GATA3 Expression in Triple Negative Breast Cancer

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Summary
Background: GATA3 (GATA-binding protein 3) belongs to the zinc finger transcription factor family. It plays a role in enhancing cellular growth and differentiation across various tissues and cell types. GATA3 is an estrogen receptor (ER) regulated gene, and also present in primary and metastatic breast cancer.
Aim: To evaluate clinical significance of GATA3 expression in triple negative breast cancer patients.
Method: In this study, formalin fixed paraffin embedded tumour tissue from 100 patients with triple negative breast cancer were analysed in the study. GATA3 expression was assessed using immunohistochemistry method and its correlation with clinical and pathological factors, as well as disease status, was examined.
Results: Nuclear GATA3 positivity was seen in 61% of triple negative breast cancer patients. GATA3 expression when correlated with clinicopathological parameters, a significant higher incidence of GATA3 expression was noted in patients with T4 tumor size, lymph node positive status and advanced stage of disease as compared to their respective counter parts. Such a correlation of GATA3 expression was not observed with other clinical and pathological parameters. In univariate survival analysis, GATA3 expression did not discriminate patients with worse or better disease free survival (DFS) and overall survival (OS). In multivariate survival analysis for disease free survival, disease stage entered at step 1 as significant prognostic factor.
Conclusion: A nuclear GATA3 expression correlated with T4 tumor size, lymph node positive status, advanced disease stage suggests a marker of disease aggressiveness for TNBC.
Keywords: Breast cancer, ER, GATA3, Triple Negative Breast Cancer, DFS

Introduction

Triple negative breast cancer (TNBC) is characterized by the lack of estrogen, progesterone and HER-2 receptors. TNBC accounts for 15–20% of all newly diagnosed cases of primary breast carcinoma and has the worst overall prognosis.[1] GATA3 binding protein 3 is a group of six zinc finger transcription factor initially recognized for its role in controlling immune cell activity. It is crucial in regulating the development of various tissue, including blood cells, skin, breast, kidneys and the central nervous system. GATA3 is responsible for maintaining the quiescent state of differentiated luminal cells in the adult mammary gland, therefore it is possibly causally involved in pathogenesis of breast carcinoma.[2] Several earlier studies have demonstrated elevated level of GATA3 in ER-positive (luminal) breast cancers. They found that GATA3 and estrogen receptor (ER) are involved in a cross-regulatory loop and are therefore regularly co-expressed in breast cancers.[3-11] But GATA3 expression in TNBC is particularly significant because this subtype of breast cancer typically tests negative for most markers associated with breast tissue. Due to low sensitivities of mammaglobin and GCDFP15, GATA3 may potentially be a useful marker in TNBC.[12,13] Therefore, this study evaluated clinical significance of GATA3 in TNBC patients and its correlation with clinicopathological parameters and disease status.

Materials and Methods

Patient characteristics

This study was approved by the Institutional Scientific Review and Ethics Committees. In this retrospective study, a total of 100 TNBC patients (ER-/PR-/HER2-) diagnosed and treated at The Gujarat Cancer and Research Institute were enrolled. The detailed clinical history of patients like age, menopausal status, histopathological findings, disease stage and treatment offered was recorded from the Institutional Medical Record Department. The disease was staged using the AJCC classification. Disease status was evaluated through clinical examination, radiological investigations and biochemical investigations.

Immunohistochemical Localization

The tissue blocks were archived from histopathology department. Three µm thin sections were taken on 3-Aminopropyl-triethoxysaline (APES) coated slides. GATA3 staining was performed on Ventana Benchmark autoimmunostainer. For IHC staining Ventana reagents were used. EZ prep solution used for deparaffinization, followed by antigen retrieval using cell conditioning 1 (CC1) buffer for 30min. Ultra view DAB detection kit was used for further IHC steps. 100µl GATA3 antibody

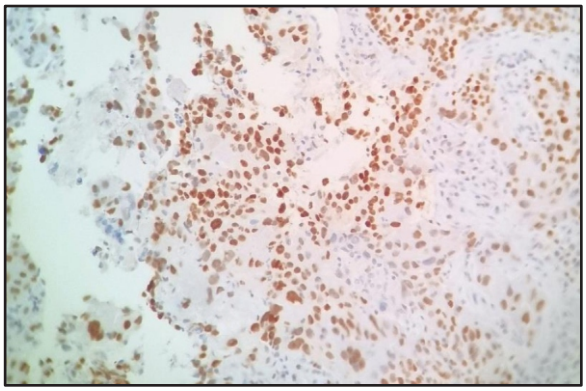

Figure 1: Nuclear GATA3 expression in tumour cells of TNBC patient (40X)

