

p-STAT3 expression in breast cancer correlates negatively with tumor size and HER2 status

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Abstract

Although some studies have reported the expression and clinical significance of phosphorylated signal transducer and activator of transcription 3 (p-STAT3) in breast cancer tissues, it is still controversial whether p-STAT3 play a role in promoting or suppressing cancer. Here, we used immunohistochemistry analysis to explore expression of p-STAT3 in 407 cases of breast cancer, and analyzed the relationship between p-STAT3 expression and the clinicopathological characteristics and prognosis of breast cancer patients. Positive p-STAT3 expression was seen in 112 cases (27.5%) of breast cancer. p-STAT3 expression was negatively correlated with tumor size, tumor stage and human epidermal growth factor receptor 2 (HER2) status, and the positive rate of p-STAT3 was lowest in HER2-enriched subtype breast cancer (15.3%), while other subtypes were luminal B (23.0%), luminal A (30.2%), and triple-negative breast cancer (TNBC) (37.5%). Logistic regression model multivariate analysis showed that the independent correlation factor of p-STAT3 expression in breast cancer was tumor size (OR=0.187, 95% CI=0.042–0.839, $P=.029$) and HER2 status (OR=0.392, 95% CI=0.216–0.710, $P=.002$). In this study, no clear relationship was observed between patients' prognosis and expression of p-STAT3. Therefore, we suggest that p-STAT3 expression in breast cancer is negatively correlated with tumor size and HER2 status, but appears to have no effect on survival.

Abbreviations: ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, OS = overall survival, PR = progesterone receptor, p-STAT3 = phosphorylated STAT3, RFS = relapse-free survival, STAT3 = signal transducer and activator of transcription 3, TNBC = triple-negative breast cancer.

Keywords: breast cancer, human epidermal growth factor receptor 2, immunohistochemistry, phosphorylated STAT3

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1. Introduction

Breast cancer is one of the most common types of cancers worldwide, and is the leading cause of cancer-related death among women.^[1] Overexpression of the human epidermal growth factor receptor 2 (HER2) protein or amplification of the HER2 gene occurs in ~15% to 20% of all breast cancers.^[2,3] HER2-positive breast cancer has a worse prognosis and having an increased risk of recurrence and a more aggressive disease course.^[4–6] Signal transducer and activator of transcription 3 (STAT3) belongs to the transcription factor family and is usually abnormally activated in malignant tumors, causing STAT3 to play an important role in tumor growth, invasion and metastasis.^[7,8] The activation of STAT3 in breast cancer has received widespread attention.^[9,10] Although several papers have previously reported on the levels of phosphorylated STAT3 (p-STAT3) expression and their clinical significance in human breast cancer tissue specimens, the evidence is controversial as to whether p-STAT3 activation promotes or suppresses cancer.^[11–16]

In this study, we performed an immunohistochemical analysis to detect p-STAT3 expression in breast cancer tissue samples obtained from 407 Chinese Han women. We sought to determine the clinicopathological and prognostic significance of p-STAT3 expression levels in breast cancer.

2. Materials and methods

2.1. Patients and tissue samples

Breast cancer tissue samples were obtained from 407 untreated Chinese Han women who underwent breast cancer surgery in the

Affiliated Dongyang Hospital of Wenzhou Medical University (Dongyang, Zhejiang, China) between 2007 and 2019. Inclusion criteria: patients diagnosed with invasive breast cancer by pathological diagnosis; exclusion criteria: anti-tumor therapy such as chemotherapy, immunotherapy and radiotherapy before surgery. Patients aged 26 to 90 years, with a median age of 50 years. A pathohistological diagnosis was made according to the breast tumor classification criteria of the World Health Organization.^[17] Histological grading was based on the Scarff-Bloom-Richardson system.^[18] According to estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 status, the samples were classified into 4 subtypes^[19–21]: luminal A (ER⁺/PR⁺ [≥20%]/HER2⁻, Ki67 <14%); luminal B, containing hormone receptor-positive cases that did not meet the conditions of luminal A; HER2-enriched (ER⁻, PR⁻, HER2⁺); or triple-negative breast cancer (TNBC) (ER⁻, PR⁻, HER2⁻). Follow-up information was available for 235 patients with breast cancer with a median follow-up time of 60 months (range, 6–72 months). The Ethics Committee of the Affiliated Dongyang Hospital of Wenzhou Medical University approved this study. All study methods satisfied the relevant guidelines and regulations issued by the Affiliated Dongyang Hospital of Wenzhou Medical University.

