Prognostic Significance of TRPS1, GATA3, and CYP4Z1 Expression in Triple-Negative Breast Cancer Patients: An Immunohistochemical Study

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Abstract
Background: Triple-negative breast cancer (TNBC) is characterized by a poor prognosis. Consequently, the current research aims to identify new therapeutic targets for TNBC patients. Trichorhinophalangeal syndrome type 1 (TRPS1), a novel GATA transcription factor, and CYP4Z1, a fatty acid hydroxylase, present potential targets for novel therapies.
Method: This retrospective study included 70 TNBC patients. The immunohistochemical expression of TRPS1, GATA3, and CYP4Z1 was evaluated. Data regarding the patient's clinical and pathological responses to chemotherapy were collected and analyzed for response assessment. SPSS version 20 software facilitated the data analysis. Quantitative variables were described using means and standard deviations, whereas categorical variables were examined using the Chi-square test or Fisher's exact test as appropriate. Survival analysis was performed using Kaplan-Meier plots, including disease-free and overall survival. A *P*-value of less than 0.05 was considered statistically significant.
Results: The analysis showed that 37 cases (53%) were positive for TRPS1 expression, 45 cases (65%) for GATA3, and 40 cases (57%) for CYP4Z1. There was a significant association between the expression of GATA3, TRPS1, and CYP4Z1 and various prognostic factors such as tumor size, grade, stage, lymphovascular invasion, recurrence, and mortality. Notably, the overall recurrence and progression rates were significantly correlated with the overexpression of GATA3, TRPS1, and CYP4Z1.
Conclusion: The overexpression of TRPS1, GATA3, and CYP4Z1 in TNBC patients may be novel prognostic factors for predicting tumor prognosis. Furthermore, these markers

could assist in selecting patients for future therapies in both localized and metastatic breast carcinoma settings.

Introduction

Breast cancer (BC) can be divided into 4 main clinical subtypes depending on the absence or presence of specific molecular markers: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).[1]

These subtypes include (ER/PR positive occurring nearly in 70% of patients), HER2-positive type occurring nearly in 12% of patients), and triple-negative breast cancer (TNBC), occurring nearly in 15% of patients.[2] TNBC is the most aggressive type in breast carcinoma with a high relapse rate and early metastasis. It is the most undifferentiated phenotype.[3]

GATA3 (GATA-binding protein 3) is a zinc finger transcription factor. Its expression is essential for luminal breast epithelium development. It also has been detected in many tumor types, such as BC and urothelial carcinomas.[4] GATA3 mutation has been implicated in breast carcinoma, representing a tumor suppressor role.[5]

The GATA3 gene is placed on chromosome 10p15, consisting of six exons. Though it was described as a transcription factor implicated in the maturation of T cells and the production of helper T cell cytokines, it has recently been proved to have a part in iNK T (invariant natural killer T cells) development.[6]

Though GATA3 has been associated with ER expression in luminal breast carcinomas, many studies document its expression in TNBC.[7]

Trichorhinophalangeal syndrome type 1 (TRPS1) is a new GATA transcriptional factor. It is also a significant modulator in the mesenchymal-to-epithelial transition (MET), so many types of tissue, including normal mammary epithelial cells, kidneys, cartilage, bone, and hair follicles, can develop and differentiate.[8]

It was found to be implicated in the development of BC, including basal-type/TNBC; however, its positivity was so scarce in other tumor types such as salivary duct carcinoma, ovarian cancer, UC, pancreatic adenocarcinoma, melanoma, gastric adenocarcinoma, and renal cell carcinoma.[9] CYP4Z1, "a fatty acid hydroxylase," has a role in steroid metabolism. It is also a subtype of CYP4 that is expressed in mammary epithelial tissue. Also, it has a role in BC development by forming the signaling molecule 20-Hydroxyeicostatetraenoic acid (20-HETE); therefore, it affects hormonal control, so it could suppress or promote tumors that are sensitive to estrogen hormones.[10]

CYP4Z1 can help cancer development by activating food compounds and converting procarcinogens to carcinogens, so it has a diverse potential and significant role in tumor biology.[11]

lately, a diversity of CYPs has been expressed in a rising number of tumor tissue and normal tissues.[12]

The study aims to evaluate the expression of TRISP1, GATA3, and CYP4Z1 in TNBC to select patients for future therapies.

