Mammaglobin, GATA-binding protein 3 (GATA3), and epithelial growth factor receptor (EGFR) expression in different breast cancer subtypes and their clinical significance

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ABSTRACT

Increasing evidence has shown that mammaglobin, GATA-binding protein 3 (GATA3), and epithelial growth factor receptor (EGFR) have unique clinical implications for breast cancer subtyping and classification, as well as for breast cancer targeted therapy. It is particularly important to clarify the correlation between their expression and different molecular breast carcinoma subtypes to better understand the molecular basis of the subtypes and to identify effective therapeutic targets for the disease. This study aimed to evaluate mammaglobin, GATA3, and EGFR expression in different breast cancer subtypes, as well as their clinical significance. Subjects of the study included 228 patients with breast cancer at The First Affiliated Hospital of University of Science and Technology of China. They were divided into triple negative (TN), Luminal A, Luminal B, and HER-2 positive (HER-2.P) breast cancer groups based on molecular classification. Immunohistochemical methods were used to detect mammaglobin, GATA3, and EGFR expression in cases of different molecular subtypes before determining the correlation between protein expression and subtype. Mammaglobin and GATA3 expression levels were found to significantly vary with respect to histopathological grade, lymph node status, and molecular subtype; EGFR expression was significantly correlated with breast cancer histopathological grade and molecular subtype. For breast cancer, the expression levels of mammaglobin and GATA3, as well as mammaglobin and EGFR, were significantly correlated. In addition, there was a significantly negative correlation between the expression levels of GATA3 and EGFR in breast cancer tissue samples, especially in HER-2.P samples. These findings provide a theoretical basis for assessing breast cancer clinical prognosis based on the cancer subtype, and hence, have significant practical value.

Key words: breast cancer; mammaglobin; GATA-binding protein 3; epithelial growth factor receptor; molecular subtype.

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Introduction

Breast cancer has become one of the most common types of malignant tumours among women worldwide. It has been gaining public attention owing to its association with high morbidity and mortality rates that have become a major public healthcare issue. With rapid advancements in biology and medicine, individualised treatments for different breast cancer molecular subtypes have become the focus in breast cancer research.[1,2] Studies have shown that mammaglobin, GATA-binding protein 3 (GATA3), and epithelial growth factor receptor (EGFR) are differentially expressed in different tumour types. Mammaglobin is expressed in breast tumours, the endometrium, and sweat and salivary glands, and it might be involved in the regulation of steroid metabolism.[3,4] Mammaglobin overexpression is associated with breast cancer occurrence and metastasis.[5,6] The protein GATA3 has also been found to participate in oestradiol regulation *via* a transcriptional circuitry that links it to oestrogen receptor α (ERα).[7,8] Abnormal GATA3 non-expression may cause ERα to not accumulate and induce excessive accumulation of factors critical in epithelial-to-mesenchymal transition and metastasis; this promotes tumour cell metastasis and induces aggressive breast cancer phenotypes and poor prognosis.[9-11] Several studies have shown that high GATA3 expression is a good prognostic factor for breast cancer. Takaku *et al*. experimentally demonstrated *in vitro* that molecular alterations in GATA3 led to the expression of highly aggressive tumour phenotypes.[12] Warrick *et al*. found high GATA3 expression and fascin negativity to be associated with better clinical prognosis in breast cancer patients.[13] However, GATA3 has not yet been identified as an independent prognostic factor. Voduc *et al*. found a significant correlation between GATA3 expression and ER in a cohort study involving 3119 breast cancer patients; through an ER-positive subgroup analysis, they found GATA3 to have no independent prognostic value.[14] The importance of EGFR in epithelial cell proliferation and survival is well-recognized as it is one of the most widely used drug targets for malignant tumour treatment.[15] Burness *et al.* reported that breast cancer occurrence and development could be promoted through the action of EGFR on cancer stem cells.[16]

The present study explored the sensitivities of mammaglobin, GATA3, and EGFR, and it evaluated the correlation between expression of those proteins and the clinical characteristics of 228 breast cancer patients with different subtypes of the disease, who were treated at The First Affiliated Hospital of University of Science and Technology of China (USTC) in 2019. Furthermore, we explored the application of the proteins as molecular markers for the clinicopathological diagnosis and classification of breast cancer and provided novel strategies for the treatment of the disease.

