Prognostic Value of Signal Transducers and Activators of Transcription 3 in Breast Cancer

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Abstract

Introduction: **Constitutively activated signal transducers and activators of transcription (STAT) proteins are found in various types of tumors. However, there is still very limited information about the role of STATs in breast cancer. The power of tissue microarray technique is the capability of doing a series of analyses of thousands specimens in a parallel fashion with minimal damage to the origin blocks. This study was designed with the application of tissue microarray to analyze the STAT3 status in breast cancer.**
Materials and Methods: **Archival tissue specimens from 102 patients with primary invasive breast cancer were selected, and STAT3 expression was analyzed by immunohistochemical staining with tissue microarray. The data of primary tumor staging, age, estrogen receptor status, lymph node status, histologic grading, and tumor-node-metastasis staging were also collected.**
Results: **By multivariate analysis, the STAT3 expression turned out to be significantly related to the overall 5-year survival rate (P = 0.024).**
Conclusion: **Immunohistochemical staining with tissue microarray was convenient and feasible for the analysis of STAT3 expression status in breast cancer. Our preliminary results are promising and deserve further evaluation.** (Cancer Epidemiol Biomarkers Prev 2008; 17(9):2286–90)

Introduction

Signal transducers and activators of transcription (STAT) constitute a family of latent transcription factors whose activation is dependent on tyrosine phosphorylation at a site in their C-termini (1). Tyrosine phosphorylation of STAT proteins induces dimer formation, followed by translocation to the nucleus, where they bind DNA response elements and thereby regulate gene expression (2). It has been suggested that STAT proteins have primarily evolved to mediate cytokine signaling, particularly in cells of the immune system (3). Indeed, gene knockout experiments in mice indicate a pivotal role for STAT proteins in the development and regulation of the immune system (4).

Although originally discovered as effectors of normal cytokine signaling, subsequent studies have shown the participation of STATs in signaling by polypeptide growth factors and oncoproteins. Precise regulation of STAT activation is critical with regard to eliciting the appropriate responses to extracellular signals. In the event that control of STAT activation is deregulated, for example through constitutive ligand or receptor engagement or oncogenic tyrosine kinase activity, aberrant STAT signaling may contribute to malignant transformation by promoting cell cycle progression and/or cell survival (5–11). Because STATs directly regulate gene expression, implicit in the constitutive activation of STATs observed during oncogenesis is the acquisition of a permanent alteration in the genetic program. Moreover, better understanding of the mechanisms underlying aberrant STAT signaling during oncogenesis may lead to the development of novel cancer therapies based on interrupting key steps in this pathway (12–17).

Constitutively activated STAT protein are found in various types of tumors, including leukemia, prostate cancer, and neck tumors (18–20). However, there is still limited information about the role of STATs in breast cancer (21, 22).

The creation of tissue microarray allows for the rapid immunohistochemical analysis of thousands of tissue samples in a parallel fashion with minimal damage to the origin blocks (23, 24).

This study was designed with the application of tissue microarray to analyze the STAT3 status in breast cancer and with the hope to elucidate the possible relationship between STAT3 expression and breast cancer.

Materials and Methods

Specimen Selection and Data Collection. Archival tissue specimens from 102 patients with primary invasive breast cancer were selected from the pathology files of Chang Gung Memorial Hospital at Kaohsiung between January 1994 and December 1998. All the patients underwent modified radical mastectomy due to invasive breast cancer, defined as carcinoma with

