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Syk Tyrosine Kinase Expression during Multistep Mammary Carcinogenesis

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Aim To analyze the expression of Syk tyrosine kinase, recently implicated as a tumor suppressor in mammary carcinogenesis, during the multi step development of human breast carcinoma.

Methods Syk expression was examined in invasive carcinomas of the breast and in corresponding premalignant epithelial lesions in 50 women, using immunohistochemical method and semi-quantitative scoring system (H-score). The results were correlated with the expression of Syk in the lymph node metastases.

Results Syk was strongly and uniformly expressed in normal mammary epithelium (H score = 2.36 ± 0.75), and no significant reduction in Syk expression was observed in hyperplastic lesions (H = 2.31 ± 0.87), carcinoma in situ (H = 1.90 ± 0.93), or invasive carcinomas (H = 1.83 ± 0.88). Loss of Syk expression (defined as H≤1.0) was seen in approximately 1/5 of invasive breast carcinomas, but the majority of metastatic carcinomas (15/21) still strongly expressed Syk, irrespective of Syk expression in primary tumor.

Conclusion The loss of Syk characterizes a subset of breast carcinomas but does not apparently contribute to the development of the metastatic potential of these carcinomas, limiting the potential diagnostic utility of immunohistochemical test in the prediction of malignant behavior.

Syk (spleen tyrosine kinase) is involved in coupling activated immunoreceptors to downstream signaling events in hematopoietic cells, leading to proliferation, differentiation, and phagocytosis (1-4). However, the expression of Syk is not restricted to hematopoietic cells and many epithelial cell lines express this kinase (5). Moreover, mouse mammary tissue was found to strongly express Syk in an immunohistochemical assay (5). The function of Syk in non-hematopoietic cells is not known. It has been recently postulated by Coopman et al (6) that SYK gene might act as a tumor suppressor in human breast carcinomas. These authors found that Syk was strongly expressed in normal human breast tissue and mammary carcinoma cell lines of low tumorigenic potential, but was undetectable in highly tumorigenic breast cancer cell lines. Transfection of wild-type SYK into SYK-negative breast cancer cell line caused the reduction of tumor growth and metastasis formation. This finding prompted us to investigate the expression of this protein in patients with breast carcinoma and how it correlates with the stages of cancer progression. The development of breast carcinoma is believed to progress through multiple steps characterized by cumulative genetic alterations associated with the development of characteristic morphologic lesions (7-9). Investigation of the expression of Syk kinase in these lesions could identify the key step characterized by the loss of this protein and determine if this loss affects the rate of metastatic disease in humans.

Material and Methods

Patients

The study group consisted of a cohort of 50 women admitted to the John Sealy Hospital of

the University of Texas Medical Branch in Galveston between January 1997 and December 2001 and treated with mastectomy for mammary carcinoma. The sample was a convenient sample, representing about an eighth of all patients receiving this treatment during the study period. The patients were further divided into 2 groups: with metastasis in the axillary lymph nodes (33 patients) and without pathologic evidence of lymph node metastasis (14 patients); 3 patients had no axillary lymph nodes resected.

Tissues

Formalin-fixed, paraffin-embedded tissues were used in the study. Histomorphologic criteria used to classify epithelial lesions were previously published (9-12). These included normal ductal and lobular epithelium, ductal and lobular hyperplasia, atypical ductal hyperplasia, lobular and ductal carcinoma in situ, and invasive ductal and lobular carcinoma. Axillary lymph node metastases were available for evaluation in 21 cases.

Immunohistochemical Staining

Rabbit polyclonal anti-Syk IgG antibody, sc-1077 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used at 1:150 dilution. Slides were deparaffinized, subjected to heat-induced epitope retrieval (1 mmol/L EDTA, pH 8.0) and stained using automated procedure (9). Antigen blocking experiment to test the specificity of the antibody was performed using the blocking peptide sc-1077P, according to the manufacturer's instructions (Santa Cruz Biotechnology).

Scoring of Immunohistochemical Assays

Tumor heterogeneity in Syk expression was assessed using semi-quantitative histo-score method (H-score) (9). The staining intensity (I) was graded as 0 – no staining, 1 – weak, 2 – moderate, and 3 – strong and the proportion (P) of cells with the observed intensity was recorded, (from 0 – none to 1.0 – the entire population). H-score for each histologic category was determined by the sum of all I×P products. Therefore, an H-score of 0.0 was obtained if no staining (I=0) was observed in the entire population of cells (P=1.0). The maximum H score of 3.0 would be obtained if all of the cells (P=1.0) in the lesion stained with maximal intensity (I=3).

Estrogen Receptor Status and DNA Tumor Flow Cytometry

Estrogen receptor status (determined as positive or negative), as well as tumor DNA flow analysis results were retrieved from the pathology files, and were not repeated in this study.