Materials and Methods

This retrospective study analyzed 70 cases of TNBC, selected from the archives of the Clinical Oncology and Pathology Departments at the Faculty of

Medicine, Zagazig University, covering the period from 2017 to 2021. Comprehensive clinicopathological data and follow-up, recurrence, and survival information were meticulously compiled from patient files housed within the same institution's Clinical Oncology and Nuclear Medicine Department. Paraffin-embedded tissue blocks from these cases were prepared at the Pathology Department for histopathological examination.

The Institutional Review Board (IRB) of Zagazig University (ZU-IRB#: 996651–11-10-2022) granted this study's ethical clearance. Before participation, informed consent was obtained from all study participants.

Patient cases were categorized as TNBC, excluding other histopathological types. Clinicopathological parameters—including age, tumor grade and size, lymph node (LN) status, and lymphovascular invasion—were extracted from medical records and oncological assessments. Tissue sections, cut at 4 to 5 microns and stained with hematoxylin and eosin (H&E), facilitated the confirmation of diagnoses and the determination of tumor grades. All slide evaluations adhered to the WHO classifications for breast and female genital system tumors.[13] Staging was conducted in alignment with the International Union Against Cancer (UICC) TNM Classification.[14]

The follow-up regimen, extending over five years, was conducted through the Clinical Oncology Department at the Faculty of Medicine, Zagazig University.

Immunohistochemical staining

The Autostainer Link 48 (Agilent) was utilized.[15] Paraffin-embedded blocks cut into 4-micron thick sections. Deparaffinization was done using a series of xylene, followed by rehydration with descending grades of alcohol to block endogenous peroxidase activity. Subsequently, the sections were immersed in 0.5% hydrogen peroxide in methanol for 10 minutes to facilitate antigen retrieval. Primary antibodies targeting TRSPI (Anti-TRPS1 antibody produced in rabbit, Product Number: SAB4300902, Brand: Sigma), GATA (Monoclonal Anti-GATA3 antibody produced in mouse, Product Number: SAB5300458, Brand: Sigma), and CYP4Z1 antibodies (CYP4Z1-Specific Polyclonal Antibody for WB, ELISA, Host/Isotype: Rabbit/IgG) were employed.

Scoring of marker immunostaining

For TRPS1 and GATA, only nuclear staining was considered positive. The calculation of immunoreactivity scores was performed by multiplying a number representing the percentage of immunoreactive cells (zero, less than 1%; 1, 1 to 10%; 2, 11 to 50%; 3, 51 to 100%) by another representing the intensity of staining (zero, negative; one, weak; two, moderate; three, strong). The immunoreactivity scores were classified as unfavorable (zero to one), low positive (two), intermediate positive (three to four), or high positive (six and nine).[9]

CYP4Z1 scoring

The percentage of tumor cells exhibiting cytoplasmic or membranous staining for CYP4Z1 was determined, and the degree of positivity was scored from 0 to 5 as follows: zero, none; 1, less than 1%; 2, 1% to 10%; 3, 11% to 33%; 4, 34% to 66%; and 5, more than 67%. The staining intensity was graded from 0 to 3: 0, negative; 1, weak; 2, moderate; and 3, strong. The final score was calculated by summing the percentage and intensity scores. Negative CYP4Z1 expression was defined; as a result, a score less than two,

while result scores between three and eight characterized positive CYP4Z1 expression.[16]

Statistical analysis

Data analysis was performed using SPSS version 20 software. Quantitative variables were described using means and standard deviations (SDs). The description of categorical variables utilized the Chi-square test and Fisher's exact test when appropriate. A chi-square test was employed to compare two groups concerning ordinal categorical data. The strength of association between two categorical variables was assessed using the Phi correlation coefficient. Disease-free survival (DFS) and overall survival were measured using survival analysis and Kaplan-Meier plots. Statistical significance was set at 5% ($P < 0.05$).

