and a consensus score agreed upon. These scores were then analysed against clinicopathological variables such as age, ethnicity, tumour size, histological grade, lymph node status, lymphovascular invasion and associated ductal carcinoma *in situ* (DCIS) grade, which were retrieved from the patients' pathology reports.

All patients underwent surgery for their breast cancer. Surgery included a lumpectomy or mastectomy with axillary dissection. Based on the final histology findings, patients underwent adjuvant therapy, as per recommendations discussed at multidisciplinary tumour board meetings. Immunoreactivity scores of triple-positive breast carcinoma were correlated with recurrence and survival follow-up till 17 May 2011. Overall survival was calculated from the date of histological diagnosis of breast malignancy to the date of death from any cause or to the last known follow-up date. Recurrence was calculated from the date of histological diagnosis of breast malignancy to the date of recurrence either as locoregional or distant metastases.

All analyses were performed using the Statistical Package for the Social Sciences version 17 (SPSS Inc, Chicago, IL, USA) and R version 2.11.1. The relationship between the clinicopathological variables and immunohistochemical scores was tested using

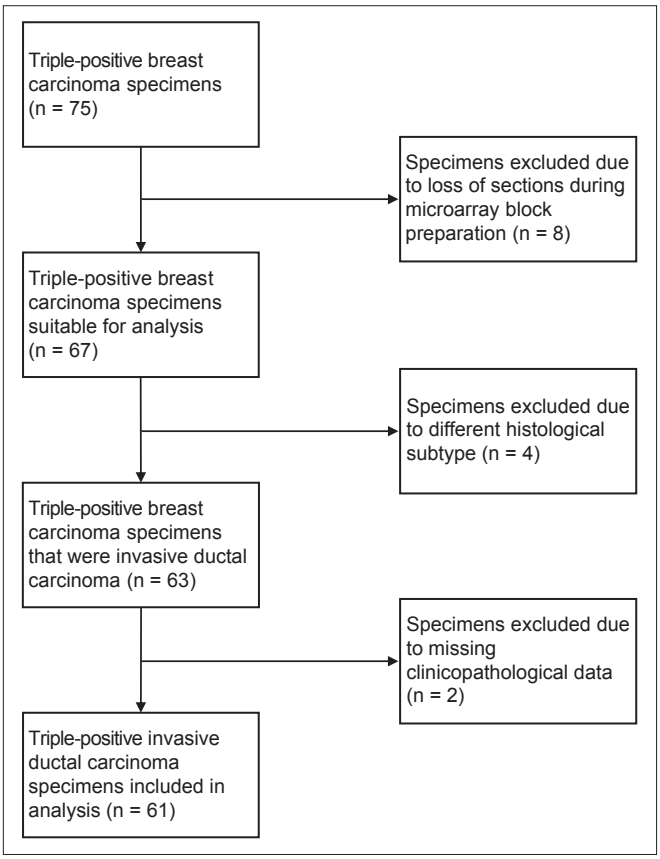

Fig. 1 Schematic representation of patients' recruitment.

t-test, chi-square test or Fisher's exact test. Multivariate analysis using logistic regression was performed to assess for any independent relationship between the immunohistochemical scores and clinicopathological variables, and also between the immunohistochemical scores and overall survival and recurrence after adjusting for the clinicopathological variables. A p-value < 0.05 was considered to be statistically significant. The survival data was analysed using the Kaplan-Meier curve and log-rank test.

RESULTS

Of the 75 patients with triple-positive breast carcinoma, only 67 patients with breast cancer and 61 normal-matched breast tissues were suitable for analysis. The remaining specimens were rendered unsuitable due to loss of some sections of the individual cores during cutting of the tissue microarray blocks (n = 8). Of the 67 patients with breast cancer, 63 patients had invasive ductal carcinoma (IDC). The remaining four patients had invasive breast cancer of different histological subtypes, such as lobular (n = 2), papillary (n = 1) and mucinous (n = 1) carcinoma, and were thus excluded. Clinicopathological data were missing for two patients. Hence, 61 triple-positive IDC specimens and their microarrays were eventually analysed (Fig. 1).

The mean and median ages of the final cohort were 52.2 (range 28–90) and 50.0 years, respectively (Table I). A majority of the patients was of Chinese (n = 49, 80.3%) ethnicity. Mean tumour size was 31.5 mm and a majority of the patients (n = 44, 72.1%) had high-grade IDC.

Table I. Demographic characteristics of patients with triple-positive invasive ductal carcinoma (n = 61).

