Expression of Syk in Invasive Breast Cancer: Correlation to Proliferation and Invasiveness

KATERINA REPANA1, Konstantinos Papazisis2, Periklis Foukas3,4, Rozalia Valeri5, Alexandros Kortsaris6, Eleni Deligiorgi3 and Dimitrios Kyriakidis1

1Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124, Thessaloniki; 2Department of Medical Oncology and 3Department of Cytopathology, Theagenion Cancer Hospital, Al. Simeonides St. 2, 54007, Thessaloniki; 3Department of Pathology, Henry Dunant Hospital, Mesogion St. 107, 11526, Athens; 4Department of Histopathology and Molecular Pathology, Locus Medicus Laboratories, Riangour Ave. 53, 11523, Athens; 5Laboratory of Biochemistry, Department of Medicine, Democritus University of Thrace, 68100, Alexandroupolis, Greece

Abstract. Background: Spleen tyrosine kinase (Syk) kinase has recently been considered as a tumor suppressor gene in breast cancer. Materials and Methods: Syk expression in patients with invasive breast cancer was immunohistochemically assessed. Results: Decreased expression was found in 26% of the specimens examined. In cases with vascular invasion, expression of Syk was lost in the intravascular emboli. A significant relationship between increased proliferation levels (as estimated by the proliferative index, Ki67) and decreased Syk expression (p<0.05) was found. Conclusion: Our data suggest that Syk protein expression inversely correlates with the proliferation and invasive capacity of breast cancer.

Spleen tyrosine kinase (Syk) is a non-receptor protein tyrosine kinase that is widely expressed in hematopoietic cells and is one of the two members of the Syk family (Syk and ZAP-70). Syk contains two Src homology 2 domains and multiple autophosphorylation sites (1). Syk is activated and assembled into signalling complexes via the binding of its tandem SH2 domains to the phosphorylated immunoreceptor tyrosine-based activating motif (ITAM) (2). In blood cells, it couples immunoreceptors to signal transduction pathways regulating cell proliferation, differentiation, motility, degranulation, phagocytosis and cytotoxicity (3-6). Besides hematopoietic cells, Syk is widely expressed in other cell types, like epithelial cells, hepatocytes, fibroblasts, neuronal cells and vascular endothelial cell lines, where it plays a variety of roles that are not yet completely understood (7).

Syk is also expressed in the mammary glands (8). There are multiple lines of evidence that Syk plays the role of tumor suppressor in breast tissue. Syk mRNA is reduced in invasive breast carcinoma tissue and cell lines (9-11). A decrease or loss of Syk expression seems to be associated with malignant phenotypes such as increased motility and invasion (9). Loss of Syk was linked to poor prognosis and distant disease-free survival in breast cancer patients (10). Loss of Syk expression was also detected in gastric carcinoma. This event was associated with lymph node metastasis and the malignant property of gastric cancer (12, 13). Syk was found to regulate cellular proliferation, as the levels of Ki67 protein increased while Syk mRNA was decreased, possibly through the inhibition of the Src kinase activity (11). Moreover, 5 CpG hypermethylation was associated with loss or reduction of Syk gene expression in breast, as well as in gastric cancer (12, 14). Reactivation of its expression, through reversion of methylation, was accompanied by a significant decrease of breast cancer cell invasion in vitro (15). Syk was stated to be a centrosomal kinase that negatively affects cell division (16). Its role was proven to be in the control of mitosis of breast cancer cells (9, 11, 16). Recently, Syk was also found to exhibit transcription repressor activity, down-regulating the expression of the FRA1 and Cyclin D1 oncogenes (17).

The chemokines are a family of small proteins that, together with their receptors, play an important role in leukocyte growth, migration and host inflammatory responses (18). Chemokines have been implicated in the process of tumor cell migration, invasion and metastasis (19). Specific chemokine receptors were found to be highly expressed on breast cancer cells, whilst their ligands were expressed at high
levels in all target organs for breast cancer metastasis (20). IL8, the first chemokine to be characterized, has been found to promote metastatic potential in several studies. It promotes the growth and migration of human melanoma, liver, pancreatic and breast cancer cells (21-23). Moreover, it acts as an autocrine (21, 22) and angiogenic (24) growth factor.