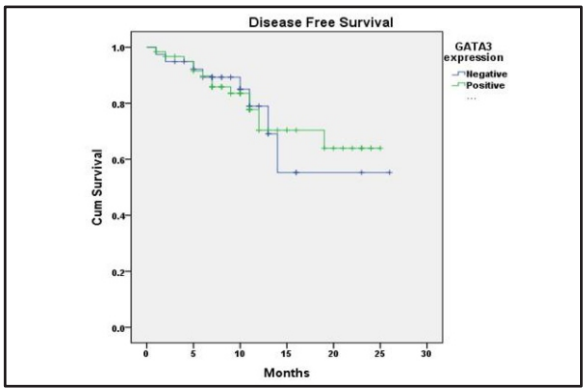

Figure 2: (A) Kaplan - Meier survival analysis for disease free survival (DFS)

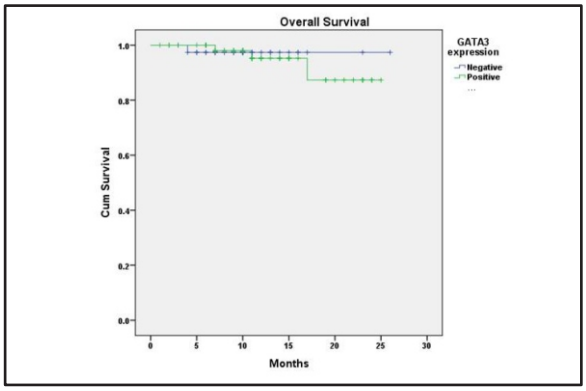

Figure 2: (B) Kaplan - Meier survival analysis for overall survival (OS)

(Clone: L50-823; dilution 1:100) was used from cell marque company. Slides were mounted with DPX and viewed under microscope.

Scoring

For GATA3 evaluation H-score method was used. Nuclear staining pattern was observed for GATA3.

Statistical analysis

Statistical analysis was done by SPSS statistical software version 20. Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. Univariate survival analysis, Kaplan Meier and Log Rank tests, was conducted to assess the prognostic impact on disease-free survival (DFS) and overall survival (OS). Multivariate survival analysis was carried out using Cox regression model with forward stepwise (likelihood ratio) approach. P values ≤ 0.05 were considered to be statistically significant.

Results

GATA-3 expression in breast carcinoma

Nuclear GATA3 expression was detected in 61% (61/100) of TNBC patients. Among these patients, 17% showed an H score of +1, 25% had a score of +2, and 19% exhibited a score of +3 staining (Figure 1).

Correlation of GATA3 expression with clinicopathologic parameters

A significant higher incidence of GATA3 expression was noted in patients with T4 tumor size (100%; 04/04; p = 0.04) as compared to patients with T3, T2, and T1 tumor size (79%; 11/14), (58%; 24/41), and (46%; 12/26), respectively. (Table 1). Further, a significant higher incidence of GATA3 was detected in patients with lymph node positive status (76%; 29/38; p = 0.011) than lymph node negative status (49%; 21/43) (Table 1). Similarly, significant higher incidence of GATA3 expression was noted in patients with advanced stage of disease (83%; 20/24; p = 0.011) as compared to patients with early stage of disease (34%; 35/65) (Table 1). Regarding BR Score, a higher incidence of GATA3 expression was found in patients with high BR score (61%; 40/65; p = 0.131) as compared to patients with intermediate BR score (41%; 07/17). While GATA3 expression was not associated with age at diagnosis and menopausal status (Table 1).

Correlation of GATA3 expression with disease status

Based on the univariate survival analysis, conducted by Kaplan-Meier, in relation to DFS, similar rate of disease relapse was observed in patients with GATA3 positivity (23%; 14/61) and GATA3 negative patients (21%, 08/39; Log rank = 0.008, df = 1, p = 0.929) (Table 2, figure 2A). Regarding OS, a similar incidence of death was seen in GATA3 positive patients (05%, 03/61) and GATA3 negative patients (03%, 01/39; Log rank = 0.087, df = 1, p = 0.767) (Table 2, Figure 2B). A multivariate survival analysis was conducted using a Cox regression model with forward stepwise approach to assess the prognostic importance of clinical and pathological

Table 1: Correlation of GATA3 expression with clinicopathological parameters and disease status

