Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 3 Vol 8 No 1 2023

Article

FSCN1 Gene Expression Influence on Breast Cancer in Iraqi Patients

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Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.03.85

Abstract
Our investigation aim was to ascertain how the Fascin actin-bundling protein 1 (FSCN1) gene affected breast cancer patients and how it affected the molecular type of the disease (Luminal A, Luminal B, Triple negative, Enrich Her2).By collecting blood samples from sixty patients with Breast cancer from many hospitals in Baghdad/Iraq between the periods (October 2021 to August 2022) and collecting forty volunteers heaths as Control. The volunteer's blood samples were collected and tested with Cancer antigen 15-3 (CA 15-3) to confirm absent breast cancer. The blood and control samples from these patients were collected, the RNA was extracted, and molecular methods using PCR and primers targeting the FSCN1 gene were used. The results showed that genes found in all patient samples have overexpression compared to healthy volunteers, and we also discovered differences in expression between the molecular types of breast cancer. We discovered that Triple-negative breast cancer is more affected than Luminal A, B, and Enrich Her2.

Keywords: *Breast Cancer*, Molecular classification breast cancer, Fascin Actin Bundling protein 1 genes

Introduction

The most common cancer in women and one of the main reasons for death in this group is breast cancer (BC) 1. Cancer originates in breast cells. It usually begins in the epithelium of the breast ducts or lobules that supply milk to the ducts [1]. The disease primarily affects women, but men might be affected as well.

Fascin is a member of the fascin family of globular filamentous actin-binding proteins.[2,3] The FSCN1 facilitates the creation of various cellular structures by sustaining actin bundles—additionally, actin-based protrusions like microspikes, filopodia, lamellipodia, and others below the plasma membrane [4]. Cellular motility, matrix adhesion, and intercellular communication depend on these structures. In healthy adult tissues, mesenchymal neuronal, endothelial, and dendritic cells express FSCN1, but normal epithelial cells either express FSCN1 at low levels or not. [4] Numerous malignant cancers, as well as altered epithelium cells, have it overexpressed. This suggests that it could contribute to the development of cancer. In many human cancers, elevated FSCN1 levels have been associated with poor clinical outcomes, poor prognosis, and low survival rates. It is now understood that FSCN1 overexpression in cancer cells may serve

as a biomarker for a number of cancer types as well as a therapeutic target: migration, invasion, and metastasis. [3].
Four molecular kinds of breast cancer (Luminal A, Luminal B, TNBC, and HER2 overexpression). The Luminal A type of tumors, which are low grade, have a good prognosis and are ER/PR positive, exhibit the following characteristics: ER/PR positivity, HER2 negativity, high ER-related gene content, and low proliferation-related gene content. HER2 overexpression was not observed in this group of ER and PR tumors, unlike luminal B tumors, which were either HER2 positive or HER2 negative with a Ki67 score of >14%, Triple-negative breast cancer (TNBC), which is characterized by the lack of ER and PR expression, is thought to be caused by aggressive clinical practices, subpar patient survival rates, and a lack of targeted therapy choices. It is more challenging to treat this subtype of breast cancer than others [5–7]

Materials and Methods

Study design & subjects

This study included sixty Iraqi women with breast cancer who attended Al-Andalus Hospital and the oncology teaching hospital/medicine city between December 2021 and March 2022, and whose ages ranged from 20 to >60 years. The relevant information regarding the patients, as well as the tumors' histological features, were gathered from the patient's files. All of the patients had been identified. These patients are in various stages of the disease. Molecular analysis was performed on all of the cases. TRIzol was used to preserve the materials. There were forty healthy volunteers ranging in age from 20 to >60 years old, all unrelated and had no history of breast cancer in their families.

Blood sample collection

Venous blood was taken from the patient and the healthy group. Tow milliliters (mL) All patients diagnosed with breast cancer were placed in an EDTA tube.