2.2. Tissue array preparation

We followed the methods of C-Q. Wang et al 2020.^[22] In brief, the Quick-Ray UT-06 (Unitma Co., Ltd., Seoul, Korea) tissue microarray system and the Quick-Ray premade recipient block (UB-06) wax model were used to prepare tissue specimens (1 mm in diameter). Two representative sites from each breast cancer tissue sample were selected for sampling.

2.3. IHC analysis

Immunohistochemical analysis was conducted as described previously.^[20] The primary antibodies used were anti-p-STAT3 mouse monoclonal antibody (clone M9C6; diluted at 1:75; Cell Signaling Technology, Boston, USA), Hercep Test (Dako), ready-to-use anti-ER rabbit monoclonal antibody (clone SP1, Dako), ready-to-use anti-PR mouse monoclonal antibody (clone PgR636, Dako), and ready-to-use anti-Ki-67 mouse monoclonal antibody (clone MIB-1, Dako).

2.4. Assessment of staining

The entire tissue array section was scanned and scored separately by 2 pathologists. A case was considered to be p-STAT3-positive, ER-positive or PR-positive if the percentage of positive invasive cancer cells (nuclear staining) was ≥1%.^[23] HER2 status was assessed according to the 2018 American Society of Clinical Oncology/College of American Pathologists guidelines for HER2 testing in breast cancer.^[24]

2.5. Patient follow-Up

We followed the methods of C-Q. Wang et al 2020.^[22]

2.6. Statistical analysis

Statistical analyses were conducted using SPSS software version 19.0 (SPSS Inc, Chicago, IL). Between-group differences were compared using a Pearson's Chi-Squared test for qualitative

variables. The correlation between p-STAT3 and HER2 protein expression was assessed by Spearman's correlation analysis. The independent correlation factor of p-STAT3 expression in breast cancer was assessed by logistic regression model multivariate analysis. Relapse-free survival (RFS) and overall survival (OS) rates were estimated by the Kaplan–Meier method and compared using log-rank testing. Multivariate analysis using the Cox proportional hazard model was performed to investigate independent factors prognostic of RFS and OS. $P < .05$ was considered to be statistically significant.

3. Results

3.1. Expression of p-STAT3 in breast cancer tissue and its relationship with clinicopathological characteristics of patients

p-STAT3 was expressed in the nuclei of breast cancer cells. The proportion of positive p-STAT3 expression in breast cancer tissue specimens was 27.5% (112/407). As shown in Table 1, p-STAT3 expression was significantly and negatively associated with tumor size ($P = .016$) and stage ($P = .027$). We observed significantly higher levels of p-STAT3 expression in breast

Table 1
Association of p-STAT3 expression with clinicopathological parameters in breast cancer patients.

Parameters	No. of patients	p-STAT3 positive expression, n (%)	P value
Age (yrs)			
≤35	23	6 (26.1%)	.902
35–55	242	65 (26.9%)	
>55	142	41 (28.9%)	
Tumor size (cm)			
≤2	181	61 (33.7%)	.016
2–5	205	49 (23.9%)	
>5	21	2 (9.5%)	
Lymph node metastases			
No	204	59 (28.9%)	.525
Yes	203	53 (26.1%)	
Tumor grade			
I	21	6 (28.6%)	.393
II	260	77 (29.6%)	
III	126	29 (23.0%)	
Tumor stage			
I	105	39 (37.1%)	.027
II	203	52 (25.6%)	
III	99	21 (21.2%)	
IV	0	0 (0.00)	
Estrogen receptor			
Negative	173	48 (27.7%)	.930
Positive	234	64 (27.4%)	
Progesterone receptor			
Negative	215	56 (26.0%)	.482
Positive	192	56 (29.2%)	
HER2 expression			
Negative (0–1+)	195	68 (34.9%)	.004
Equivocal (2+)	111	26 (23.4%)	
Positive (3+)	101	18 (17.8%)	
Molecular classification			
Luminal A	139	42 (30.2%)	.008
Luminal B	100	23 (23.0%)	
HER2-enriched	72	11 (15.3%)	
Triple-negative breast cancer	96	36 (37.5%)	

