GATA3 Expression in Advanced Breast Cancer
Prognostic Value and Organ-Specific Relapse

Brandi C. McCleskey, MD,¹ Thuy L. Penedo, MD,¹ Kui Zhang, PhD,² Omar Hameed, MD,¹ Gene P. Siegal, MD, PhD,¹ and Shi Wei, MD, PhD¹

From the ¹Department of Pathology, School of Medicine, and ²Department of Biostatistics, School of Public Health, University of Alabama at Birmingham.

Key Words: Breast cancer; GATA3; Subtype; Metastasis; Prognosis

Abstract

Objectives: GATA3 is a transcription factor regulating luminal cell differentiation in the mammary glands and has been implicated in the luminal types of breast carcinoma. The prognostic significance of GATA3 in breast cancer remains controversial.

Methods: In this study, we assessed the prognostic value of the molecule in a subset of 62 advanced breast cancers and 10 control breast cancers (no metastasis after follow-up).

Results: GATA3 expression levels in luminal tumors of advanced stage were significantly higher than that of the human epidermal growth factor receptor 2 (HER2) subtype and triple-negative carcinomas, as expected, but were similar to those of the luminal controls. Furthermore, 88% of nonluminal tumors showed variable GATA3 expression, for which the HER2 subtype had significantly higher GATA3 expression than that of the triple-negative carcinomas. Interestingly, GATA3 levels were significantly lower in carcinomas with lung relapse compared to those with metastatic recurrence to other organs, thus reflecting the findings in animal models. No significant difference was observed between tumors with bone relapse and those metastasized to nonskeletal sites. Moreover, high GATA3 expression was significantly associated with favorable relapse-free survival and overall survival.

Conclusions: These findings suggest that GATA3 may not act solely as a luminal differentiation marker, and further uncovering the molecular pathways by which GATA3 regulates the downstream targets will be crucial to our understanding of breast cancer dissemination.

GATA3 is a member of the GATA family of six zinc finger–containing transcription factors that play a crucial role in the gene regulatory networks governing the specificity of cell fates. In addition to its regulatory functions in hematopoietic cells, skin, kidney, and the central nervous system, GATA3 has emerged as the most highly enriched transcription factor in the mammary epithelium, and its expression is thought to be restricted to the luminal cells and absent in the myoepithelial cells.¹,² The essential role of GATA3 has been further demonstrated by the profound defects seen in mammary morphogenesis induced by mammary epithelium-specific knockout of Gata3.¹,² Recent findings also strongly suggest that GATA3 plays a pivotal role in tumor differentiation.³ One of the key downstream targets of GATA3 in the luminal epithelium is FOXA1, an important regulator of estrogen receptor (ER) expression.¹ Thus, it is not surprising that the highest levels of GATA3 have been observed in the luminal types of breast cancers, and its expression decreases with increasing tumor...
grade.\textsuperscript{4-7} Overwhelmingly maintained GATA3 expression has been found in paired metastases and, most notably, in all “estrogen/progesterone receptor loss” metastases.\textsuperscript{8} Furthermore, somatic mutations of GATA3 are mostly seen in ER-positive tumors, suggesting the role of GATA3 mutation in the pathogenesis of luminal breast cancers.\textsuperscript{9,10}

While these findings hint at GATA3’s usefulness as a relevant marker in predicting clinical outcomes, the prognostic significance of GATA3 in breast cancer remains controversial. There have been conflicting results regarding the prognostic value of GATA3, with some studies demonstrating it as an independent favorable prognosticator\textsuperscript{4,6} but others showing a lack of prognostic benefit.\textsuperscript{5,7,11-13} There have also been inconsistent observations regarding the protective effect of GATA3 expression among patients with ER-positive tumors.\textsuperscript{5,11,13} Furthermore, high GATA3 expression was significantly associated with improved survival outcomes in premenopausal women with ER-positive breast cancer but not in postmenopausal patients.\textsuperscript{14} Moreover, the molecule has been shown to negatively correlate with pathologic response in the neoadjuvant setting.\textsuperscript{15,16}

While the reason for these conflicting observations is likely multilayered, lack of standardization in defining GATA3 positivity may be a key contributing factor for this discrepancy. The varied cutoffs used in the previous studies ranged from 1%\textsuperscript{12} to 5%,\textsuperscript{8,13,17,18} 10%,\textsuperscript{15,16} 20%,\textsuperscript{11} and 30%.\textsuperscript{5} Only two studies used a score that incorporated both the intensity scale and the proportion of staining cells.\textsuperscript{4,6} The marked variation in determining GATA3 expression thus makes it problematic in comparing the studies and all but impossible in attempting to render meaningful conclusions. To further complicate the plight, some studies combined patients with and without systemic therapy, along with others in which the treatment modalities were not documented.\textsuperscript{4,6,11} Given the contradictory findings, the aim of the present study was to explore the prognostic significance of GATA3 in a well-characterized cohort of patients with advanced breast cancer with distant metastases who received systemic therapy. Furthermore, the association of GATA3 expression with established clinicopathologic factors and the sites of distant relapse were also investigated.