Materials and Methods

Tissue samples

In this study, 228 cases of breast cancer, treated in our hospital from December 2018 to December 2019, were selected for the research. According to international consensus,[17] breast cancer can be classified into different molecular subtypes based on expression levels of human epidermal growth factor receptor 2 (HER2), ER, and progesterone receptor (PR) in breast cancer tissues as determined using immunohistochemical methods. Clinicopathological data were obtained from pathology reports. The breast cancer tissues were fixed in 4% paraformaldehyde for 48 h and subsequently embedded in paraffin to prepare them for formalin-fixed paraffin-embedding (FFPE).

Antibodies and immunohistochemistry

The two-step EnVision method[18] was used for immunohistochemical analysis. The positive control was prepared according to the reagent instructions; secondary antibody alone was used as the negative control.

The immunohistochemistry was performed using rabbit anti-human mammaglobin (1:500, clone EP249, Zhongshan Biotechnology, China), mouse anti-human GATA3 (1:100, clone EP368, Zhongshan Biotechnology, China), and mouse anti-human EGFR (1:150, clone EP22, Zhongshan Biotechnology, China) antibodies as the primary antibodies. The FFPE slides were deparaffinized, antigen retrieved, and blocked for endogenous peroxidase (3% H_2O_2). After pretreatment, the sections were incubated with the primary antibodies overnight at 4°C. Then, the slides were rinsed with PBS and incubated with a secondary antibody (PV600, Zhongshan Biotechnology, China) for 50 min at room temperature. The slides were visualized using an ultra-View Universal DAB detection kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). All slides were counterstained with haematoxylin.

The immunohistochemical staining results were determined by a pathologist using a semi-quantitative method, with the percentage of positive or negative cells calculated based on the following criteria: mammaglobin >10% is positive, mammaglobin ≤10% is negative;[19] GATA3 >10% is positive, GATA3 ≤10% is negative;[20] EGFR ≥10% is positive, and EGFR <10% is negative.[2] The magnification of the microscope was 200x, and the yellow-brown area was randomly selected as the observation field. Three areas were selected from each slide for interpretation. Two deputy chief physicians from the pathology department independently evaluated the staining results on two tissue chips, and the average value of the evaluation results of the same case was taken as the result for that case.

Statistical analysis

Data analysis was performed using the SPSS 20.0, Python (version 3.8.2), and Prism (version 8.4.3) software packages. Measurement data were expressed as the number of cases and compared using the *t*-test, while data involving counts were expressed as percentages (%) and compared using the chi-square (χ^2) test. Pearson correlation test was used for correlational analysis and for all comparisons, and p-values <0.05 indicated statistically significant differences. The correlation between mammaglobin, GATA3, and EGFR expression and clinicopathological parameters was determined using the χ^2 test.

Results

Correlation of molecular subtypes of breast cancer with clinicopathological characteristics

Table 1 shows the clinicopathological characteristics of breast cancers of four molecular subtypes. In this study, 32 cases of triple negative (TN) breast cancer, 41 cases of Luminal A, 103 cases of Luminal B, and 52 cases of HER-2 positive (HER-2.P) were detected. The average age of the patients was 52.48±12.34 years. Lump sizes were between 1 and 11 cm, with an average of 5.45±1.25 cm. As for histopathological grades, 55 cases were grade Ⅰ, while 173 cases were grade Ⅱ and Ⅲ. The most common histological type of all the breast cancer was the invasive ductal carcinoma with no special type (range: 80.4%–82.9%). There were 32 cases of TN patients, with an average age of 53.7 years (range: 35–98) and an average tumour size of 17.5 cm^3 (1.5–120 cm^3). The number of cases in grades Ⅰ, Ⅱ, and Ⅲ were 2 (6.25%), 10