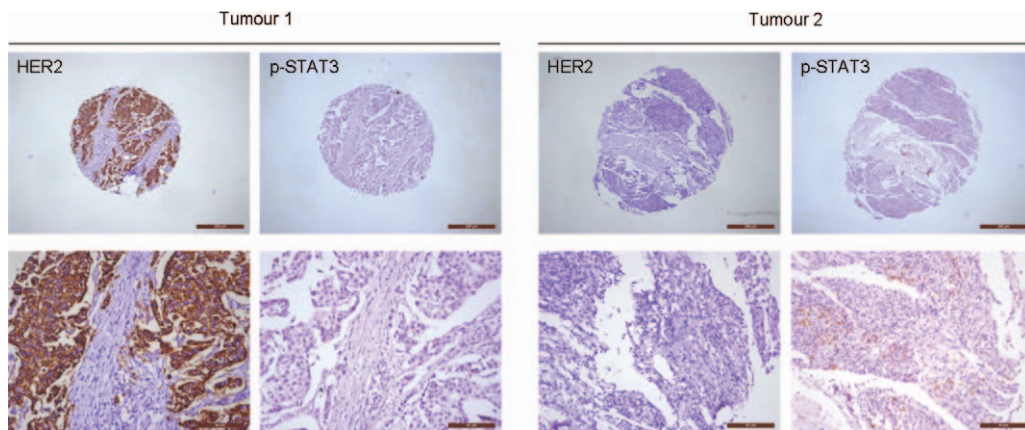


Figure 1. A tendency of negative protein levels between HER2 and p-STAT3 in breast cancer. Human breast cancer tissue microarrays were immune-stained with anti-HER2 and anti-p-STAT3 antibodies. Representative staining pictures of tumors are shown.

cancer tissue specimens from cases that were HER2-negative (0–1+) (34.9%, 68/195) compared with those that were HER2-equivocal (2+) (23.4%, 26/111) or HER2-positive (3+) (17.8%, 18/101; $P = .004$) (Fig. 1). Spearman correlation analysis revealed a significantly negative correlation between HER2-positive and p-STAT3-positive expression in breast cancer tissue specimens ($R = -0.164$, $P = .001$). p-STAT3-positive rates were lowest in HER2-enriched breast cancer (15.3%, 11/72), and the rates were 23.0% for luminal B (23/100), 30.2% for luminal A (42/139), and 37.5% for TNBC (36/96) ($P = .008$).

Logistic regression multivariate analysis showed that the independent predictors of p-STAT3 expression in breast cancer included larger tumor size (OR=0.187, 95% CI=0.042–0.839, $P = .029$) and HER2-positive status (0.392, 0.216–0.710, $P = .002$).

We analyzed the relationship between p-STAT3 expression and tumor size and HER2 status in different stages of breast cancer. In stage II tumors, we also observed significantly higher levels of p-STAT3 expression in tissue specimens from cases that were HER2-negative (32.7%, 34/104) compared with those that were HER2-equivocal (22.2%, 12/54) or HER2-positive (13.3%, 6/45; $P = .036$). Spearman correlation analysis revealed a significantly negative correlation between HER2-positive and p-STAT3-positive expression in stage II breast cancer tissue specimens ($R = -0.180$, $P = .010$). Higher levels of p-STAT3 expression were observed in stage II breast cancer tissue

specimens from cases that tumor size were ≤ 2 cm (36.6%, 15/41) compared with those that were 2 to 5 cm (23.2%, 36/155) or > 5 cm (14.3%, 1/7), but the between-group difference was not statistically significant ($P = .172$). Logistic regression multivariate analysis showed that the independent predictor of p-STAT3 expression in stage II breast cancer was HER2-positive status (0.317, 0.122–0.821, $P = .018$). In stage I and stage III tumors, there is no correlation between p-STAT3 expression and tumor size and HER2 status, and logistic regression multivariate analysis showed that tumor size and HER2 status are not the independent risk factors for p-STAT3 expression.

3.2. No association between p-STAT3 expression and survival of patients with breast cancer

To assess the potential impact p-STAT3 expression on patient survival, we analyzed p-STAT3 expression in relation to RFS and OS rates in patients with breast cancer. As shown in Figures 2A and 2B, no clear associations were observed between p-STAT3 expression and these survival variables ($P > .05$ for each comparison).

In Cox proportional hazards regression analysis, patient age (hazard ratio [HR]=0.124, 95% CI=0.043–0.355, $P < .001$) and tumor stage (8.161, 1.840–36.206, $P = .006$) were independent predictors for RFS. Patient age (HR=0.184, 95% CI=0.049–0.683, $P = .011$) and tumor stage (12.519, 1.554–100.868, $P = .018$) also independently predicted OS.

