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Original article

GATA-3 expression is not associated with complete pathological response in triple negative breast cancer patients treated with neoadjuvant chemotherapy

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ABSTRACT

Objective: The prognosis for patients with triple negative breast cancer (TNBC) is poor, however, a subset will demonstrate complete pathological response (pCR) to chemotherapy. GATA-3 and AR may be a negative predictors for pCR although it is unclear if these results apply to TNBC.
Methods: Patients diagnosed with TNBC and treated with neoadjuvant chemotherapy were identified. Immunohistochemistry was performed for GATA-3 and AR. Both were scored using a composite of staining intensity and percentage cells stained. The primary outcome was pCR.
Results: Twenty-four patients were included and 7 achieved pCR. There was no difference in the pre-chemotherapy tumor size (44 ± 28 mm vs. 54 ± 30 mm; $p = 0.764$) or lymph node status (86% vs. 71%; $p = 0.629$) between patients with and without pCR. GATA-3 expression was present in 20 cases (83%) while AR was present in 6 cases (25%). No AR expression was seen in 15 cases (63%) with GATA-3 positivity. There was no difference in either GATA-3 (4.3 ± 2.7 vs. 3.6 ± 2.5; $p = 0.549$) or AR (1.4 ± 2.5 vs. 1.1 ± 2.4; $p = 0.778$) expression between patients with and without pCR.
Conclusions: GATA-3 expression is frequent in TNBC even in the absence of AR. However, neither GATA-3 nor AR are associated with pCR after neoadjuvant chemotherapy.

1. Introduction

Triple negative breast cancer (TNBC) is defined by the lack of Estrogen Receptors, Progesterone Receptors, and HER-2 expression. TNBC accounts for 15–20% of all newly diagnosed cases of primary breast carcinoma and compared to all other subtypes of breast cancer, has the worst overall prognosis. Moreover, patients with TNBC are more likely be under 40 years old and to suffer early relapse. Indeed, survival of patients with triple negative disease is independent of tumor grade, lymph node status, tumor, size, and treatment [1].

Despite its aggressive behavior, TNBC appears to be more sensitive, at least initially, to chemotherapy compared to non-TNBC [2–4]. Indeed several studies have shown that patients with TNBC were more likely to show complete pathological response (pCR) compared to patients with non-TNBC although patients with TNBC still had decreased disease free survival and overall survival. However, almost all patients with TNBC who had pCR achieved greater long-term survival than patients with non-TNBC. In contrast, patients with TNBC who had residual disease faired much worse overall than patients with non-TNBC [3]. Taken together, these studies suggest that although TNBC has a worse overall prognosis compared to non-TNBC, there is a subset of patients with TNBC who respond favorably to chemotherapy and have excellent prognosis. At present however clinicians have no way of identifying who these patients will be prior to treatment.

One protein that has shown potential as a novel predictive marker is GATA-3, a transcription factor critical in mammary gland development and a key regulator of luminal cell differentiation [5]. GATA-3 is expressed in breast tissue and is used to identify metastatic lesions when other markers are absent [6]. The expression of GATA-3 is associated with the estrogen receptor (ER) as GATA-3 is a downstream effector of hormone signaling [7]. Indeed most GATA-3 positive tumors are also ER positive and share the favorable prognosis of hormone receptor positive tumors. Moreover, like ER, the presence of GATA-3 in tumor cells is associated with a poor response to chemotherapy [8]. Indeed, GATA-3 may limit the effectiveness of chemotherapy by activating downstream targets of estrogen, even in the absence of ER expression,

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possibly under the influence of the androgen receptor (AR) [9]. The fact that GATA-3 expression has been demonstrated in a subset of TNBC raises the possibility that triple negative/GATA-3 positive tumors represent a separate subtype of TNBC with unique clinical behavior. Specifically, the expression is GATA-3 may help explain why some TNBC respond so well to neoadjuvant chemotherapy while others respond in a manner more consistent with hormone receptor positive tumors [2–4,10].

In this study, we examine for the first time the expression of GATA-3 in both AR positive and negative TNBC. We hypothesized that the absence of GATA-3 would be associated a higher rate of complete pathological response (pCR) and by extrapolation to increased overall survival.

2. Materials and methods

2.1. Patient population

This retrospective study was approved by the Ottawa Hospital Research Ethics Board (study 20130836-01H). The pathology database of the Department of Anatomical Pathology was searched for all patients diagnosed with TNBC at The Ottawa Hospital between January 2005 and March 2014. Tumors were defined as hormone receptor negative if the percentage of positive cells was less than 1%. Patients who underwent surgery for locally advanced or inflammatory breast cancer after neoadjuvant chemotherapy were identified and included in this study. Patients with positive axillary nodes at presentation who underwent neoadjuvant chemotherapy prior to surgical resection of the primary tumor were also included. Cases without sufficient tissue for subsequent immunohistochemical analysis were excluded.