(31.25%), and 10 (31.25%), respectively. Eight cases (25%) had positive axillary lymph nodes. For Luminal A, there were 41 patients, with an average age of 51.9 years (range: 27–84). The average tumour size was 16.53 cm^3 (0.24–102 cm^3), and the number of cases in grades Ⅰ, Ⅱ, and Ⅲ were 2 (4.88%), 18 (43.90%), and 4 (9.76%), respectively. Of patients in this group, 15 (36.6%) had positive axillary lymph nodes. Among the 104 cases of Luminal B, patients had an average age of 49.7 years (31–81), the average tumour size was 15.25 cm^3 (0.36–230 cm^3), and the cases in grades Ⅰ, Ⅱ, and Ⅲ were 2 (1.92%), 55 (52.88%), and 13 (12.50%), respectively, with 35 cases (33.7%) of positive axillary lymph nodes. For the last group of Her-2.P, there were 51 patients, with an average age of 48.9 years (22–75). The average tumour size was 20.67 cm^3 (0.64~117.6 cm^3), and the cases in grades Ⅰ, Ⅱ, and Ⅲ were 1 (1.96), 23 (45.10), and 11 (21.57), respectively. Fifteen cases (29.4%) had positive axillary lymph nodes.

Expression patterns of mammaglobin, GATA3, and EGFR in breast cancer tissues

In this study, 228 breast cancer samples were analysed to determine mammaglobin, GATA3, and EGFR protein expression. The findings of the immunohistochemical analysis conducted on the breast tumour specimens are shown in Figure 1. Two immunostaining patterns of mammaglobin were observed in the breast cancer cells: cytoplasmic and membranous patterns (Figure 1 A,D,G,J). The proteins GATA3 (Figure 1 B,E,H,K) and EGFR (Figure 1 C,F,I,L) were found to be expressed in the nucleus and cytoplasm, respectively. Notably, EGFR expression was not observed in the Luminal A group (Table 2) in which negative EGFR staining was observed (Figure 1F). Staining of the three markers in normal tissues as controls is shown in Supplementary Figure 1.

Correlation of mammaglobin, GATA-3, and EGFR expression with clinicopathological characteristics

The correlation between mammaglobin, GATA3, and EGFR expression and clinicopathological parameters was analysed using SPSS20.0. We found the expression levels of these proteins to be significantly correlated with histopathological grade, ER status, PR status, and breast cancer molecular subtype ($p<0.05$). Mammaglobin expression was detected in 132 (57.9%) samples and was significantly correlated with lymph node status, HER2 status, and Ki-67 expression levels ($p<0.01$). Expression of GATA3 was detected in 193 (84.6%) samples and was significantly correlated with lymph node status and HER2 status ($p<0.01$); however, there was no correlation between its expression levels and those of Ki-67 ($p=0.054$). Expression of EGFR was detected in 31 (13.6%) samples and was significantly correlated with Ki-67 expression levels ($p<0.001$) but was not correlated with lymph node status ($p=0.069$) or HER2 status ($p=0.349$). Of all the tissue samples, 84.6% (193/228) were GATA-3-positive, and this was significantly higher than the proportion of mammaglobin-positive (57.9%) and EGFR-positive (13.6%) samples. These data are presented in detail in Table 2.

Table 1. Clinical and pathological characteristics of breast cancer tissues analysed in this study.