Molecular detection:

RNA extraction and quantification

RNA was isolated from the sample by using the TRIzolTM Reagent(Thermo Fisher Scientific USA) according to the manufacturer's protocol, which included Sample lysis by mixing Each tube for 400 µl of blood and 600 µl of TRIzolTM Reagent, Purification After being incubated for two to three minutes at room temperature, The mixture was centrifuged for 10 minutes at a speed of 12,000 rpm to separate it into three phases: a lower organic phase, interphase, and an upper, colorless aqueous phase. Each lysate tube was filled with 0.2 mL of chloroform before being carefully screwed on the tube top.
After being moved to a separate tube, the aqueous phase containing the RNA was combined with 0.5 mL of isopropanol, pause (ten min.) before being centrifuged (12,000 rpm /ten min). A pellet of a white, gel-like material resembling RNA precipitated as total RNA at the tube's bottom. The washing process follows. Each tube received 0.5mL of 70% ethanol to clear out any debris, which was then vortexed briefly before being centrifuged at 10,000 rpm for 5 minutes.
Afterward, the pellet was aspirated and dried with air, then put in an Incubator at (55–60 degrees/ten min.) in a water bath. After that, I rehydrated the pellet in 20µl of Nuclease Free H_2O.
The final concentration of extracted RNA was measured using a Quantus Fluorometer to gauge the sample quality for further use. 199µl of diluted QuantyiFluor Dye were combined with 1µl of RNA. RNA concentration data

were calculated during a 5-minute incubation period at room temperature and in the dark.

Quantitative Real-Time PCR (RT-q PCR):
Primer and probe design and preparation
The GenBank National Center for Biotechnology Information database provided the cDNA sequences for the (FSCN1) gene. The Primer Premier 3 program was used to create RT-qPCR primers with melting temperatures ranging from 58 to 62 degrees Celsius., primer lengths ranging from 18 to 23 nucleotides, and PCR amplicon lengths ranging from 90 to 115 base pairs, with the primer and probe sequences shown in the table (1) (2) as examples

Primer name	Sequence	Annealing Temp. (ºC)
FSCN1-F	Forward TCAAAGACTCCACAGGCAAATA (Sense)	58
FSCN1-P	Probe AAGAAGAAGTCCACAGGAGTGTCGC (Anti-Sense)	
FSCN1-R	Reverse CCACCTTGTTATAGTCGCAGAA (AntiSense)	

Table 1. Parameter Set: RT-qPCR (Primers with Probe)

Sequence Name: FSCN1

Amplicon Length: 111

Primer name	Sequence	Annealing Temp. (ºC)
TEGT-F	Forward 5`-TGCTGGATTTGCATTCCTTACA-3`	60
TEGT-R	Reverse 5`-ACGGCGCCTGGCATAGA-3	

Table 2. Parameter Set: RT-qPCR (Housekeeping Primers) Sequence Name: TEGT

The Macrogen Company provided the primers in lyophilized form, which are known as lyophilized primers. They disintegrated in water devoid of nucleases to create a stock solution with a final concentration of (100 pmol/ µl). In order to create the working primer solution, 10 µl of the primer stock solution (which had been kept at a freezer temperature of -20 C) was added to 90µl of nuclease-free water

One step Quantitative Real-time PCR Assay (qRT-PCR) protocol FSCN1 genes

The precision, sensitivity, and speed of reverse transcription quantitative PCR (RT-qPCR) set it apart from other gene expression analysis techniques. The use of this technique in gene expression analysis has become the norm. It is crucial to understand that in a relative quantification investigation, the studies typically compare the amount of expression of a particular gene among several sample

targets [8], so the use of GoTaq® 1-Step RT-qPCR System kit from Promega (Table 3) company with Taqman prob a fluorophore, which identifies specific stranded cDNA, is used in real-time PCR quantification in the current investigation.

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
qPCR Master Mix	2	X	1	X	12.5
RT mix	225	µl			0.8
MgCl2					0.1
Forward primer	10	µM	1	µM	1.0
Reverse primer	10	µM	1	µM	1.0
Nuclease Free Water					7.7
RNA		ng/µl		ng/µl	2.0
Total volume					25
Aliquot per single rxn	23µl Master mix /tube + 2µl of Template				

Table 3. Reaction Configuration and Thermal Cycling Protocol

Steps	°C	m: s	cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	50
Annealing	58	00:20	
Extension	72	00:20	

Table 4. Real-Time PCR program.

Statistical analysis

The data analysis was finished using IBM SPSS for Windows, version 26 (SPSS Inc. Chicago, Illinois, United States).

The prerequisites for data normalcy were examined using the following techniques: Skewness, Kurtosis, and Q–Q plot. Normality tests like the Kolmogorov-Smirnov test and the Shapiro-Wilk test were employed. For analysis, the investigated gene was first turned to log10 and then returned to conventional units for display. In categorical variables, frequencies and proportions were used as expressions. The mean and standard deviation expressed continuous variables (SD).