Results

Clinical and pathological characters of studied TNBC cases

The clinicopathological characteristics of seventy TNBC patients are summarized in table I. 15 (21.4%) cases were under 60, while 55 (78.6%) were over 60. Large-sized tumors predominated (68.6%). Low-grade tumors were identified in 10 cases, whereas high-grade tumors were observed in 60 of the studied cases.

Relation between TRISPI, GATA3, CYP4Z1 expression in the examined TNBC cases (Table 1)

TRISPI immune expression was positive in 52.8% of TNBC cases, significantly correlating with tumor size, stage, grade, lymphovascular invasion, recurrence, and mortality. GATA3 expression was positive in 64% of the examined TNBC cases and also significantly correlated with tumor size, stage, grade, lymphovascular invasion, recurrence, and mortality. Positive CYP4Z1 expression

was noted in 57% of the studied cases, significantly correlating with tumor size, stage, grade, lymphovascular invasion, recurrence, and mortality. Furthermore, there was a positive correlation between TRISPI, GATA3, and CYP4Z1 expression in the examined TNBC cases. Follow-up was conducted at the Clinical Oncology Department, Zagazig University Hospitals, for all cases included in the study. This follow-up involved radiological and biochemical assessments, including full laboratory and tumor marker evaluations every 3 months during the first 2 years, followed by evaluations every 6 months over the next 3 years and annually after that to detect any radiological or biochemical progression and assess overall recurrence, progression rates, and DFS.

Correlation of TRISPI, GATA3, and CYP4Z1 expression in the examined TNBC cases (Table 2)

A significant positive correlation was observed between GATA, TRSPI, and CYP4Z1 among the studied patients. Additionally, overall recurrence and progression rates were significantly correlated with overexpression of TRSPI, GATA, and CYP4Z1. A strong relationship between TRSPI, GATA, and CYP4Z1 expression was detected.

Prognostic value of TRISPI, GATA3, and CYP4Z1 expression in the examined TNBC cases (Table 3)

DFS was stratified according to TRISPI, GATA3, and CYP4Z1 expression in the examined TNBC cases and demonstrated in Kaplan-Meier plot curves. The Kaplan-Meier curve revealed that high TRISPI, GATA3, and CYP4Z1 expression were associated with shorter DFS. There was a statistically significant association between DFS and the expression of all TRSPI, GATA, or CYP4Z1 markers.

Patients with positive expression of any marker had significantly lower DFS.

Discussion

In the study, a high expression of GATA (GATA-binding protein 3) was found in 64% of the TNBC cases studied, TRSPI (Trichorhinophalangeal syndrome type 1) was expressed in 52.8% of the cases, while CYP4Z1, a fatty acid hydroxylase, was observed in 57% of the cases. Their expression was significantly associated with increased tumor size, high stage, and grade, the occurrence of LN metastasis, lymphovascular invasion, increased tumor recurrence rate, and poor overall patient survival.

These results follow other results that reported positivity for the GATA-3 receptor in 48% of TNBC patients.[17] Ashley et al. also found GATA3 expression in 74.6% of triple-negative carcinomas.[18] while some older studies have mainly evaluated GATA3 expression in triple-negative breast carcinomas and found its expression in 5% of cases as reported by Yang and Nonaka,[19] and expression was found in 16% of cases as reported by Albergaria et al.[20] These different degrees of expression may be owing to diverse methodology used such as number of cores per tumor, core size and, as well as the various cutoffs that were used to define GATA3 positivity in previous studies.

The results obtained in this study were consistent with other findings, indicating that TRPS1 had a high sensitivity in both metaplastic and non-metaplastic TNBC, which was significantly higher than that of GATA.[9,21] TRPS1 showed little to no expression in urothelial carcinomas or other tumor types.[9] So TRPS1 is an excellent marker for TNBC and can prove the breast origin of Metastases.

Several studies indicated the expression of CYP4Z1 enzyme in certain types of tumors, such as cervical carcinoma, where its expression was significantly found in patients with the late stage of the disease, LN metastasis, and high tumour invasion.[22]

Despite the growing evidence for the involvement of CYP4Z1 in tumor malignancy, there have been restricted studies investigating its expression in breast carcinoma. Therefore, its expression in TNBC was studied. In comparison to our results, Al-Sawi et al. reported that CYP4Z1 overexpression was detected in 70.8% of Iraqi female patients with breast carcinoma, with a higher prevalence in T3 tumors (80%).[23,11] This observation underscores the association between CYP4Z1 expression and tumor development, contrasting with benign tumors exhibiting no CYP4Z1 expression. Moreover, its overexpression correlates with the advanced stage, indicating a poor prognosis. These findings are consistent with our study results.