Results

Patients and Tumor Characteristics

The average age (±standard deviation) of the patients was 54.3±11.5 years. There were 45 invasive ductal and 5 invasive lobular carcinomas. Average tumor size was 3.4±2.18cm (n=43). Forty seven patients had axillary nodal dissection; 33 of them with evidence of nodal disease. No significant difference in age (56.4±12.2 vs 52.8±10.8 years) was observed between node positive and node negative patients. Primary tumor size was expectedly larger in the node positive tumors, but the difference did not reach statistical significance (3.79±2.26 vs 2.48±1.40 cm, $P>0.10$, *t* test).

Syk Expression in Non-Neoplastic Mammary Epithelium

Syk was strongly expressed in normal luminal cells of ducts and lobules (mean H±standard deviation=2.36±0.75; n=38), whereas myoepithelial cells did not stain or were weakly positive (Fig. 1). In addition, lymphocytes, periductal fibroblasts, endothelial cells, nerve sheath cells, and scattered adipocytes were also expressing Syk with various intensities. Luminal cells with apocrine and lactational changes also strongly expressed this antigen. No staining was observed with the blocked antibody, confirming the specificity of the reaction.

Syk Expression in Invasive Carcinoma and Precursor Lesions

Ductal hyperplasia showed no significant reduction in Syk expression (H=2.31±0.87; n=17) in comparison to the surrounding nonproliferative epithelium. A small, not statistically significant reduction in the expression of Syk was seen with in situ carcinomas (H=1.90±0.93; n=19). Nearly identical expression of Syk (H=1.83±0.88; n=50) was seen in invasive carcinomas (Fig. 2). Overall, 11 out of 50 (22%) carcinomas showed marked reduction in Syk expression (defined as H less than or equal to 1.0), due to the various proportions of neoplastic cells without

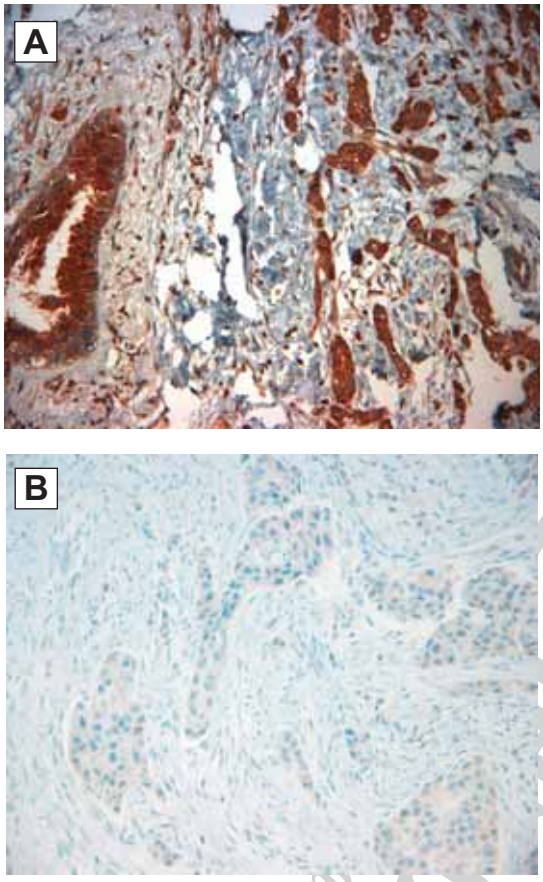

Figure 1. A. Strong expression of Syk in luminal cells of normal ducts (left side) and invasive ductal carcinoma (right side). **B.** An example of Syk-negative invasive ductal carcinoma. Immunohistochemical stain with DAB as a chromagen, ×20 magnification.

any expression of this antigen (I = 0; Fig. 1). One out of five lobular carcinomas and 10 out of 45 ductal carcinomas showed Syk expression H≤1.0.

Estrogen receptor (ER) expression status of primary carcinomas was available in 44 patients; no significantly different expression of Syk was seen between cases deemed ER positive or negative (H = 1.95 ± 0.85 in ER+ carcinomas vs H = 1.78 ± 0.91 in ER- carcinomas).

We further evaluated the relationship between Syk H-score in primary tumors and the percentage of tumor cells in S-phase (using flow cytometry, data were available in 12 patients) and found negative trend (correlation coefficient r = -0.43) that did not reach statistical significance (Figure 3).