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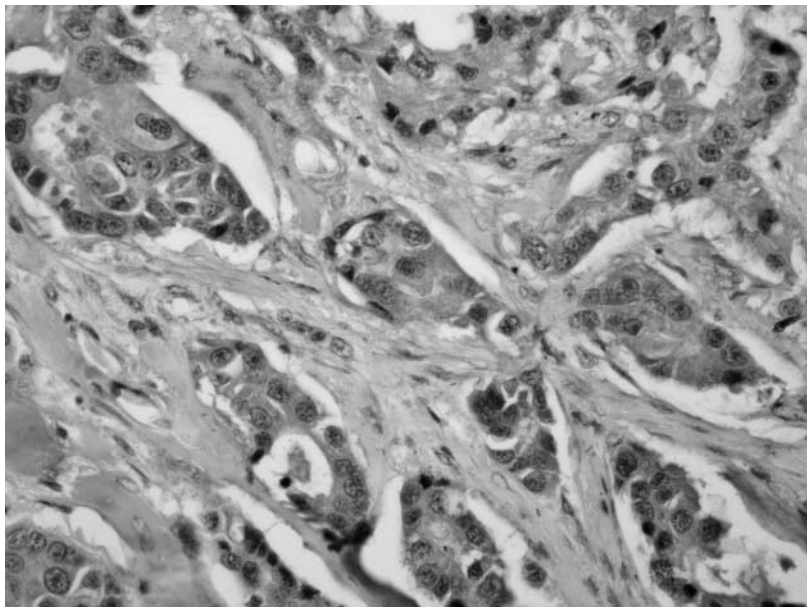

Figure 1. Immunostaining with the STAT3 antibody on the tissue microarray slides of the breast cancers. The representative 3+ case reveals strong cytoplasmic immunoreactivity, as well as focal nuclear immunoreactivity in the tumor cells. Original magnification, ×400.

invasion to or beyond the basement membrane, regardless of histologic classification (ductal or lobular; ref. 25). The data of primary tumor staging, age, estrogen receptor status (26–31), lymph node status, histologic grading, and tumor-node-metastasis (TNM) staging were also collected. The H&E-stained slides of the paraffin-embedded tumor specimens were reviewed by our pathologists to confirm the accuracy of the histologic diagnoses and lymph node status.

Tissue Microarray Assembling. The representative areas of tumor and nontumor parts for each case were selected and circled to match the blocks for the tissue microarray. Then, the blocks matching the circled slides were retrieved to prepare the recipient block for the microarray. To assure the representation of the selected cores, three areas each for tumor and nontumor parts per case were determined for assembling the recipient blocks. Each target area on the selected blocks was punched to form a 0.6-mm-diameter tissue core and placed consecutively on the recipient blocks of ~3 × 2 cm with a precision instrument (Beecher Instruments), as described elsewhere (32).

Immunohistochemical Analysis. The rabbit polyclonal antibody against human STAT3 (RB-9237) was purchased from NeoMarkers, Inc., and was diluted 1:50 in PBS. Five-micrometer sections were cut from the recipient blocks of the tissue microarray, incubated overnight in a 37°C oven, dewaxed in xylene, and

Table 1. STAT3 expression in relation to clinicopathologic variables

STAT3 expression	1	2	3	*P*
Age (y)				
<50	16 (26.23%)	29 (47.54%)	16 (26.23%)	0.881
≥50	9 (21.95%)	21 (51.22%)	11 (26.83%)	
Estrogen receptor status				
Negative	18 (28.57%)	31 (49.21%)	14 (22.22%)	0.327
Positive	7 (17.95%)	19 (48.72%)	13 (33.33%)	
Histologic grading				
1	3 (23.08%)	5 (38.46%)	5 (38.46%)	0.861
2	15 (25.42%)	29 (49.15%)	15 (25.42%)	
3	7 (23.33%)	16 (53.33%)	7 (23.33%)	
Primary tumor staging				
T_1	7 (35.00%)	9 (25.00%)	5 (25.00%)	0.871
T_2	13 (24.53%)	26 (49.06%)	14 (26.42%)	
T_4	2 (22.22%)	4 (44.44%)	3 (33.33%)	
Lymph node status				
N_0	14 (29.16%)	21 (43.75%)	13 (27.09%)	0.113
N_1	2 (10.53%)	15 (78.94%)	2 (10.53%)	
N_2	5 (22.73%)	8 (36.36%)	9 (40.91%)	
N_3	4 (30.77%)	6 (46.15%)	3 (23.08%)	
TNM staging				
I	4 (30.77%)	5 (38.46%)	4 (30.77%)	0.619
II	10 (21.28%)	27 (57.45%)	10 (21.28%)	
III	11 (26.19%)	18 (42.86%)	13 (30.95%)	