	TN (%)	Luminal A (%)	Luminal B (%)	HER-2.P (%)
Total cases	32	41	104	51
Median age (y)	53.7	51.9	49.7	48.9
Minimum	35	27	31	22
Maximum	98	84	81	75
Median tumor size (mm)	17.50	16.53	15.25	20.67
Minimum	1.5	0.24	0.36	0.64
Maximum	120	102	230	117.6
Grade				
Ⅰ	2 (6.25)	2 (4.88)	2 (1.92)	1 (1.96)
Ⅱ	10 (31.25)	18 (43.90)	55 (52.88)	23 (45.10)
Ⅲ	10 (31.25)	4 (9.76)	13 (12.50)	11 (21.57)
Unknown	11 (34.38)	17 (41.46)	32 (30.77)	16 (31.37)
Histological type				
Invasive ductal carcinoma	26 (81.3)	34 (82.9)	84 (80.8)	41 (80.4)
Intraductal carcinoma	1 (3.1)	1 (2.4)	8 (7.7)	5 (9.8)
Poorly differentiated carcinoma	0 (0.0)	0 (0.0)	6 (5.8)	3 (5.9)
Secretory carcinoma	0 (0.0)	1 (2.4)	3 (2.9)	1 (2.0)
Mucinous adenocarcinoma	0 (0.0)	5 (12.2)	0 (0.0)	0 (0.0)
Papillary carcinoma	1 (3.1)	0 (0.0)	1 (1.0)	1 (2.0)
Invasive lobular carcinoma	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Medullary carcinoma	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
Apocrine carcinoma	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)
Other	2 (6.3)	0 (0.0)	1 (1.0)	0 (0.0)
Hormonal receptor and Her2 status				
ER negative	32 (100.0)	0 (0.0)	18 (17.3)	45 (88.2)
PR negative	32 (100.0)	0 (0.0)	2 (1.9)	44 (86.2)
Her2 negative	32 (100.0)	41 (100.0)	27 (26.0)	0 (0.0)
Lymph node status				
Positive	8 (25.0)	15 (36.6)	35 (33.7)	15 (29.4)
Negative	11 (34.4)	16 (39.0)	20 (19.2)	16 (31.4)
Unknown	14 (43.8)	10 (24.4)	47 (45.2)	20 (39.2)

Correlation of mammaglobin, GATA3, and EGFR expression in different breast cancer molecular subtypes

Figure 2 shows the proportions of positive mammaglobin, GATA3, and EGFR expression in different breast cancer molecular subtypes. The percentages of mammaglobin-positive samples in the TN, Luminal A, Luminal B, and Her2.P groups were 9.4%, 56.1%, 71.2%, and 62.7%, respectively; there was no correlation between mammaglobin expression in the different groups (*results not shown*). The percentages of GATA-3-positive samples in the TN, Luminal A, Luminal B, and Her2.P groups were 43.8%, 100%, 97.1%, and 72.5%, respectively, indicating that GATA3 was highly expressed in luminal cancers. The percentages of EGFR-positive samples in the TN, Luminal A, Luminal B, and Her2.P groups were 50%, 0%, 5.8%, and 17.6%, respectively, suggesting the possibility of a correlation between EGFR expression and breast cancer molecular subtype. To verify our findings, the RNA-seq data for 117 breast cancer samples were collected and analysed on the cBioPortal website (https://www.cbioportal.org/datasets),[22] which was developed based on the work of Krug *et al*.[23] We found GATA3 expression to be significantly associated with breast cancer subtype ($p<0.001$, Figure 3A); similar results were obtained for EGFR expression ($p<0.001$, Figure 3B). These findings support the differential expression of GATA3 and EGFR with respect to breast cancer tissue subtype.

Correlation between GATA3 and EGFR expression in breast cancer tissues

We analysed the correlation between mammaglobin, GATA3, and EGFR expression in breast cancer tissues and found that there was a significant correlation between mammaglobin and GATA3 expression in breast cancer tissues ($p=0.002$, Table 3); similar results were obtained for GATA3 and EGFR ($p<0.0001$, Table 3). We used the GEPIA database (http://gepia.cancer-pku.cn/) to evaluate the correlation between the gene expression levels of mammaglobin, GATA3, and EGFR in breast cancer tissues. As shown in Figure 4A, there was a significant negative correlation between GATA3 and EGFR gene expression levels in breast cancer samples ($p=0.0002$). The correlation between mammaglobin and GATA3 gene expression levels were not significant; thus, the results are not shown.

Previous studies have shown that GATA3 and EGFR expression significantly vary with breast cancer subtype. There was a sig-

Table 2. Mammaglobin, GATA3, and EGFR expression levels and their correlation with clinicopathological parameters, biomarker expression levels, and primary tumour molecular subtypes.