Clinicopathological parameter	No. (%)
Age* (yr)	52.2 (28–90); 50.0
Ethnicity	
Chinese	49 (80.3)
Malay	9 (14.8)
Indian	2 (3.3)
Other	1 (1.6)
Tumour size* (mm)	31.5 (5–100); 30.0
Lymphovascular invasion	
Absent	37 (60.7)
Present	24 (39.3)
No. of lymph nodes	
0 nodes	20 (32.8)
1–3 nodes	17 (27.9)
> 3 nodes	14 (22.9)
Not available	10 (16.4)
Histological grade	
IDC grade 1	2 (3.3)
IDC grade 2	15 (24.6)
IDC grade 3	44 (72.1)
Associated DCIS	
Absent	5 (8.2)
Low grade	5 (8.2)
Intermediate grade	7 (11.5)
High grade	44 (72.1)
Syndecan-1 epithelial staining†	
TP score	65.4 (0–90)
IRS	83.5 (0–180)
WAI score	1.13 (0–2)
Syndecan-1 stromal staining†	
TP score	9.3 (0–70)
IRS	9.3 (0–70)
WAI score	0.74 (0–1)

*Data is presented as mean (range); median. †Data is presented as mean (range). DCIS: ductal carcinoma *in situ*; IDC: invasive ductal carcinoma; IRS: immunoreactivity score; TP: total percentage; WAI: weighted average intensity

Table II. Correlation of clinicopathological parameters with syndecan-1 epithelial immunoreactivity scoring in patients with triple-positive invasive ductal carcinoma (n = 61).

Clinicopathological parameter	Syndecan-1 epithelial component staining*		p-value†
	IRS ≤ 100	IRS > 100	
Mean age (yr)			0.309
≤ 45 (n = 19)	17 (89.5)	2 (10.5)	
> 45 (n = 42)	32 (76.2)	10 (23.8)	
Ethnicity			0.198
Chinese (n = 49)	40 (81.6)	9 (18.4)	
Malay (n = 9)	7 (77.8)	2 (22.2)	
Indian (n = 2)	2 (100)	0 (0.0)	
Other (n = 1)	0 (0.0)	1 (100)	
Mean tumour size			1.000
≤ 55 mm (n = 56)	45 (80.4)	11 (19.6)	
> 55 mm (n = 5)	4 (80.0)	1 (20.0)	
Lymphovascular invasion			0.019‡
Absent (n = 37)	26 (70.3)	11 (29.7)	
Present (n = 24)	23 (95.8)	1 (4.2)	
No. of lymph nodes			0.065
0 (n = 21)	16 (76.2)	5 (23.8)	
1–3 (n = 17)	16 (94.1)	1 (5.9)	
> 3 (n = 14)	12 (85.7)	2 (14.3)	
NA (n = 9)	5 (55.6)	4 (44.4)	
Histological grade			0.711
IDC 1 (n = 2)	2 (100)	0 (0.0)	
IDC 2 (n = 15)	11 (73.3)	4 (26.7)	
IDC 3 (n = 44)	36 (81.8)	8 (18.2)	
Associated DCIS grade			0.025‡
Absent (n = 5)	3 (60.0)	2 (40.0)	
Low (n = 5)	2 (40.0)	3 (60.0)	
Intermediate (n = 7)	5 (71.4)	2 (28.6)	
High (n = 44)	39 (88.6)	5 (11.4)	

*Data is presented as no. (%). †p-value is calculated for difference between IRS ≤ 100 and IRS > 100. ‡p < 0.05 is considered statistically significant. DCIS: ductal carcinoma *in situ*; IDC: invasive ductal carcinoma; IRS: immunoreactivity score; NA: not available

There was statistically significant syndecan-1 staining of the malignant epithelial component in tumour tissue from the triple-positive breast cancer group (Fig. 2) compared to the benign epithelium of normal tissue (TP: 65.4% vs. 16.3%, $p < 0.001$). The stromal component of tumour tissue also showed statistically significant syndecan-1 staining when compared to normal breast tissue (WAI score: 0.74 vs. 0.49, $p = 0.018$). Fig. 3 shows minimal syndecan-1 immunohistochemical staining in normal breast tissue.

The IRS of syndecan-1 epithelial staining in triple-positive breast cancer specimens correlated inversely with lymphovascular invasion ($p = 0.019$) and associated DCIS grade ($p = 0.025$) (Table II). Multivariate analysis revealed no significant association between syndecan-1 epithelial IRS score and the clinicopathological parameters, although lymphovascular invasion showed a mild association ($p = 0.07$). The stromal component of the triple-positive breast cancer group did not show any statistical association with clinicopathological variables.