**Materials and Methods**

**Patients and Tissue Samples**

After approval by the institutional review board, the Tumor Registry and Surgical Pathology database at the authors’ institution were searched to identify cases of breast carcinomas with metastatic recurrence at distant organs between 1997 and 2004. Of 337 advanced breast carcinomas identified, 62 consecutive cases of excisional breast specimens with sufficient tumoral tissue were retrieved. In parallel, 10 consecutive breast carcinoma cases with no distant relapse after a minimum of a 10-year follow-up (all diagnosed in 2003) were also included as nonmetastatic controls. All patients received systemic hormonal therapy, targeted therapy, and/or chemotherapy. Tissue microarrays (triplicate 1-mm cores/case) were constructed from these cases. The median follow-up time for survival outcomes of all patients was 4.6 years.

**Immunohistochemical and In Situ Hybridization Analyses**

The 5-µm deparaffinized tissue sections were exposed to heat-induced epitope retrieval in sodium citrate buffer (0.02 mol/L; pH 9.0), at 97°C, for 20 minutes. Sections were treated in 3% H\textsubscript{2}O\textsubscript{2} for 30 minutes to quench superoxide, blocked with 5% goat serum and 0.3% Triton X-100 in phosphate-buffered saline, and incubated with a mouse monoclonal antibody raised against GATA3 (L50-823, 1:400; Cell Marque, Rocklin, CA). The immunostaining was accomplished with an automated immunostainer (AutoStainer Link 48; DAKO, Carpinteria, CA). Diaminobenzidine tetrachloride was used to visualize the antibody-antigen complex. The tissue was counterstained with hematoxylin.

Appropriate positive controls were included, and negative controls were tested by replacing the primary antibody with mouse immunoglobulin G. The tissue adequacy was confirmed by the H&E-stained sections of the final tissue microarray blocks before immunostaining. The intensity of the GATA3 nuclear labeling was scored as negative (0), weak (1+), moderate (2+), or strong (3+). An H-score was determined by multiplying the intensity by the percentage of tumor cell nuclei stained, giving a range of 0 to 300. The H-score of GATA3 was given as the mean of the three cores. ER and progesterone receptor (PR) status was assessed by immunohistochemistry, and human epidermal growth factor receptor 2 (HER2) protein overexpression and/or gene amplification was evaluated by immunohistochemistry or in situ hybridization in the primary tumor sections as previously described.\textsuperscript{19}

**Classification of Breast Carcinoma Subtypes**

Breast carcinoma subtypes were classified by a combination of hormonal receptors and HER2 status as previously described.\textsuperscript{19} In brief, tumors were defined as luminal (ER+ and/or PR+), HER2 (ER–/PR–/HER2+), or triple-negative carcinoma (ER–/PR–/HER2–). While some other investigators further classified the ER+ and/or PR+ tumors into luminal A and luminal B subtypes, as well as divided triple-negative tumors into basal-like and non–basal-like carcinomas, these subsets of tumors were not further subclassified in this study given the lack of reliable surrogates for such classifications.\textsuperscript{20-22}
Statistical Analysis

The continuous variables obtained were analyzed using the Student t test, while the categorical data were evaluated using either the $\chi^2$ test or the Fisher exact test, as appropriate. Overall survival and relapse-free survival were mapped on Kaplan-Meier curves. The time of patients who survived or were lost to follow-up was considered censored data in the analysis. Comparison between groups was conducted by the log-rank test, followed by a Cox proportional hazards regression analysis to test if a factor was significantly associated with survival. The cutoff for high/low GATA3 score was dichotomized by maximally selected log-rank statistics. The R.3.0.1 (The R Project for Statistical Computing, Vienna, Austria) software was used for statistical analysis.

Results

Patient and Pathologic Characteristics

All 72 patients included in the study were women. Fifty-five (76%) were white, and the remaining patients were African American. The age at diagnosis ranged from 30 to 83 years, with a median age of 52 years. As in the general patient population, the overwhelming majority of the breast cancers were those of invasive carcinoma of no special type (64/72 [89%]), with the remaining cases representing lobular carcinoma. There was a higher proportion of tumors with higher histologic grade and clinical stage and a lower percentage of luminal subtypes, thus skewing for a worse overall clinical outcome in this cohort. The key clinicopathologic factors are summarized in Table I.

