

Research Article

GATA3 Expression in Breast Cancers Is Strongly Associated With AR Expression in a Large TMA Study

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Abstract

GATA3 is a protein that regulates the transcription of proteins from multiple lineages. It is used in anatomic pathology as a marker of breast or urothelial differentiation because it exhibits sensitivity and specificity for staining these tissue types over others. GATA3 expression has been correlated with ER (estrogen receptor) and PR (progesterone receptor) expression in breast carcinomas. Additionally, GATA3 expression was recently strongly correlated with AR expression in triple negative breast cancers, showing particularly high expression in the molecular apocrine type of triple negative breast cancer which is predominantly AR positive. This study shows that AR is strongly correlated with GATA3 not just in triple negative breast cancers but in all breast cancers. Furthermore, the correlation is stronger than the correlation of GATA3 with ER or PR, suggesting that GATA3 may play a role in AR signaling as it does with ER and PR.

Keywords: Androgen Receptor; Breast Cancer; GATA3

expression and lower histologic grade [6].

Introduction

Breast cancer is the second most lethal cancer among women, responsible for approximately 40,000 deaths in 2015 [1]. The prognosis of breast cancer has improved significantly with the introduction of immune histochemical subtypes of breast cancer that have predicted response to therapy and prognosis. Thus, for each patient there is an abundance of immune histochemical data that can be correlated with progression or expression of other proteins in an attempt to better understand molecular pathways. Physiologically, GATA3 is a transcription factor that has a role in the development of several tissues including the human breast, embryo, and hematopoietic tissues [2-4]. In the human breast, GATA3 expression is seen primarily in ductal epithelial cells with no staining on myoepithelial cells [5]. Consistent with its role in the development of the breast, GATA3 knockout mice show abnormalities in nipple development [4]. Because GATA3 is strongly expressed and functional in mammary ductal epithelial cells, it is possible that it could have a role in carcinogenesis which often arises from ductal epithelial cells and features ductal cells with different stages of differentiation. Thus far, GATA3 has been associated with ER

These findings support the hypothesis that GATA3 expression is reflective of a more well-differentiated status and ER positivity in breast ductal carcinomas. However, other studies paradoxically have shown that GATA3 expression does not predict response to tamoxifen [7]. Molecularly, GATA3 colocalizes with the ER enhancer, promoting estrogen receptor expression while simultaneously ER can bind to the enhancer of GATA3 [8]. This mutual enhancement is likely linked to why ER and GATA3 expression is seen to be correlated in tumors. If GATA3 expression could be correlated with another receptor besides ER and PR, it would suggest the existence of new molecular pathways which GATA3 could interact with either directly or indirectly.

Another receptor that has a well-documented role in breast development is the AR receptor. Signaling through the AR receptor is thought to be the reason why males have less breast tissues than females in whom ER signaling predominates. Similar to AR, ER activation involves increased transcription on nearby noncoding RNAs [9,10]. In addition, both signaling pathways are enabled by FOXA1-mediated DNA binding [11]. Similar to GATA3, AR expression is seen in a large number of triple negative cases (25%)

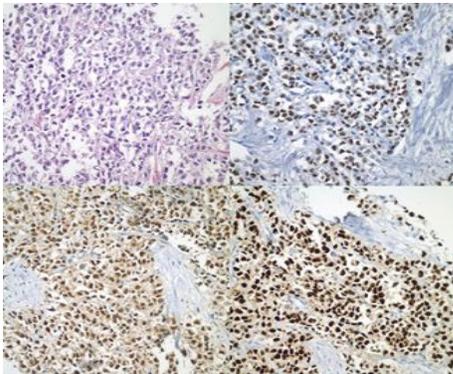
and is seen in approximately half of autopsy metastatic lesions [12]. Since AR is better preserved in the metastatic setting than ER, similar to GATA3, it is possible that the two signaling pathways intertwine. AR has been documented to be associated with ER and PR as well as lower disease grade [13]. Among ER positive breast cancers, AR has been shown to be a positive prognosticator and most ER positive breast cancers are AR positive [14]. Recently, GATA3 expression has been shown to correlate with AR expression in a cohort of triple negative breast cancers [15].

This study seeks to determine whether GATA3 expression is correlated with AR expression in all breast cancers or if the previous results apply strictly to the triple negative breast cancer type. If this correlation is present, it could associate GATA3 with another large transcriptional regulatory unit. The study also seeks to verify that GATA3 expression is correlated with ER and to correlate GATA3 expression with the expression of other known breast markers such as GCDFP-15 and mammaglobin.