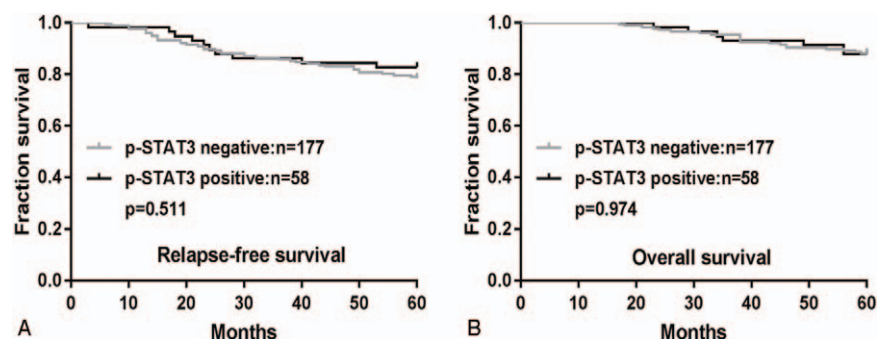


Figure 2. p-STAT3 expression not associated with the survival of patients with breast cancer. The associations of p-STAT3 expression with relapse-free survival (RFS) (A) and overall survival (OS) (B) are shown.

We analyzed the effect of p-STAT3 expression on the prognosis of stage I, stage II, and stage III tumors. The prognosis of tumors that were p-STAT3-positive, did not differ significantly from p-STAT3-negative group in stage I, stage II, and stage III disease.

4. Discussion

Several papers have previously described different levels of p-STAT3 expression in breast cancer tissue specimens, but without confirming whether p-STAT3 plays a role in promoting or suppressing cancer.^[11–16] Some reports have shown that p-STAT3 expression is significantly associated with a good prognosis and features such as smaller tumor size, lower grade, ER-positivity, PR-positivity, and the luminal A subtype.^[13,14,16] Other reports have found that p-STAT3 expression is positively correlated with adverse prognostic factors such as lymph node metastasis and higher tumor stage^{11,12}, while still other researchers have found no correlations between p-STAT3 expression and patients' prognosis or tumor size.^[12,15] We speculate that differences between the research populations and the evaluation criteria used to determine p-STAT3 expression is the main reason for these inconsistent findings.

In this study, we determined levels of p-STAT3 expression in 407 breast cancer tissue samples obtained from Chinese Han women. p-STAT3 expression was significantly lower in women whose tumors were larger, of a higher stage, HER2-positive, or HER2-enriched. The lower expression of p-STAT3 in HER2-enriched and luminal B tumors compared with levels of p-STAT3 expression in luminal A and TNBC tumors is because all HER2-enriched and some luminal B cases are HER2-positive. Further analysis showed that tumor size and HER2 status are independent correlation factor for p-STAT3 expression in breast cancer. A survival analysis revealed no apparent association between p-STAT3 expression and survival of patients with breast cancer. These results suggest that p-STAT3 expression in breast cancer is negatively correlated with tumor size and HER2 status. This study echo other reports describing tumor size is not an independent prognostic factor for breast cancer.^[25,26] Therefore, although p-STAT3 is negatively correlated with tumor size, it does not translate to improved overall survival.

We speculate that activation of STAT3 signaling pathway in breast cancer may be mutually exclusive with the HER2 pathway, and that in HER2-negative breast cancer, other signaling pathways may be mutually exclusive with the STAT3 pathway. Therefore, in primary untreated breast cancer, the STAT3 pathway may not play a major role in its development. Studies have confirmed in vitro that activation of STAT3 mediates HER2 targeted therapy resistance, including trastuzumab or trastuzumab-emtansine (T-DM1).^[27,28] So we think when a patient's oncology treatment inhibits the mutually exclusive pathway with STAT3, such as HER2, this may lead to activation of the STAT3 pathway and increase p-STAT3 expression, with eventual drug resistance. Solving these problems has enormous value for understanding drug resistance mechanisms and improve the efficacy of breast cancer treatment in the future.

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Author contributions

CQW designed the experiments. YW, QW, and LLJ performed the experiments. CQW, HDC, and XFD analyzed the data. GNH and JKS were responsible for management of the clinical samples. The manuscript was written by CQW and revised by CHT. All authors have read and approved the final manuscript.

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