A recent study by Al-Saraireh et al.[24] reported near results as overexpression of CYP4Z1 was in 83.3% of TNBC compared to its negative expression in normal breast tissues. Its expression was in patients with high grades and advanced stages. Also, its expression was strongly interrelated with the survival of TNBC patients.[25]

A recent study aided the development of the first selective and potent mechanism-based inhibitor of cytochrome p450 4z1. This inhibitor competently blocks CYP4Z1 production in positive BC cells, in contrast to little inhibitory profiles against other CYPs. These recent advances are essential for the molecular targeted therapy of CYP4Z1 functional

activities and its incorporation in TNBC.[26]

Evaluation of the expression of three novel biomarkers for the first time in TNBC, assessment of their expression regarding clinicopathological features, correlation between their expression and prognostic parameters, and the long follow-up time of about 5 years were considered points of strength in the study. Additionally, a correlation between their expression and the presence of hormonal status, which is recently implicated in the development of BC, will aid prospective studies in discovering novel targeted therapies. However, there were limitations in the study, as it was retrospective, had a relatively small sample size, and immunohistochemical methods solely evaluated marker expression without genetic assessment. Prospective cohort studies on large sample sizes utilizing genetic and immunohistochemistry assays are recommended to substantiate further the prognostic roles of TRISP1, GATA3, and CYP4Z1 markers.

Conclusion
The overexpression of TRISP1, GATA3, and CYP4Z1 in TNBC represents novel prognostic factors that aid in tumor prognosis prediction. This information can be invaluable in selecting appropriate therapies for patients with both localized and metastatic BC. TNBC patients expressing these markers should be treated aggressively with full-course chemotherapy regimens, incorporating various treatment modalities specific to TNBC, such as immunotherapy (e.g., pembrolizumab or atezolizumab), platinum-based chemotherapy, or PARP inhibitors like Olaparib, tailored according to disease staging.

Conflict of Interest
None declared.

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Table 1. Relation between TRSPI, GATA, and CYP4Z1 expression and clinic-pathological features of the studied patients