The differences between the groups were examined using Levene's test variances are equal to those of the Student's t-test(STT). The (ANOVA) is utilized to set if there are statistically significant differences among treatment groups. After analyzing the results of the ANOVA, a post hoc test (Tukey HSD) for multiple comparisons was conducted. A 0.05 or lower p-value was regarded as statistically significant [9]

Results

When data from breast cancer patients were analyzed (60 cases) and Control (40 Volunteers), we found that FSCN1gene was overexpressed in breast cancer patients, and the result was significant (P=0.001). The patients were 4.56 times more overexpressed than Control (Figure 1).

Our results are consistent with previous research provided by Li, Z. published in 2022[11], which papeared the FSCN1 related to being available in abundant types of human tumors. It has a challenging clinical trajectory and a bad prognosis. By altering the indication of FSCN1 in tumor cell lines in vitro, it has been demonstrated that FSCN1 encourages development, invasion, and cancerous cells' ability to migrate and spread.

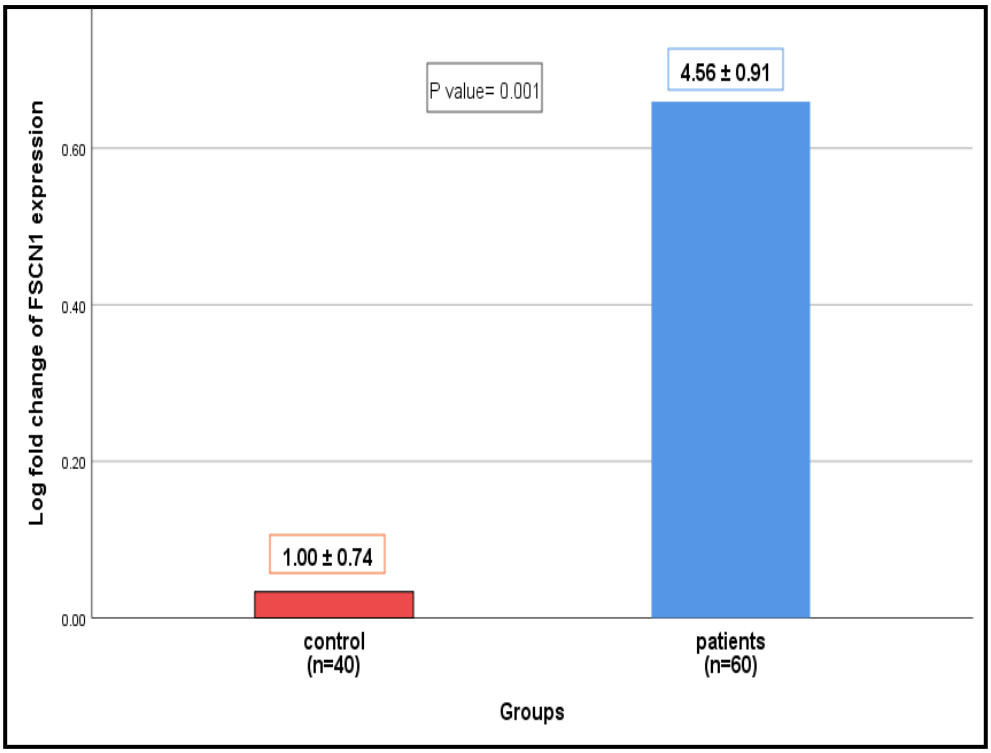

Figure 1. Log fold change ($2^{-\Delta\Delta ct}$) of FSCN1 expression of controls and patients with breast cancer.

The luminal A, B, HER2-enriched, and TNBC subtypes had favorable fascin-1 expression rates of 4.87, 3.01, 3.21, and 16.59, respectively (Figure 2). Fascin-1 appeared at much higher rates of positive and strong positive presentation in TNBC patients than other molecular types.