	Mammaglobin		p-value	GATA3		p-value	EGFR		p-value
	Positive (57.9%)	Negative (42.1%)		Positive (84.6%)	Negative (15.4%)		Positive (13.6%)	Negative (86.4%)	
Total cases	132	96		193	35		31	197	
Lymph node status									
Positive	52	21	0.009	69	4	0.008	12	61	0.069
Negative	31	32		50	13		4	59	
Grade									
I-II	69	44	0.033	100	13	0.028	13	100	0.028
III	16	22		28	10		10	28	
ER									
Positive	99	50	<0.0001	147	2	<0.0001	6	143	<0.0001
Negative	33	46		46	33		25	54	
PR									
Positive	90	44	<0.0001	130	4	<0.0001	6	128	<0.0001
Negative	42	52		63	31		25	69	
Her2									
Positive	93	35	<0.0001	117	11	0.002	15	113	0.349
Negative	39	61		76	24		16	84	
Ki-67									
High	73	26	<0.0001	89	10	0.054	30	69	<0.0001
Low	59	70		104	25		1	128	
Subtype									
TN	3	29	<0.0001	14	18	<0.0001	16	16	<0.0001
Luminal A	23	18		41	0		0	41	
Luminal B	74	30		101	3		6	98	
HER-2.P	32	19		37	14		9	42	

Table 3. Correlation between mammaglobin, GATA3, and EGFR expression in breast cancer tissues.

		Mammaglobin		p-value	EGFR		p-value
		Positive	Negative		positive	negative	
GATA3	Positive	120	73	0.002	18	175	0.052
	Negative	12	23		13	22	
EGFR	Positive	13	18	<0.0001			
	Negative	119	78				

nificantly negative correlation between GATA3 and EGFR expression in breast cancer tissues. Thus, we analysed the correlation between their expression levels in the different breast cancer subtypes. Our findings showed that GATA3 and EGFR exhibited significantly different expression levels in the TN, Luminal A, Luminal B, and HER-2.P breast cancers subtypes (Table 4, $p<0.0001$). The RNA data described above were used to perform linear regression analyses on GATA3 and EGFR expression levels