Table 2. Overall 5-y survival rate for each category

Variable	Category	5-y Survival rate (%)
Age	<50	78.6
	≥50	63.4
TNM stage	I	100
	II	91.4
	III	42.9
Estrogen receptor status	Negative	66.7
	Positive	81.8
Histologic grading	1	61.5
	2	76.2
	3	70.0
STAT3 expression	1	80.0
	2	75.9
	3	59.0
STAT3 expression	1-2	77.2
	3	59.0

dehydrated in a series of graded alcohols. The sections were then treated with 3% hydrogen peroxide for 10 minutes to deprive the endogenous peroxidase activity and microwaved in 10 mmol/L citrate buffer (pH 6.0) to unmask the epitopes. After antigen retrieval, the sections were incubated with diluted STAT3 antibody for 1 hour, followed by PBS wash. Horseradish peroxidase-Fab polymer conjugate (PicTure-Plus kit, Zymed) was then applied to the sections for 30 minutes. After washing, the sections were incubated with peroxidase substrate diaminobenzidine for 5 minutes and counterstained with hematoxylin.

Grading for STAT3 Immunoreactivity. For evaluating the immunoreactivity of STAT3, it was classified into a four-grade scale: 0, absence of staining in tumor cells; 1+, weak nuclear and/or cytoplasmic staining in tumor cells; 2+, an intermediate staining intensity between 1+ and 3+ in tumor cells; and 3+, strong cytoplasmic staining in tumor cells (Fig. 1).To assure the representation of the selected cores, three areas for each tumor were picked up to assemble the recipient block. On evaluating the immunoreactivity of STAT3, the intensity of each spot was respectively evaluated. The grading was determined when two or more spots showed the same staining intensity for each case. No significant intratumoral variation in expression was found in these cases.

Patients and Follow-up. All of the patients were women from 26 to 76 years old, with a mean age of 48.2 ± 10.5 years. The mean follow-up was 69.7 ± 25.8 months (range, 6-95 months). Follow-up was usually done every 3 months for the first 2 years and then every 6 months for the next 3 years. After 5 years, follow-up became annual. Chest radiography, serum alkaline phosphatase level, and detailed physical examination were usually done at follow-up. Annual mammography or breast sonography (for the younger patient) was done. Radionuclide bone scan, abdominal sonography, or other image studies were done if specific symptoms, signs, or elevated serum alkaline phosphatase level were noted. Data about patient survival, clinical status, and clinicopathologic factors were obtained from medical records, from contact with the patients at the outpatient clinics or by telephone, or from both.

Statistical Analyses. Comparisons between groups were done using χ^2 test. For survival analyses, the end point was overall survival. Survival differences were compared using the log rank test. To assess the relative influence of the potential prognostic variables on survival, all clinicopathologic and genetic variables with $P = 0.1$ in univariate (log rank) analyses were entered into the final Cox's proportional hazards model for multivariate analysis. Statistical analyses were conducted using SPSS software (version 13.0). Statistical significance was set at $P < 0.05$. All Ps were estimated from two sided tests.

Results

There were 25 patients (24.5%) with 1 expression in STAT3, 50 patients (49.0%) with 2 expression in STAT3, and 27 patients (26.5%) with 3 expression in STAT3 (Table 1). By using $\chi 2$ test, comparisons between groups were done. There was no significant relationship between STAT3 expression and age ($P = 0.881$), STAT3 expression and estrogen receptor status ($P = 0.327$), STAT3 expression and histologic grading ($P = 0.861$), STAT3 expression and primary tumor staging ($P = 0.871$), STAT3 expression and lymph node status ($P = 0.113$), and STAT3 expression and TNM staging ($P = 0.619$). For survival analyses, the end point was overall survival. The overall 5-year survival rates for different categories were listed in Table 2. By multivariate analysis, the STAT3 expression turned out to be significantly related to the overall 5-year survival rate ($P = 0.024$; Table 3).