GATA3 Expression and Clinicopathologic Factors

Nuclear labeling of GATA3 was seen in 80% of all breast cancer cases in this cohort, with an H-score range from 10 to 300. All control cases were luminal subtypes. While GATA3 expression was not associated with age at diagnosis, race, tumor type, tumor size, nodal status, or specific organ relapse, it was significantly lower in those breast cancers with distant metastases than in controls. Among advanced breast carcinomas, expression of the molecule was strongly correlated with hormonal receptor status and inversely associated with histologic differentiation. Interestingly, GATA3 expression levels in luminal tumors of advanced stage were similar to those of the luminal controls, indicating that the molecule is not a marker of progression/aggressiveness in this subset of luminal breast cancers. As expected, GATA3 expression in luminal tumors was significantly higher than that of the HER2 subtype and the triple-negative cancers. It is of further interest that 88% (23/26) of the ER-negative tumors showed greater GATA3 expression (H-score range, 10-275) and that the HER2 subtype, in turn, had a significantly higher level of GATA3 expression than the triple-negative tumors.

GATA3 Expression and Organ-Specific Relapse

Given that luminal carcinomas demonstrate a strong association with bone relapse, we next explored the relationship between GATA3 expression and the common sites of distant metastasis, including bone, lung, liver, and brain. To that end, a broad range of GATA3 expression levels was found in breast cancers associated with all common organs of relapse. Surprisingly, the levels of GATA3 expression in tumors with associated bone relapse did not differ significantly from those metastasized to nonskeletal sites. It is of great interest that GATA3 tumor expression was significantly lower in those patients who developed lung metastasis compared to those with other organ relapse (Table 2). We next asked if this finding could be secondary to the distributions of luminal tumors in the two groups, since these subtypes were less frequently associated with lung relapse compared with the nonluminal tumors. However, a significant difference was not found in the proportion of luminal tumors between these groups in this cohort ($P = .1$), thus rendering it less likely to be a significant contributing factor for this phenomenon. There was a trend of lower GATA3 expression in those with brain relapse, although this did not reach statistical significance.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Clinicopathologic Factors$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Pathologic Factors</td>
<td>Value</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
</tr>
<tr>
<td>No special type</td>
<td>64 (89)</td>
</tr>
<tr>
<td>Lobular</td>
<td>9 (11)</td>
</tr>
<tr>
<td>Nottingham histologic grade</td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>36 (50)</td>
</tr>
<tr>
<td>III</td>
<td>36 (50)</td>
</tr>
</tbody>
</table>

$^a$ Values are presented as number (%) unless otherwise indicated.

$^b$ All control cases were luminal subtypes.

$^c$ Site of metastasis is not exclusive; 29 patients developed multiple-organ metastases.

HER2, human epidermal growth factor receptor 2.
The log-rank test revealed that a high GATA3 score (>210) was significantly associated with a favorable relapse-free survival as a dichotomized variable (hazard ratio [HR], 0.5086; 95% confidence interval [CI], 0.2455-0.8262; \( P = 0.01 \)) \( \text{Figure 2A} \), as well as a significant prognosticator for overall survival as a continuous variable \( (P = 0.03) \) or dichotomized variable (HR, 0.3991; 95% CI, 0.1968-0.6462; \( P = 0.002 \)) \( \text{Figure 2B} \). Moreover, the levels of GATA3 expression did not significantly affect the survival outcomes in the subset of patients with ER-positive carcinomas or those with ER-negative tumors, although a trend of protective effect associated with high GATA3 expression was seen in the latter group of patients (median relapse-free survival, 671 vs 415 days, \( P = 0.2 \)). Multivariate analysis identified tumor size \( (P = 0.009) \) and ER status \( (P = 0.003) \) as independent factors for overall survival among the clinicopathologic parameters analyzed in this cohort.