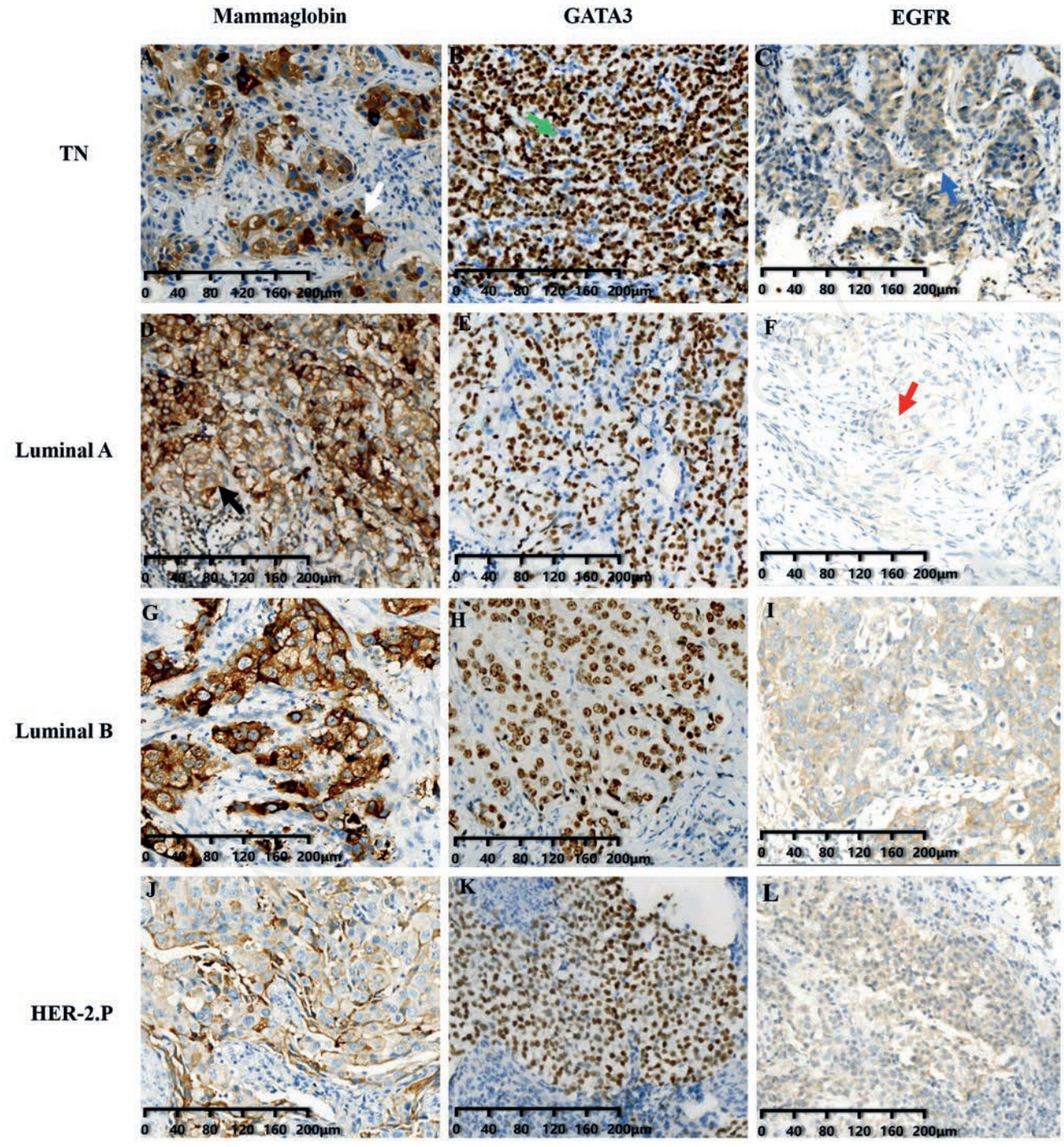

Figure 1. Immunohistochemical analysis of mammaglobin, GATA3, and EGFR expression in four breast cancer subtypes. A-C) Expression of the three markers in the TN subtype. D-F) Expression of the three markers in the Luminal A subtype. G-I) Expression of the three markers in the Luminal B subtype. J-L) Expression of the three markers in the HER-2.P subtype. The white arrow indicates the cytoplasmic pattern of mammaglobin expression (A). The black arrow indicates the membranous pattern of mammaglobin expression (D). The green arrow indicates the nuclear pattern of GATA3 expression (H). The blue arrow indicates the cytoplasmic pattern of EGFR expression (C). The red arrow indicates negative EGFR staining in tumour cells (F). Magnification: 10×; scale bar: 200 μm.

in the four breast cancer subtypes. Expression of the proteins was found to be significantly negatively correlated only in the HER-2.P breast cancer subtype (p=0.0001, Figure 4B).

Discussion

Breast cancer is a commonly encountered malignant tumour in clinical practice.[24] Because of its high degree of heterogeneity, its disease progression and treatment options significantly differ for different molecular subtypes. This heterogeneity also affects disease prognosis in patients, affecting the physical and mental health, life, and property safety of most patients. Therefore, pathological diagnosis at the molecular level is important for early breast cancer detection, and it is particularly critical in developing more accurate detection methods and identifying effective indicators for diagnostic and prognostic purposes.

Mammaglobin is a well-known specific marker for breast cancer, and the high expression of mammaglobin is associated with tumour stage,[9] histological grading,[3,6] lymph node metastasis,[3,19] and endocrine state.[5,6] In this study, we found mammaglobin expression to be significantly associated with lymph node status, histopathological grade, and Ki-67 expression, despite its low sensitivity. These findings agree with those reported by previous studies, in which mammaglobin overexpression was found to be correlated with lymph node metastasis and was identified as a potential protein metastasis marker in breast cancer patients.[3,19]

As a member of the GATA transcription factor family, GATA3 plays a key role in the regulation of breast tissue growth and promotes differentiation.[10,25,26] In addition, GATA3 is an emerging diagnostic molecular marker for breast cancer[27] and is more sensitive than traditional tumour markers, such as mammaglobin and GCDFP15.[18] Previous studies have demonstrated the value of GATA3 as an early diagnostic and prognostic marker for breast cancer;[12,28,29] however, its differential expression in breast cancer molecular subtypes is unclear. This study demonstrated that GATA3 expression is significantly associated with prognostic