2.2. Immunohistochemistry

All specimens analyzed were core needle biopsies performed prior to neoadjuvant chemotherapy. Fixation and ischemic times were optimized according to CAP/ASCO guidelines [11]. Immunohistochemical analysis was performed on paraffin-embedded sections using the Leica Bond automated platform. Tissue sections were incubated with primary antibodies against GATA-3 (Santa Cruz, clone HG3-31, 1:50), AR (Vector, clone 2F12, ready to use), ER (VECTOR, clone 6F11, 1:300), or PR (Novacastra, clone PR16, ready to use). GATA-3 and AR were incubated with 40 minute HIER using Bond Epitope Retrieval Solution 2. ER and PR were incubated with 20 minute HIER using Bond Epitope Retrieval Solution 2. Cell proliferation was assessed by immunohistochemistry for Ki-67 (Dako, clone MIB-1, 1:100) and the Ki-67 labelling index (LI) was assessed using the 'hot-spot' method. Using this method, areas of high Ki-67 labelling were identified at scanning magnification and the percentage of positive cells was assessed using image assisted analysis (Leica LAS V3.8) A total of 1000 cells was counted in % of cases and in all cases more than 300 cells were available.

2.3. HER2 analysis

HER2/neu was tested using HERCEPTEST according to manufacturers' specifications and if indeterminate was reflexed to fluorescent in situ hybridization (FISH). HER2 copy number was determined by FISH using the FDA approved PathVysion HER2 DNA probe kit (Vysis, Dowers Grove, IL). Testing was performed according to the manufacturer's specifications. Specimens were scored on 60 cells and were scored positive if the HER2/CEP17 ration was ≥ 2.0.

Table 1
Modified Allred score used to assess GATA-3 and AR staining in TNBC.

Staining intensity	Intensity score	Proportion of cells labelled	Proportion score
Negative	0	None	0
Weak	1	<1/100	1
Intermediate	2	≥1/100 to <1/10	2
Strong	3	≥1/10 to <1/3	3
		≥1/3 to <2/3	4
		≥2/3	5

2.4. Scoring GATA-3 and AR

Both GATA-3 and AR were scored using a modified version of the Allred scoring system which assesses both the proportion of labelled cells and the average staining intensity of the positively labelled cells (Table 1). Briefly, the proportion of labelled cells was scored from 0 to 5 and the average intensity of the labelled cells was scored from 0 to 3. Only nuclear staining was considered positive for both GATA-3 and AR and > 1% of all neoplastic cells had to be labelled for the tumor to be considered positive. This conservative model was used to mirror that used for ER and PR.

2.5. Outcome

The primary outcome was pCR, defined as the absence of invasive disease in both breast tissue and lymph nodes. Clinicopathological parameters and the expression of GATA-3/AR were compared in patients with and without pCR.

2.6. Statistical analysis

An *a priori* power analysis was performed to determine the sample size required to detect a statistically significant difference in GATA-3 expression between patients with and without pCR. Consistent with previously published results, we predicted that 30% of our patients would demonstrate pCR. Based on this assumption, our study would require 26 total patients (8 with pCR and 18 without pCR) and have a power of 95% to detect a two-fold difference in GATA-3 expression between the groups. However, the study would require 58 total patients (17 with pCR and 41 without pCR) and have a power of 95% to detect a one and a half-fold difference in GATA-3 expression between the two groups.

Statistical analyses were performed using SPSS version 20 for Windows. Proportions were compared using the Chi-square test or Fisher's exact test where appropriate. Continuous variables were compared using the Mann-Whitney U test. Spearman's rank coefficient was used to test the correlation between GATA-3 and AR. All tests were two-sided and a p-value of < 0.05 was considered significant.

3. Results

Twenty-four patients were included in this study. The median age at diagnosis was 48 years (range, 30–70 years) and the median time of follow-up was 17.5 months (range, 1–88 months). All cases were diagnosed as invasive ductal carcinoma. Prior to chemotherapy the median tumor size was 54 mm (range, 8–130 mm); 18 patients had lymph node positive disease. In two cases, hormone receptor and HER2 expression analysis was performed on biopsies derived from metastatic lymph node tissue.

Twenty-two out of 24 patients (92%) in our study received adriamycin and cyclophosphamide, followed by paclitaxel. One patient received 5-fluorouracil–epirubicin–cyclophosphamide followed by docetaxel. The final patient was enrolled in a clinical trial and received paclitaxel plus a notch inhibitor. Following