Discussion

As a well-established regulator in mammary development, GATA3 expression is limited to the luminal epithelial cells and is absent in myoepithelial cells of the terminal duct lobular units. The differentiated cell types are derived from a multipotent progenitor population. Deletion of Gata3 in the mammary glands in adult mouse models results in dedifferentiation, decreased cell-cell adhesion, and increased cell proliferation of the luminal cells, whereas introduction of
**Table 2**

**Association of GATA3 Expression With Clinicopathologic Factors**

<table>
<thead>
<tr>
<th>Variable</th>
<th>H-Score, Mean (Range)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (no distant metastasis)</td>
<td>237 (25-300)</td>
<td>.04</td>
</tr>
<tr>
<td>Breast cancers with distant metastasis</td>
<td>177 (0-300)</td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>226 (0-300)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>III</td>
<td>135 (0-300)</td>
<td></td>
</tr>
<tr>
<td>Hormonal receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+/ER−</td>
<td>229/124 (175-300/0-275)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>PR+/PR−</td>
<td>234/152 (175-300/0-285)</td>
<td>.0008</td>
</tr>
<tr>
<td>Subtypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal</td>
<td>229 (100-300)</td>
<td>.7 (vs control)</td>
</tr>
<tr>
<td>HER2</td>
<td>165 (85-265)</td>
<td>.0006 (vs luminal)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>83 (0-275)</td>
<td>&lt;.0001 (vs luminal); .009 (vs HER2)</td>
</tr>
<tr>
<td>Nonluminal</td>
<td>124 (0-275)</td>
<td>&lt;.0001 (vs luminal)</td>
</tr>
<tr>
<td>Site of distant relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone/nonbone</td>
<td>188/171 (0-290/0-285)</td>
<td>.5</td>
</tr>
<tr>
<td>Lung/nonlung</td>
<td>138/203 (0-285/0-300)</td>
<td>.007</td>
</tr>
<tr>
<td>Liver/nonliver</td>
<td>187/181 (100-285/0-300)</td>
<td>.8</td>
</tr>
<tr>
<td>Brain/nonbrain</td>
<td>145/192 (0-285/0-300)</td>
<td>.1</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Gata3 into the mammary progenitor-enriched cell population induces luminal cell differentiation.1,2 Given the fundamental role of GATA3 in maintaining the integrity of the luminal epithelium, it is likely that the molecule is causally involved in breast cancer pathogenesis. Attesting to this hypothesis, loss of GATA3 in a mouse model correlated with loss of differentiation genes and marked progression from adenoma to early carcinoma and onset of tumor dissemination, whereas restoration of GATA3 in late carcinomas induced tumor differentiation and suppressed tumor dissemination.27 In keeping with these findings, a number of previous studies, along with our data, have demonstrated that the highest GATA3 levels are seen in the well-differentiated/ER-positive/luminal breast cancers, and its expression is inversely correlated with histologic grade/tumor differentiation.4-7

While it is without doubt that GATA3 expression is strongly associated with ER status, the reported GATA3 expression in ER-negative breast carcinomas historically ranged from 5%28 to 16%,5 50%,8 and 69%,18 using varied cutoffs. Yet, we found that nearly 90% of nonluminal tumors expressed variable levels of GATA3, the highest percentage of positivity among all published studies. This argues against the previous findings that GATA3 acts solely as a luminal differentiation marker. A novel observation in this cohort was that carcinomas of the HER2 subtype had a significantly higher level of GATA3 expression than that of triple-negative tumors, although its expression level was significantly lower than that of luminal tumors. These observations may provide new insight into the pathogenesis of breast cancer and further support the recent hypothesis that breast cancer may be viewed as a hierarchical disease derived from two main cell types of origin; that is, basal-like breast cancers arise from the basal/myoepithelial cell compartment, whereas non–basal-like carcinomas emerge from a more luminal-like cell compartment.29

Whether GATA3 is associated with organ-specific relapse is another topic of interest. Given that ER is strongly associated with GATA3 and that ER-positive/luminal breast cancers demonstrate a significant bone-seeking phenotype,24 it is natural to hypothesize that tumors with high GATA3 expression would be associated with a strong bone-homing characteristic. To our surprise, the levels of GATA3 expression in tumors with bone relapse did not differ significantly from those that metastasized to other organs in this cohort. Rather, GATA3 expression was significantly lower in those that spread to the lungs, thus suggesting a potential role of this molecule in suppressing breast cancer pulmonary

**Figure 1** GATA3 expression stratified by organ-specific relapse.
metastasis. Interestingly, the aforementioned previous study showed that reintroduction of Gata3 in a mouse model with targeted deletion of the gene in the mammary gland resulted in a striking suppression of breast cancer metastases to the lungs by 25-fold. Using an aggressive breast cancer cell line generated by in vivo selection for lung tropism, another study demonstrated that expression of GATA3 led to a reduced tumor outgrowth in the mammary fat pad of nude mice and, engagingly, a lower lung metastatic burden by inhibiting breast cancer cell expansion inside the lung parenchyma. Furthermore, gene microarray analysis revealed that Id1 and Id3, both downregulated in GATA3-expressing cells, had been previously shown to mediate reinitiation during breast cancer lung metastases, thus suggesting a direct regulatory role of these genes by GATA3.