For survival analysis, six patients with triple-positive breast carcinoma were found to be lost to follow-up. After a median follow-up of 54 months, triple-positive patients had an overall median survival of 55 (range 21–74) months (Fig. 4). The overall survival was worse with increased staining scores, along with epithelial TP, IRS and WAI scores on univariate analysis. Of these, epithelial TP and epithelial IRS scores showed the most statistical significance ($p = 0.01$). Multivariate analysis of the staining scores, after adjusting for other clinicopathological factors, revealed statistically significant correlation between epithelial TP and epithelial IRS scores and survival ($p = 0.02$). About 20% of our patients developed recurrence, with distant metastasis and local recurrence occurring in nine and two patients, respectively. Statistical analysis of these patients' epithelial scores, however, did not reveal any correlation with recurrence. Stromal staining scores also did not reveal any statistical correlation with survival or recurrence.

DISCUSSION

Syndecan-1 is a transmembrane heparan sulphate proteoglycan that is involved in cell-cell cohesion, regulation of cell-matrix adhesion [13] and control of growth factor signalling. As a result, stromal syndecan-1 staining has been reported to be induced in invasive breast cancer associated with a reduction in epithelial

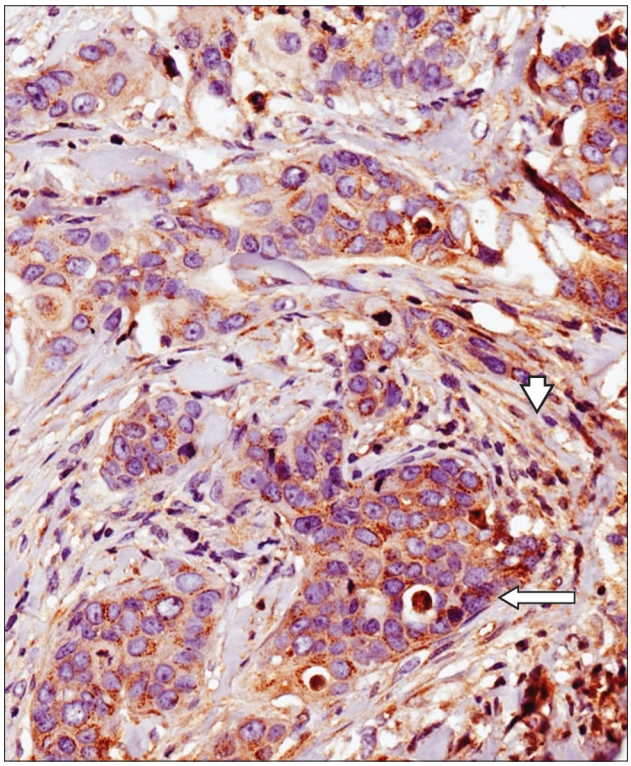

Fig. 2 Photomicrograph of invasive ductal breast carcinoma specimen shows positive syndecan-1 staining of epithelial cytoplasmic (arrow) and stromal (arrowhead) components (Immunohistochemical staining, × 40).

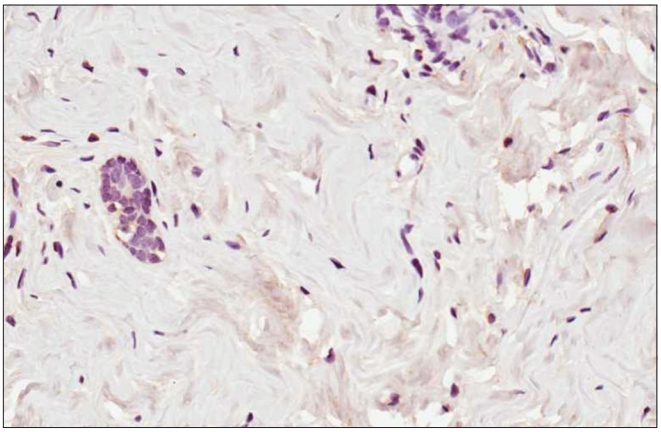

Fig. 3 Photomicrograph of normal breast tissue shows minimal syndecan-1 staining (Immunohistochemical staining, × 20).

syndecan-1 staining.[14] However, Leivonen et al have shown differing results, with epithelial syndecan-1 staining being associated with worse survival.[15] While data from Caucasian populations suggests that overexpression of syndecan-1 could be a biomarker of invasive breast cancer, little is known about the expression of syndecan-1 in Asian women, especially among patients with the triple-positive subtype of breast carcinoma.