Parameters	GATA3 expression			
	N(%)	Negative N(%)	Positive N(%)	P
Age(years)	**100(100)**	**39(39)**	**61(61)**	
≤50 years	53(53)	17(32)	36(68)	0.132
>50 years	47(47)	22(47)	25(53)	
Menopausal Status	**100(100)**	**39(39)**	**61(61)**	
Premenopausal	29(29)	10(35)	19(65)	0.554
Postmenopausal	71(71)	29(41)	42(59)	
Tumor Size	**85(100)**	**34(40)**	**51(60)**	
T1	26(31)	14(54)	12(46)	0.040
T2	41(48)	17(42)	24(58)	
T3	14(16)	03(21)	11(79)	
T4	04(05)	00(00)	04(100)	
Lymph node Status	**81(100)**	**31(38)**	**50(62)**	
Negative	43(53)	22(51)	21(49)	0.011
Positive	38(47)	09(24)	29(76)	
Stage	**89(100)**	**34(38)**	**55(62)**	
Early (Stage + IA + IIA + IIB)	65(73)	30(66)	35(34)	0.011
Advanced (Stage IIIA + IIIB)	24(27)	04(17)	20(83)	
Histopathology	**100(100)**	**39(39)**	**61(61)**	
IDC	99(99)	38(38)	61(62)	0.209
IDC + DCIS	01(01)	01(100)	00(00)	
Histological Grade	**100(100)**	**39(39)**	**61(61)**	
Grade II	19(19)	07(37)	12(63)	0.830
Grade III	81(81)	32(40)	49(60)	
BR Score	**82(100)**	**35(43)**	**47(57)**	
6-7 (Intermediate)	17(21)	10(59)	07(41)	0.131
8-9 (High)	65(79)	25(39)	40(61)	
Disease Metastasis	**100(100)**	**39(39)**	**61(61)**	
Yes	22(22)	08(36)	14(64)	0.774
No	78(78)	31(40)	47(60)	
Disease Status	**100(100)**	**39(39)**	**61(61)**	
Alive	96(96)	38(48)	58(52)	0.558
Dead	04(04)	01(25)	03(75)	

Table 2: Univariate analysis of GATA3 expression

GATA3 expression	N	DFS in months Mean ± SE	Remission N (%)	Relapse N (%)
Negative	39(39)	19.066 ± 2.144	31(79)	08(21)
Positive	61(61)	19.609 ± 1.224	47(77)	14(23)
			Log = 0.008, df = 1, p = 0.929	
GATA3 expression	**N**	**OS in months Mean ± SE**	**Alive N (%)**	**Dead N (%)**
Negative	39(39)	25.421 ± 0.571	38(97)	01(03)
Positive	61(61)	23.620 ± 0.783	58(95)	03(05)
			Log = 0.087, df = 1, p = 0.767	

Table 3: Multivariate analysis of GATA3 expression

							95.0% CI for Exp(B)	
Patients	**Steps**	**Variables**	**Wald Statistics**	**Df**	**P**	**Exp(B)**	**Lower**	**Upper**
DFS	1	Stage	5.640	1	0.018	3.985	1.273	12.475

factors, including age, menopausal status, tumour size, lymph node status, disease stage, histological grade, BR score and GATA3. Disease stage entered at step 1 as significant factor (Wald statistic = 5.650, df = 1, Exp (B) = 3.985, p = 0.018; Table 3).

Discussion

In this retrospective study, we evaluated the expression of GATA3 in TNBC patients and observed nuclear GATA3 expression in 61% of tumor tissues. Similar incidence of GATA3 expression was observed in earlier studies which reported GATA3 expression ranging from 40% to 90% in TNBC patients.[12, 14-19] In this study, GATA3 expression was not correlated with clinical parameters such as age and menopausal status. Significant association was not observed with age and menopausal status which is in concordance with many studies which showed no correlation between the GATA3 expression with age and menopausal status.[19-22]

Regarding pathological parameters, a significant higher incidence of GATA3 expression was noted in T4 tumor size as compared to their counterparts which is in concordance with the study of Lahari Banik et al.[22] Furthermore, with respect to lymph nodes status, GATA3 expression was significantly higher in lymph node positive patients which is similar with the study of Kouros-Mehr et al.[2] This study also represents a significant higher incidence of GATA3 expression with advanced stage of disease as compared to early stage of disease which is supported by the study of Singh et al.[18] While no significant correlation was noted with histopathology subtypes this was similar with the studies of Suri et al.[21] and Albergaria et al.[23] Also, with respect to high grade tumors no significant correlation was observed which is in concordance with the studies of Ahadi et al. [19] and Kim et al. [24]

In present study, a similar incidence of GATA3 expression was noted in patients with disease relapse and patients who undergone disease remission. A significant correlation was not observed between GATA3 expression and disease metastasis which is in concordance with the study of McCleskey et al.[7] Further, significant correlation was not found between DFS and OS in TNBC patients this was supported by the study of Albergaria et al.,[23] and Ciocca et al.[25] In multivariate survival analysis with respect to DFS, disease stage is found to be independent prognostic factor in step 1 among the clinicopathologic parameters analysed in this cohort. Also, multivariate analysis of Mehra et al.[3] revealed that low GATA3 expression was an independent predictor of DFS in prognostic feature.

Conclusion

In summary, nuclear GATA3 protein expression correlated with T4 tumor size, lymph node positive status and advanced disease stage suggests a marker of disease aggressiveness. Further investigation of GATA3 related pathway will help to understand disease metastasis and may also provide novel therapeutic targets in TNBC.

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