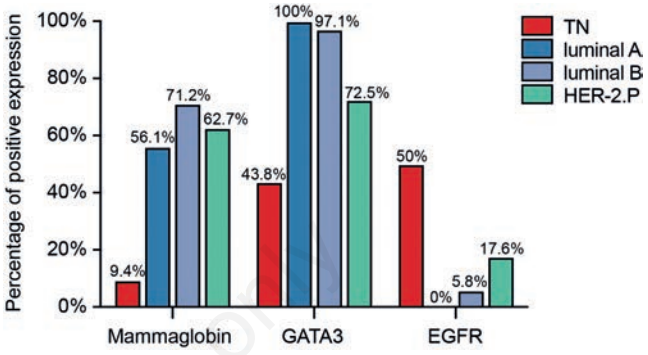

Figure 2. Percentages of mammaglobin GATA3 and EGFR-positive expression in four breast cancer subtypes.

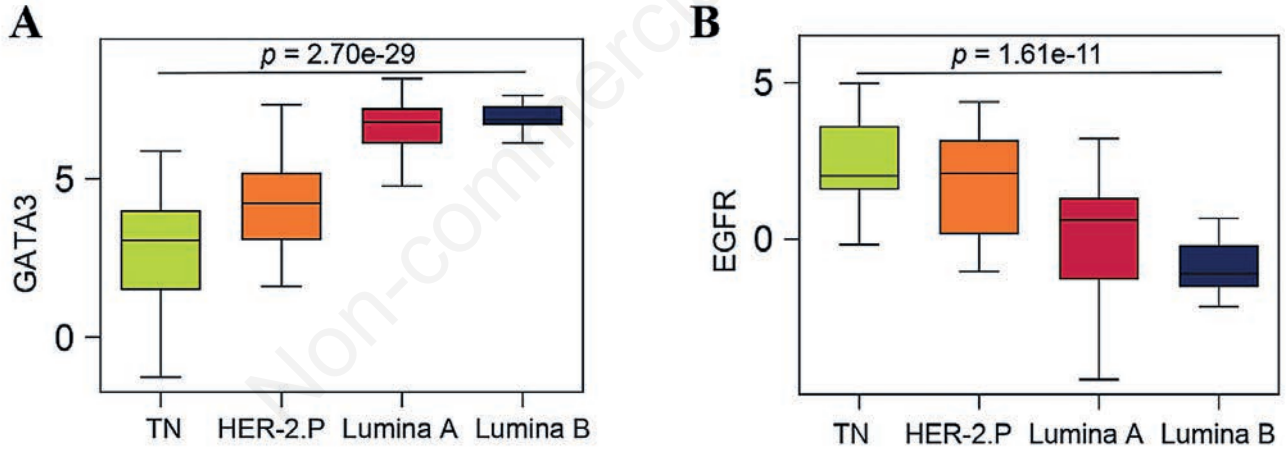

Figure 3. RNA expression of GATA3 and EGFR in four breast cancer subtypes. A: GATA3 RNA expression in four breast cancer subtypes. B: EGFR RNA expression in four breast cancer subtypes. The RNA-seq data used for analysis were obtained from 29 TN samples, 57 Luminal A samples, 17 Luminal B samples, and 14 HER-2.P samples. The p-values for differences in gene expression between the four breast cancer groups were determined using one-way ANOVA, and p<0.05 was considered statistically significant.

Table 4. Correlation between GATA3 and EGFR expression in different breast cancer molecular subtypes.

	GATA3 positive		GATA3 negative	
	EGFR positive	EGFR negative	EGFR positive	EGFR negative
TN	6	8	10	8
Luminal A	0	41	0	0
Luminal B	6	95	0	3
HER-2.P	6	31	3	11
p-value	<0.0001			