Materials and Methods

Patients

A microarray with tumor cores from 259 patients was assessed for GATA3, ER, PR, AR, Her2, GCDFP15, and mammaglobin expression. Samples were retrieved from Yale New Haven Hospital pathology specimens collected between 1953 and 1983. Patients lacking sufficient tissue in the core to assess all seven proteins were excluded. Otherwise, all patients in the tissue microarray were included. The average followup time was 4,602 days. The types of cancers studied included 9 lobular carcinomas, 2 mammary carcinomas with medullary features, 7 mucinous adenocarcinomas, and 241 ductal carcinomas.



H&E (A), GATA3 (B), AR (C), and ER (D) of an Invasive Ductal Carcinoma Case Showing Strong Nuclear Staining for all Three Markers.

Specimen Characteristics

All specimens were formalin fixed and paraffin embedded from the collection date until the tma preparation date.

Assay Methods

Expression of GATA3 (Biocare, catalog # CM405A), AR (Dako, catalog # M3562), GCDFP15 (Biocare, catalog #PM113AA), and mammaglobin (Dako, catalog #M3625) was assessed using their respective antibodies per the manufacturers' instructions. ER, PR, and Her2 expression was quantified by immunofluorescence and given a score of 0, 1, 2, or 3 as previously described [16]. GATA3 (nuclear), AR (nuclear), GCDFP15 (cytoplasmic), and mammaglobin (cytoplasmic) expression was scored semi quantitatively as 0, 1, or 2, corresponding to the absence of staining, weak/focal staining, and strong/diffuse staining, respectively.

Study Design

The cases were selected retrospectively and no stratification or matching was used. The expression levels measured included GATA3, ER, PR, AR, Her2, GCDFP 15, and mammaglobin. Nuclear grade and tumor size were also measured as part of the study.

Statistical Analysis Methods

Expression of GATA3, ER, PR, AR, Her2, GCDFP15, and mammaglobin were correlated with each other and with nuclear grade and size using a Spearman two-tailed correlation test. The significance threshold was set at an α value of 5%. Correlation coefficients were compared to each other with the use of a Fisher transformation and subsequent inverse Fisher transformation to calculate confidence intervals. The initial cohort was much larger but was pruned to exclude patients for which complete information on all 9 variables was not present to attain the final number.

Results

Validation of Known Correlations

We re-validated the previously reported correlations between GATA3 and ER ($r=0.46$) and GATA3 and PR ($r=0.38$). GATA3 was negatively correlated with nuclear grade ($r=-0.2$). ER was positively correlated with PR ($r=0.34$) while it was negatively correlated with nuclear grade ($r=-0.18$). PR was negatively correlated with nuclear grade ($r=-0.13$) and size ($r=-0.13$). AR was positively correlated with both ER ($r=0.33$) and PR ($r=0.14$). Her2 was positively correlated with both size and nuclear grade ($r=0.1$, 0.21 , respectively). Nuclear grade was positively correlated with size ($r=0.12$).

	GATA3	ER	PR	AR	Her2	GCDFP	Mamma	NG	Size
GATA3	1.00								
ER	0.46	1.00							
PR	0.38	0.34	1.00						
AR	0.61	0.33	0.34	1.00					
Her2	-0.07	-0.08	-0.06	-0.02	1.00				
GCDFP	-0.05	0.02	0.00	0.10	0.02	1.00			
Mammaglobin	0.03	0.04	-0.03	0.07	0.00	0.13	1.00		
NG	-0.20	-0.18	-0.13	-0.08	0.21	-0.07	-0.12	1.00	
Size	0.00	-0.02	-0.13	-0.14	0.10	-0.08	-0.07	0.12	1.00

Correlations between staining for different markers statistically significant correlations (absolute value >0.1) are in colored boxes. Positive statistically significant correlations are in green boxes. Negative statistically significant correlations are in red boxes.

New Associations

AR was positively correlated with GATA3 (r=0.61). This correlation was stronger (r=0.61; 95% CI 0.52-0.68) than the second strongest correlation in the study between GATA3 and ER (r=0.46; 95% CI 0.36-0.55). AR was correlated with GCDFP15 (r=0.1) and negatively correlated with size (r=-0.14). GCDFP15 was positively correlated with mammaglobin (r=0.13). Mammaglobin was negatively correlated with nuclear grade (r=-0.12).