In the present study, Syk protein expression in human invasive breast carcinomas and the relationship between its expression and clinicopathological factors were estimated. Additionally, we estimated the levels of expression of the two IL8 receptors, CXCR1 and CXCR2, in the same samples and explored their correlation with Syk protein expression.

Materials and Methods

Tissue. Thirty-nine formalin-fixed and paraffin-embedded breast tissue samples were obtained from the Henry Dunant Hospital, Athens, Greece. Samples were derived from patients with invasive breast carcinoma, of ductal or lobular histology, who underwent surgery with curative intent. The patients consisted of 38 females and 1 male, with ages ranging from 36 to 73 years (mean, 55 years). The size of the tumors ranged between 0.7 and 8.5 cm. The histological grade was estimated according to the criteria developed by Elston and Ellis (25).

Immunohistochemistry. Formalin-fixed, paraffin-embedded 5-μm tissue sections were deparaffinized, rehydrated and subjected to a microwave antigen retrieval method, by boiling in 10 mmol/L sodium citrate buffer (pH 6.0) (26) for 15 min and allowing to cool at room temperature. Non-specific immunoglobulin binding was blocked by incubating the sections in blocking reagent (Biocare Medical, CA, USA) at room temperature for 15 min. The slides were then incubated at 4°C, overnight with primary antibodies (Anti-Syk, anti-CXCR1, anti-CXCR2; Rabbit Polyclonals, Santa Cruz, CA, USA) in a 1:50 dilution. Following extensive washing, the avidin-biotin-peroxidase method was used for detection of the bound antibody (27). Slides were then incubated with biotinylated secondary antibody for 45 min and finally with horseradish peroxidase-streptavidin complex (Dako Norden A/S, Glostrup, Denmark). Slides were stained with 3,3′-diaminobenzidine (Dako Norden A/S, Glostrup, Denmark) and finally slightly counterstained with hematoxylin. All tissue sections were immunostained simultaneously. Incubation and development times were the same for all sections.

The intensity and pattern of cytoplasmic staining for Syk and membrane staining of CXCR1, CXCR2 and HER2 were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity). Estrogen (ER) and progesterone (PgR) receptors were scored on a scale of 0 to 3 (0-negative; 1-faint; 2-moderate; 3-strong positivity).

### Table I. Immunohistochemical levels of Syk protein expression of three breast cancer patients with intravascular disease.

<table>
<thead>
<tr>
<th>Patient</th>
<th>In situ</th>
<th>invasive</th>
<th>intravascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Moderate</td>
<td>Faint</td>
<td>Negative</td>
</tr>
<tr>
<td>B</td>
<td>Moderate</td>
<td>Faint</td>
<td>Negative</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>Faint</td>
<td>Negative</td>
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Statistical analysis. The Chi-square test and Student’s t-test were used to compare Syk protein expression levels between groups of various clinicopathological factors. A p-value of <0.05 was considered to indicate statistical significance.

Results

Expression of Syk. A moderate to strong expression of Syk in the normal mammary gland of the cases examined was observed (Figure 1).

Similar levels of Syk protein were found in the in situ breast carcinoma specimens as in the normal mammary gland tissue. In some cases, there was increased Syk expression in the in situ cancers compared to that of the matched, non-cancerous tissue (Figure 2).

Strong (20%) and moderate (54%) staining for Syk were observed in the majority of the invasive breast cancers examined. The remaining 26% showed a faint staining for Syk (Figure 3).

Interestingly, in patients with intravascular tumor emboli, a pattern of decreased expression was found, with the highest expression in the in situ component, lower expression in the invasive cancer and complete loss of expression in the vascular emboli (Figure 4 and Table I).