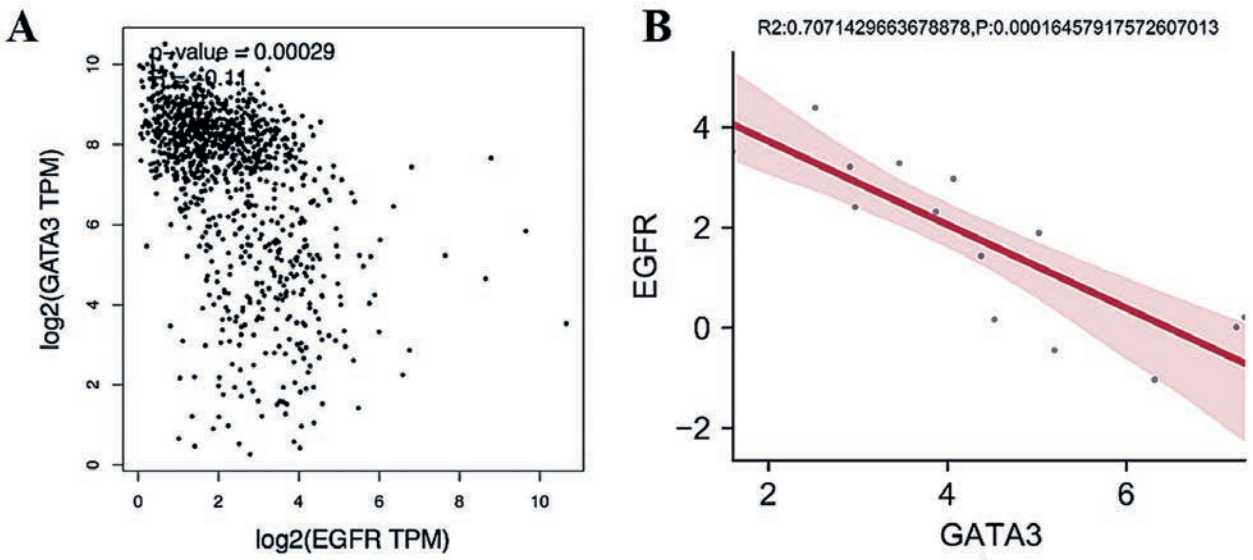

Figure 4. Correlation between GATA3 and EGFR expression. A) Correlation between GATA3 and EGFR expression in breast cancer tissues. B) Correlation between GATA3 and EGFR expression in the HER-2.P breast cancer subtype. The shaded portions represent the confidence interval. The solid line was fit from linear regression; the p value and the square of the coefficient of correlation (R^2) were calculated using the "OLS" function in the "stats models" package in Python, and $p<0.05$ was considered statistically significant.

markers, such as lymph node metastasis and histopathological grade. This further demonstrated the importance of GATA3 expression in the identification of different breast cancer subtypes. Our findings showed a significant difference in GATA3 expression between the TN, Luminal A, Luminal B, and HER-2.P breast cancer subtypes, suggesting that GATA3 expression may be related to breast cancer subtype.

The protein EGFR is highly expressed in a variety of tumours and plays an important role in tumour occurrence and progression. It promotes tumour cell proliferation, adhesion, and metastasis, and it induces angiogenesis, inhibits apoptosis, and promotes tumour progression.[30] Nicholson *et al*.[31] found that when compared to tumour patients with low EGFR expression, those with high EGFR expression were more prone to tumour metastasis and had a shorter tumour recurrence time, a higher tumour recurrence rate, and a shorter survival period. In breast cancer patients, high EGFR expression is often accompanied by poor prognosis and rapid disease development,[32] as evidenced by our findings, which showed EGFR expression to be significantly correlated with histopathological grade and Ki-67 expression; however, only a few studies have reported EGFR expression to be related to breast cancer subtype. Our study demonstrated a significant difference in EGFR expression between the TN, Luminal A, Luminal B, and HER-2.P breast cancer subtypes. Through the correlation analysis conducted on the three biomarkers, we found a significant correlation between GATA3 and EGFR expression in breast cancer patients. This result was confirmed using the "OLS" function in the "stats models" package in Python, which showed that EGFR and GATA3 expression levels in breast cancer tissues were significantly negatively correlated. Through public RNA-seq database analysis, we found that the expression levels were significantly negatively correlated in HER-2.P breast cancer tissues. Therefore, these two biomarkers are expected to show high clinical value as reference indicators for determining breast cancer molecular subtype and predicting disease prognosis.

The findings of this study showed that GATA3 and EGFR expression levels were significantly different in different breast cancer subtypes and were negatively correlated in the HER-2.P subtype. In subsequent studies, we will further explore the specific signalling pathways involved in GATA3 and EGFR expression in breast cancer tissues.

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