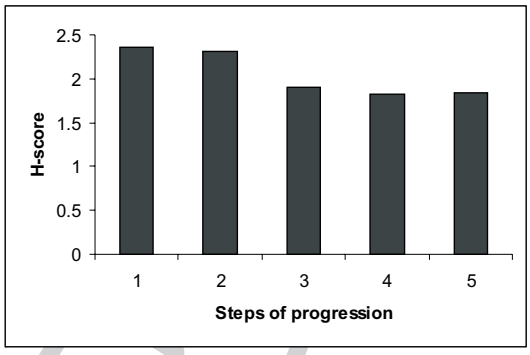

Figure 2. Expression of Syk in normal mammary epithelium during the multistep progression of mammary carcinoma. 1 – normal luminal epithelium, 2 – ductal hyperplasia without atypia, 3 – carcinoma in situ, 4 – invasive carcinoma, 5 – lymph node metastasis of mammary carcinoma.

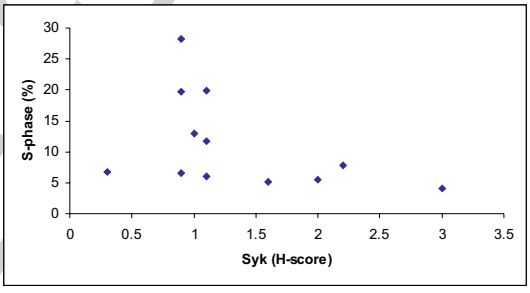

Figure 3. Percentage of cells in S-phase as a function of the H-score in primary carcinoma.

Syk in Metastatic Carcinomas

Lymph node metastases were available for evaluation in 21 cases. Five cases (24%) showed H score less than 1.0. There was no statistical difference between SYK expression in metastatic carcinomas (H = 1.8) and corresponding invasive carcinomas (H = 2.1), nor was there a difference in Syk expression between invasive carcinomas with or without concomitant lymph node metastases (H = 1.95 ± 0.9 and 1.72 ± 0.8, respectively). In one case, characterized by a significant loss of Syk expression in primary tumor (H = 1.0) and further loss in the lymph node metastasis (H = 0.5), distant bone metastasis was detected one year later but showed strong and uniform (H = 3.0) expression of the antigen.

Discussion

Syk is a non-receptor type protein tyrosine kinase that participates in transduction of re-

ceptor activated signals inside the cells. Human SYK locus was mapped to chromosome 9q22. This enzyme is essential for the development and function of hematopoietic cells. It participates in clonal expansion of pre-B cells, early development of T-cells, and plays a critical role in FcγRs signaling in macrophages and neutrophils essential for phagocytosis. In non-hematopoietic cells the function of SYK is less well understood. So far, SYK has been shown to be involved in the differentiation of mouse adipocytes, and in endothelial cell function (13,14). Early studies on Syk showed that mouse mammary gland strongly expressed this protein (5); however it was not until recently that this observation was explored in human breast cancer models. Coopman et al (6) found that Syk was absent in highly tumorigenic breast cancer cell lines, but was easily detected in normal human mammary gland tissue and several mammary carcinoma cell lines characterized by low tumorigenic potential in atymic nude mice. Furthermore, ability to form lung metastases after injection in the tail vein of mice was much higher in Syk- than in Syk+ cells.

The loss of expression of SYK appears to be primarily achieved through an epigenetic pathway of 5′ CpG hypermethylation (15,16). This silencing was found in approximately 32% of random breast carcinomas (15). Alternative splicing has been recently described and occurs exclusively in invasive carcinomas but not in matched normal tissues (17). The alternatively spliced variant lacks nuclear localization signal and does not affect the cell invasion potential.

The loss of Syk protein kinase expression observed in our study is less dramatic than the loss of Syk mRNA observed in a recently published study (18) using similar approach (semi quantitative in-situ hybridization evaluation). The methodological differences are likely the cause, as mRNA levels do not necessarily correlate with the concentration of proteins.

The inverse relationship between Syk mRNA expression and cellular proliferation (Ki-67 positive cells) was previously observed (18) during the tumor progression (from normal epithelium to invasive carcinoma) but no difference between the groups of Syk+ and Syk- carcinomas was found. Our results on the percentage of cells in S-phase as a function of Syk expression corroborate the observation, but a larger scale investigation is needed for confirmation.

Toyama et al (19) showed that patients who had Syk mRNA levels in primary carcinomas lower than 50% of corresponding normal tissues are more likely to develop distant metastasis and to die of the disease. However, our study showed that, despite the loss of Syk in a subset of invasive mammary carcinomas (both ductal and lobular), this loss did not increase the likelihood of a lymph node metastasis, and determinations of the Syk status may not predict the lymph node involvement. The detection of specific splice variants may improve on this diagnostic requirement (17). It is also possible that the loss of Syk enhances the ability of mammary carcinoma cells to establish distant growth (e.g. lung metastasis) and that this characteristic does not play a critical role in establishment of metastasis in a milieu of a lymph node (20).

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