Expression of IL8 receptors and correlation with Syk. CXCR1 expression was scored as strong in 31%, moderate in 56% and faint in the remaining 13% of the invasive breast cancers examined. The equivalent values for CXCR2 were 23%, 56% and 21%, respectively (Figure 5).

A parallel decrease in the protein levels of CXCR1 and CXCR2 was observed, together with a decrease of Syk protein expression in breast cancer tissues (Table II). There was a relation between the level of CXCR2 expression and Syk positivity, as shown in Table II, though not significant (p=0.12).

Relationship between Syk protein expression and clinicopathological factors. There was an inverse relationship between Syk expression and tumor grade (Table II), though this was not statistically significant (p=0.25). There was no relationship between Syk expression and the status of hormone receptors, though the level of expression increases, as Syk
staining becomes more prominent (Table II). p53 staining had a reverse (though not statistically significant, \( p=0.13 \)) relation to Syk expression (Table II). Additionally, there was a significant decrease in the levels of Ki67 expression in the tumors that strongly expressed Syk (\( p=0.028 \)) (Table II). There was no significant relationship between the level of Syk protein expression and HER2 status or, the existence of lymph node metastases (Table II).
Discussion

Syk is a potential tumor suppressor gene in breast, as well as gastric cancer. In the present study, decreased Syk protein expression was found in 26% of the invasive breast cancer patients examined. Moreover, Syk was not expressed in cases where cancer cells were actively invading the intravascular compartment (Figure 4 and Table 1). Thus, a progressive loss of Syk protein expression was observed as the metastatic potential of the tumor became stronger in certain patients. Moreover, two of the patients that relapsed and died due to breast cancer had reduced Syk expression.

Some cases showed analogous or even elevated total Syk (Figure 2) in comparison with the matched, non-cancerous tissue, as also reported previously (10-11). It has been reported that a shorter spliced form of Syk is specifically expressed in the cytoplasm of the tumor cells and not in the adjacent, normal tissue (28). Only the full-length Syk acts as a tumor suppressor gene (28). Theoretically, our method may have detected the expression of both forms of Syk,
since the antibody is derived from the N-terminus of the protein. This could explain the comparable or elevated levels of Syk in the cancerous, in comparison with the matched, non-cancerous tissue. Alternatively, it could highlight the heterogeneous nature of cancer. In our opinion, determination of the localization and expression of the long and short alternative spliced form of Syk awaits utilization of specific antibodies for detection in tissue sections before contribution of each to cancerous expression can be evaluated.

The decrease in Syk expression in invasive breast cancer was not related to the levels of the IL8 receptors (Table II). A non-significant association was found between the expression levels of CXCR2 and Syk positivity ($p>0.05$). Syk is one of the proteins that are activated by an extracellular signal to promote the down-modulation, hence, lowered, cell-surface, expression of CXCR1, CXCR2 and CXCR4 chemokine receptors (29-30). Possibly, the elevated metastatic potential of a tumor could shift chemokine receptor expression, from that associated with lowered invasiveness to that promoting metastases, like CXCR4 (20). Indeed, increased Syk was associated with decreased CXCR4 expression in another group of breast cancer patients that was examined (unpublished data).

A significant relationship was found between the decrease of Syk protein expression and the increase of the proliferation index, as estimated by Ki67 staining ($p<0.05$). Hence, lower Syk expression is correlated with higher proliferation of breast cancer cells. It is also important to mention that the samples that had elevated Syk showed no p53 expression and were negative to HER-2 (Table II), an observation that again links Syk expression with a low malignant potential.

There was no association between Syk protein expression and lymph node metastases. Lymphatic and intravascular invasion of cancer utilize two different mechanisms that use different pathways for cellular movement. It is possible that decreased Syk is associated with intravascular rather than lymphatic invasion of the cancer.

In conclusion, our data support that Syk is a potential tumor suppressor gene in breast cancer. Evaluation of the level of its expression could be used as a valuable biomarker to detect the progression and the metastatic potential of a tumor.

References