Discussion

Kononen et al. (33) recently described an array-based high-throughput technique that facilitates analysis of very large numbers of tumors at once, either at the DNA, RNA, or protein level. As many as 1,000 cylindrical tissue

Table 3. Multivariate analysis for overall 5-year survival rate

Variable	Category	Odds ratio	95% Confidence interval	*P*
Age	<50	1.0	—	
	≥50	1.0	0.5-2.3	0.937
ER status	Negative	1.0	—	
	Positive	0.3	0.1-0.8	0.014
STAT3 expression	1-2	1.0	—	
	3	2.5	1.1-5.6	0.024
TNM stage	1-2	1.0	—	
	3	4.0	4.0-28.7	<0.0001

biopsy specimens from individual tumor can be arrayed in a single tissue microarray Black. The power of tissue microarray technique is the capability of doing a series of analyses of a thousand specimens in a parallel fashion with minimal damage to the origin blocks (23, 24, 33). In contrast to immunohistochemical analyses on large section, the tissue microarray allows a high level of standardizations for immunohistochemical staining because all tumor are pretreated and stained under exactly the same conditions. Being different from the reading of large sections, which always is an attempt to integrate the observations in multiple different regions of a tissue section, the morphologic classification and interpretation of immunoreactivity are based on the findings within one small, highly defined tissue area in tissue microarrays. The criteria for diagnostic decisions are therefore much easier to establish between the individual samples on the array and to compare among different observers (23, 24, 33).

Nevertheless, critique about tissue microarray arises about whether these small specimen (diameter, 0.6 mm) are really representative of their donor tumors. It has been reported that some alternations are not detected if the analysis of heterogenous tumors is restricted to samples measuring 0.6 mmol/L (34). However, Moch et al. (23) pointed out that the tissue microarray approach has been designed to examine tumor populations and not to survey individual tumors. They (23) have analyzed the impact of tissue heterogeneity on tissue microarray data compared results obtained from tissue microarray with results from large sections in multiple different studies and found that the results did show heterogeneity within tumors but suggested that this heterogeneity did not influence the identification of prognostic parameters. The reliability of tissue microarrays in detecting protein expression and gene amplification in breast cancer has been confirmed (35, 36). Our study analyzed STAT3 expression in breast cancer by immunohistochemical staining with tissue microarray and the results were obtained smoothly. To the best of our knowledge, this is probably the first report with long term follow-up about STAT3 expression in invasive breast cancer analyzed using tissue microarray.

The STATs are implicated as important regulators of the development and differentiation of multicelluar organism. Recent studies suggest that activated STATs signaling was observed in cancer and that deregulation of these factors may participate in oncogenesis (37–40). STAT3 is considered an oncogene because STAT3 brings about the activation of cyclin D1, c-Myc, and bcl-xl expression and are involved in promoting cell-cycle progression and cellular transformation and in preventing apoptosis (41).Constitutive activation of STAT3 is required for either enhancing transformation or blocking apoptosis in cancer cell lines and in human cancers, especially breast cancer cell lines and tissues (16, 42–44). Using a cohort of 68 primary breast infiltrating ductal carcinoma, Yeh et al. (45) found a positive correlation between STAT3 level and tumor stage or size and suggested that high expression of STAT3 in cancer lesions may be a useful biomarker for a poor prognosis in breast infiltrating ductal carcinoma cases. In our series, archival tissue specimens from 102 patients with primary invasive breast cancer were selected, and STAT3 expression was analyzed by immunohistochemical staining with tissue microarray. The mean follow-up was 69.7 ± 25.8 months in our series. By multivariate analysis, the STAT3 expression turned out to be significantly related to the overall 5-year survival rate ($P = 0.024$; Table 3).

Based on the results, the STAT3 expression turned out to be significantly related to the overall 5-year survival rate by multivariate analysis. However, the number of our patients is not large and the mean follow-up period is only 69.7± 25.8 months. It is probably too early to draw a substantial conclusion at present. Nevertheless, our preliminary results are promising and deserve further evaluation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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