Triple-negative breast cancer has been widely studied and advocated in several studies[16,17] to have a poor prognosis among the various breast cancer subtypes. In contrast, there is limited data on triple-positive breast cancer in the literature. Although patients with triple-positive breast cancer may benefit from the use of hormonal therapy and immunotherapy – unlike patients with triple-negative breast cancer – triple-positive

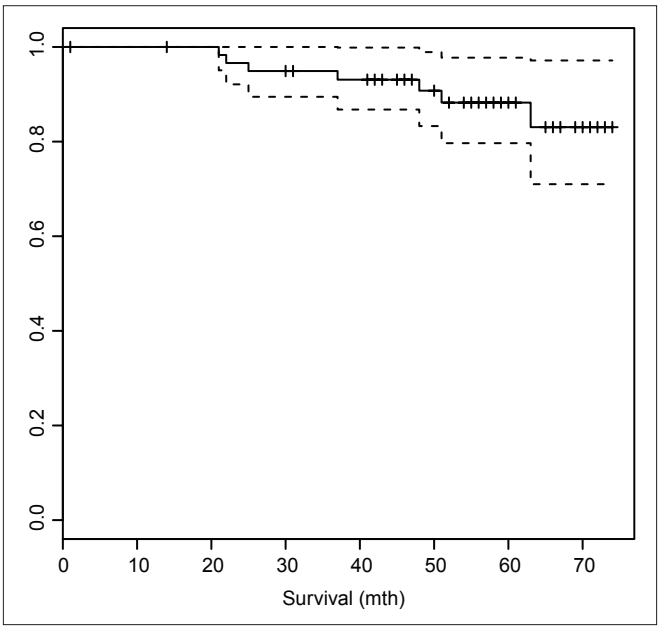

Fig. 4 Kaplan-Meier curve of triple-positive patients (solid line) with 95% confidence intervals (broken lines).

breast cancer is often not reported as the subtype with the best survival,[16,17] and its survival rate remains unpredictable. Therefore, a prognostic biomarker for triple-positive breast cancer is imperative. For this reason, the role of syndecan-1 as a survival biomarker for triple-positive breast cancer was evaluated in the present study.

In this study, there was significant syndecan-1 staining of dual epithelial and stromal components in tissues from the triple-positive breast cancer group compared to normal breast tissue. However, only the epithelial component of syndecan-1 staining in the triple-positive group showed inverse correlation with associated DCIS grade and lymphovascular invasion.

The relationship between associated DCIS and syndecan-1 is not well defined in the literature. Götte et al have shown that the expression of syndecan-1 was significantly more common in the subgroup of patients with pure DCIS than in those with DCIS and coexisting infiltrating carcinoma.[18] In the earlier study, the authors found no significant association between syndecan-1 staining and high-grade DCIS.[18] In our triple-positive breast cancer group, there was a statistically significant association of lower syndecan-1 epithelial expression in higher grades of associated DCIS in patients with IDC. The underlying mechanism is, however, unclear.

Lymphovascular invasion has been associated with an adverse outcome in breast cancer.[19] In our study, we were unable to show a positive correlation between syndecan-1 levels and the presence of lymphovascular invasion. In the current literature, little is known about the association of syndecan-1 with lymphovascular invasion, and thus, the significance of our finding will need to be confirmed by future studies.

In our study, overall survival correlated statistically with syndecan-1 epithelial TP and epithelial IRS scores, with overall survival being worse with increased epithelial TP and epithelial IRS scores. In their study on the prognostic value of syndecan-1

expression in breast cancer, Leivonen et al also demonstrated similar findings, where the overall survival for patients with epithelial expression was worse than for those without syndecan-1 epithelial expression.[15] This may suggest a prognostic role for syndecan-1 epithelial expression in patients with breast carcinoma.

However, the mechanism in which syndecan-1 is implicated in breast cancer is still unclear, although studies have shown that induction of syndecan-1 in stromal fibroblasts stimulates breast cancer cell growth,[20] regulates extracellular matrix fibre organisation and malignant cell motility,[21] as well as enhances tumour angiogenesis.[22] Syndecan-1 has also been reported to have more intense staining of the stromal component of breast cancer tissue compared to normal breast tissue,[23] in keeping with our study findings. We could not, however, demonstrate any statistical correlation between stromal scores and the clinicopathological variables studied, or with overall survival or recurrence.

To our knowledge, this is the first study that investigated the biomarker and prognostic potential of syndecan-1 in Asian women with triple-positive breast cancer. The conclusions of our study, with a median follow-up of 54 months, bear significance for populations of Asian women with the triple-positive subtype of breast carcinoma. However, the present study was not without limitations. The small sample size, its retrospective nature, the presence of incomplete data for patients who had to be consequently excluded and those who were lost to follow-up posed certain challenges. Also, the underlying mechanism by which syndecan-1 promotes breast cancer growth remains unclear and warrants further studies.

In conclusion, the intense staining scores for syndecan-1 in the epithelial component of triple-positive invasive ductal breast carcinoma tissue when compared with normal breast tissue, as well as the correlation of its epithelial TP and IRS staining scores with overall survival, suggest that syndecan-1 may have a role as a biomarker and prognostic marker for Asian women with the triple-positive subtype of breast carcinoma. Further studies are warranted in order to elucidate the underlying mechanisms by which syndecan-1 may be implicated in breast cancer.

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