With respect to its controversial role in breast cancer prognosis, we found a significant beneficial effect of GATA3 expression for both overall survival and relapse-free survival in this unique cohort of advanced breast cancers, in keeping with some early studies using different patient populations but in contrast to others. In reviewing these studies, an interesting observation was that most analyses demonstrating the prognostic significance of GATA3, including ours, used a score that incorporated both an intensity scale of immunoreactivity and the proportion of positively staining cells, thus more closely representing the expression levels. Most of those showing a lack of prognostic benefit used a single cutoff regardless of intensity. Thus, the differences in the methods may have greatly contributed to the disputed conclusions in these studies. The reported findings are further complicated by the inconsistency regarding the protective effect of GATA3 expression among patients with ER-positive tumors. Moreover, an adverse effect of GATA3 expression in the ER-negative tumors has been observed in at least one study. While this latter notion is worth further exploring, it is somewhat contrary to the suppressive effect of GATA3 in breast cancer lung metastasis, which is significantly associated with an ER-negative phenotype/nonluminal subtype and an unfavorable outcome. A significant prognostic impact of GATA3 was not found in the subset of patients with either ER-positive or ER-negative tumors in our study.

The molecular mechanisms by which GATA3 mediates breast cancer progression remain to be elucidated. Overexpression of GATA3 in GATA3-negative breast cancer cells induces E-cadherin expression through binding GATA-like motifs located in the E-cadherin promoter; this, along with the correlation of GATA3 expression and E-cadherin levels in human breast cancer, suggests that GATA3 may drive breast cancer cells to undergo a reversal of the epithelial-to-mesenchymal transition. This notion is further supported by the observation that overexpression of GATA3 in breast cancer cells induces a growth inhibitory response to transforming growth factor β, a molecule that promotes the epithelial-to-mesenchymal transition in advanced cancers but acts as a tumor suppressor in normal epithelial cells. Furthermore, GATA3 directly represses the ability of the CDK inhibitor p18INK4C to regulate the cell cycle, and low p18INK4C and high GATA3 expression were simultaneously observed in luminal A breast cancers. Mice deficient for p18INK4C spontaneously develop ER-positive luminal tumors at a high penetrance. These findings suggest an association between GATA3 and cell proliferation as well as a plausible role of p18INK4C in constraining luminal progenitor cell expansion and suppression of luminal tumorigenesis. Furthermore, GATA3 was found to suppress breast cancer lung metastasis and modulate the tumor microenvironment by regulating microRNA-29b, a discovery that opens up possibilities for therapeutic intervention. Last, the significance of GATA3 mutations in breast cancer progression needs further investigation.
It should be acknowledged that some limitations may be present in this study due to the inherent nature of tissue microarrays. Since antigens can be focally present in tumors, the semiquantitative scoring of GATA3 in tissue microarrays may not truly represent the expression level of the protein in the tumors. To ensure that the tissue microarrays better represent the lesional tissue, the H-score of GATA3 was given as the mean of three separate cores obtained from variable areas of the tumor while simultaneously reviewing other ancillary studies (including ER and PR). As expected, expression of the molecule was strongly correlated with hormonal receptor status and inversely associated with histologic grade of breast cancer. Furthermore, the proportion of GATA3+/ER−/PR− tumors in this study likely constitutes the faithful fraction of GATA3 expression in nonluminal carcinomas, if not an underestimation.

In summary, we found that the overwhelming majority of nonluminal breast cancers demonstrated variable levels of GATA3 expression. While advanced luminal tumors had significantly higher levels of GATA3 than those of the HER2 subtype and triple-negative breast cancers, they maintained similar expression levels of GATA3 compared with early stage luminal cancers. Moreover, GATA3 expression had a significant prognostic value in the subset of patients with advanced disease. Of the utmost interest, we demonstrated for the first time a significant organ-specific association of GATA3 expression in human breast cancer, thus supporting the suppressive effect of GATA3 in breast cancer lung metastasis previously observed in animal models. Therefore, GATA3 expression may be potentially used in assisting clinical decision making. Further investigation of GATA3-related pathways will be crucial to our understanding of breast cancer dissemination and may also provide novel therapeutic targets.

Corresponding author: Shi Wei, MD, PhD, Dept of Pathology, University of Alabama at Birmingham, NP 3542, 619 19th St South, Birmingham, AL 35249-7331; swei@uab.edu.

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References