Clinical features	Total N=70 (%)	TRSPI		*P* value	GATA		*P* value	CYP4Z1		*P* value
		TRSPI (-) ve 33 (%)	TRSPI (+) ve 37 (%)		GATA (-)ve 25 (25%)	GATA (+)ve 45 (%)		CYP4Z1 (-)ve 30 (%)	CYP4Z1 (+)ve 40 (%)	
Age group <60 years ≥60 years	15(21.4%) 55(78.6%)	8 (53.3%) 25(45.5%)	7 (46.7%) 30(54.5%)	0.588	6 (40%) 19 (34.6%)	9 (60%) 36(65.4%)	0.696	8(53.3%) 22 (40%)	7(46.7%) 33 (60%)	0.355
Size Small Large	22(31.4%) 48(68.6%)	15(68.2%) 18(37.5%)	7 (31.8%) 30(62.5%)	0.017*	13 (59.1%) 12(25%)	9 (40.9%) 36(75%)	0.006*	14(63.6%) 16(33.3%)	8 (37.4%) 32(66.7%)	0.018*
Stage S 1 S 2 S 3 S 4	12(17.1%) 25(35.7%) 30(42.9%) 3(4.3%)	10(83.3%) 14(56%) 9(30%) 0 (0%)	2(16.7%) 11(44%) 21(70%) 3(100%)	<0.001*	5(41.7%) 14(56%) 6(20%) 0(%)	7(58.3%) 11(44%) 24(80%) 3(100%)	0.024*	6(50%) 15(60%) 9(30%) 0(%)	6(50%) 10(40%) 21(70%) 3(100%)	0.031*
Grade Low grade High grade	10(14.3%) 60(85.7%)	8 (80%) 25(41.7%)	2 (20%) 35(58.3%)	0.025*	7 (70%) 18(30%)	3(30%) 42(70%)	0.015*	8(80%) 22(36.7%)	2(20%) 38(63.3%)	0.012*
LVI Present Absent	40(57.1%) 30(42.9%)	23(57.5%) 10(33.3%)	17(43.5%) 20(66.7%)	0.045*	20(50%) 5(16.7%)	20(501%) 25(83.3%)	0.004*	23(57.5%) 7(23.3%)	17(42.52%) 23(76.7%)	0.004*
LN Negative Positive	15(21.4%) 55(78.6%)	11(73.3%) 22(40%)	4(26.7%) 33(60%)	0.022*	9(60%) 16(29.1%)	6(40%) 39(70.9%)	0.027*	10(66.7%) 20(36.4%)	5(33.3%) 35(63.6%)	0.036*
T T1 T2 T3 T4	12(17.1%) 28(40%) 20(28.6%) 10(14.3%)	10(83.3%) 14(50%) 8(40%) 1(10%)	2(16.7%) 14(50%) 12(60%) 9(90%)	0.001*	7(58.3%) 11(39.2%) 7(35%) 0(0%)	5 (71.4%) 17(60.8%) 13(65%) 10(100%)	0.008*	8(66.7%) 12(42.9%) 9(45%) 1(10%)	4(33.3%) 16(57.1%) 11(55%) 9(90%)	0.016*
Recurrence Negative Positive	39(55.7%) 28(44.3%)	29(74.4%) 4 (14.3%)	10(25.6%) 24(85.7%)	<0.001*	23(59%) 2(7.1%)	16(41%) 26(92.9%)	<0.001*	26(66.7%) 3(10.7%)	13(33.3%) 25 (89.3%)	<0.001*
Mortality Negative Positive	50(71.4%) 20(28.6%)	32(64%) 1(5%)	18(36%) 19(95%)	<0.001*	24(48%) 1(5%)	26(52%) 19(95%)	<0.001*	29(58%) 1(5%)	21(42%) 19(95%)	<0.001*

TRPS1: Trichorhinophalangeal syndrome type 1; CYP4Z1: Cytochrome P450, family 4, subfamily Z, polypeptide 1; LVI: Lympovascular invasion; LN: L:ymph node; * *P* < 0.05 is statistically significant

Table 2. Correlation between TRSPI, GATA, and CYP4Z1 among the studied patients

	TRSPI		GATA		CYP4Z1	
	Phi	*P*	Phi	*P*	Phi	*P*
TRSPI			0.789	<0.001[*]	0.397	0.001[*]
GATA	0.789	<0.001[*]			0.439	0.002[*]
CYP4Z1	0.397	0.001[*]	0.439	0.001[*]		

TRPS1: Trichorhinophalangeal syndrome type 1; CYP4Z1: Cytochrome P450, family 4, subfamily Z, polypeptide 1; [*]*P* < 0.05 is statistically significant

Table 3. Kaplan– Meier survival curves illustrating disease-free survival time differences in patients as regards marker expressions

		Total N	N of events	Censored		Survival time, Months		*P*
						Mean		
				N	%	Estimate ±SD	95% CI	
TRSPI	Negative	33	4	29	87.9%	35.27 ± 0.43	34.44 – 36.11	<0.001[*]
	Positive	33	23	10	30.3%	25.48 ±1.45	22.65 – 28.32	
GATA	Negative	25	2	23	92.0%	35.6 ± 0.32	34.97-36.23	0.001[*]
	Positive	41	25	16	39.0%	27.21±1.31	24.63-29.78	
CYP4ZI	Negative	29	3	26	89.7%	35.72 ± 0.16	35.41 – 36.04	<0.001[*]
	Positive	37	24	13	35.1%	26.17 ± 1.37	23.49 – 28.85	
Overall		66	27	39	59.1%	30.42 ± 0.97	28.53– 32.31	

TRPS1: Trichorhinophalangeal syndrome type 1; CYP4Z1: Cytochrome P450, family 4, subfamily Z, polypeptide 1; N: Number; [*]*P* < 0.05 is statistically significant, CI: Confidence interval