LN status	
Positive	160
Negative	99
ER status	
Positive	140
Negative	119
PR status	
Negative	122
Positive	137
Her2 status	
Negative	158
Positive	111
Triple Negative	
Yes	51
No	208
Tumor grade	
1	33
2	140
3	86
Tumor size	
<=5mm	10
<= 1 cm	35
<=2 cm	73
>2 cm	141
Tumor type	

Invasive lobular carcinoma	9
Mammary carcinoma with medullary features	2
Mucinous carcinoma	7
Invasive ductal carcinoma	241
Avg diagnosis age	58.01

Clinicopathologic Data used in this Study

Lack of Association

No association was seen between GATA3, Her2, GCDFP15, mammaglobin, or tumor size. None was seen between ER and these same variables. There was no association between Her2 and GCDFP15 or mammaglobin. GCDFP15 was not correlated with nuclear grade or size. Mammaglobin was not correlated with size.

	Negative (0)	Positive (1, 2, or 3)
GATA3	25	244
ER	128	141
GCDFP15	220	49
Mammaglobin	213	56
AR	68	201
PR	125	144
Her2	163	106

Number of Breast Cancer Cases Showing Some Staining for Each of the Markers Tested.

Discussion

This study aimed to validate the documented GATA3 correlations and to see if there are any new correlations with the androgen receptor protein and other proteins used to mark breast differentiation, GCDFP15 and mammaglobin. If GATA3 is required for normal breast development and GCDFP15 and mammaglobin are markers of breast differentiation, it follows that there could be some correlation between them. As an internal control, this study validated many of the known associations between GATA3 and predictive markers. GATA3 was shown to be associated with ER and PR which are both well-known correlations. GATA3 was also negatively correlated with nuclear grade which is consistent with our knowledge of GATA3 as a marker of better differentiation. Both ER and PR were negatively correlated with nuclear grade reflecting a similar process. Her2 was positively correlated with size and nuclear grade, supporting what we know of her2, that it is a negative prognosticator. Lastly, nuclear grade was positively correlated with size, showing that tumors that are larger at the time of diagnosis are often of a higher nuclear grade and more aggressive.

GATA3 staining frequencies have been well reported in ductal carcinomas but not as well documented in lobular and mucinous carcinomas. In this study, eight of nine lobular carcinomas stained for GATA3 and all mucinous carcinomas stained for GATA3.

Along with the known correlations, this study revealed a previously undocumented correlation between GATA3 and AR that was considerably stronger than that between either GATA3 and ER or GATA3 and PR. This suggests that as well as modulating ER and PR expression by binding to their enhancers, GATA3 could also bind an AR enhancer or influence the AR regulatory network in some way. If verified, this would represent a new signaling functionality for GATA3.

Simultaneously, ER is known to regulate GATA3 expression so the GATA3 gene could have an enhancer element that is bound by AR as well. Alternatively, GATA3 along with AR could both be playing a reactive role; AR has been known to be a positive prognosticator in ER-positive tumors because it has an anti-oncogenic role, countering the effect of ER overexpression. Studies have shown AR signaling to have a buffering effect on ER-mediated c-myc oncogenic activity [18].

Experiments in monkeys have shown that testosterone has antagonistic effects on ER signaling [18]. Cell line studies have shown an inhibitory effect of AR on ER signaling. Breast cancer cell line GATA3 overexpression is associated with less metastasis in a nude mouse model [19]. Since GATA3 is more closely correlated with AR than ER there is a possibility that it too has an anti-oncogenic effect, regulating ER-mediated c-myc signaling. This heightened correlation of AR with GATA3 over ER with GATA3 could also reflect the fact that AR marks ER positive breast cancers most likely in a buffering capacity and it marks molecular apocrine AR positive triple negative breast cancers, both of which groups are likely to be GATA3 positive but only the former would be ER positive. The study also checked for several new associations of GATA3 with the known breast differentiation markers GCDFP15 and mammaglobin. No statistically significant correlation was seen between GATA3 and either of these proteins, suggesting that expression of these proteins is not heavily influenced by GATA3 expression. The study also validated the previous observation that in primary breast cancers GATA3 is a better marker for breast tumor differentiation than GCDFP15 or mammaglobin since it exhibited a sensitivity of 90.7% versus 18.2% and 20.8%, respectively.

Conclusion

GATA3 expression is strongly correlated with AR expression in breast carcinomas

Declarations

Ethics Approval

The tmAs were used without any identifying information and thus are exempt from HIC regulations according to exemption 4.

Consent for Publication

Consent is not necessary according to exemption 4.

Availability of Data

The datasets used in this study are available from the corresponding author on request.

Competing Interests

The authors declare that they have no competing interests.

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Author's Contributions

AB and MH analyzed and interpreted the data.

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