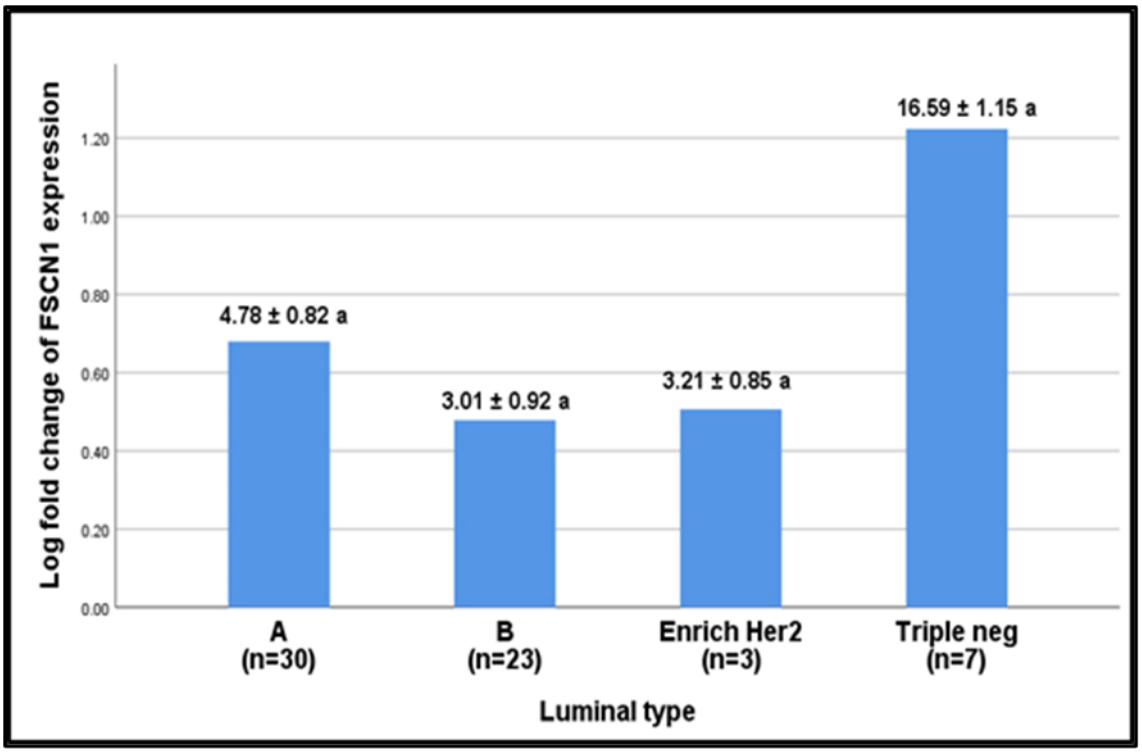

Figure 2. Log fold change ($2^{-\Delta\Delta ct}$) of FSCN1 expression of patients sub-groups depending on Luminal type.

As we have demonstrated, triple-negative breast cancer (TNBC) patients had more aggressive fascin expression than those with other subtypes of the disease because fascin plays a crucial role in the connection of cell-motility structures, which are crucial for cancer invasion and metastasis[12]. Therefore, through greater cell motility and higher metastatic potential, fascin expression in cancer cells may contribute to a more aggressive course of disease; because TNBC cannot respond to any hormone therapy and Anti-HER2 therapy results in invasion and metastasis, this study was in agreement with Wang's research in that it found that FSCN1 was ten times more overexpressed than other subtypes. As a result, FSCN1 can be used as a specific analytical indicator for TNBC.

Discussion

These findings corroborated those of Al-Alwan, M. [10], who investigated whether fascin can control molecules linked to breast cancer metastasis using many tests, including functional and immunological ones. In the study, They found that among breast cancer patients who expressed fascin, invasion, metastasis, and a worse disease-free survival rate were related. They demonstrate for the initial period that fascin reduces breast cancer expression and nuclear translocation metastasis inhibitor-1 (BRMS1), a crucial metastasis suppressor protein, while up-regulating the activity of NF-kappa B, which can be necessary for tumors. Their findings show that regulating a number of metastasis-related genes is how fascin regulates invasion. They also address a possible therapeutic strategy for treating metastatic breast cancer by targeting fascin by demonstrating that fascin expression in breast tumor cells results in a gene expression profile comparable to metastatic tumors.

Conclusion

The FSCN1 gene correlates with increased breast cancer, so FSCN1 gene expression increases in the patient compared to a control. This gene has more expression in triple-negative breast cancer than in another type because the TNBC cannot respond to therapy.

The FSCN1 gene has an increased probability of metastasis because it is responsible for the invasion and migration of breast cancer cells; this gene can act as a diagnostic test.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Rifaat , R.M.; Nader , M.I. FSCN1 Gene Expression Influence on Breast Cancer in Iraqi Patients. Revis Bionatura 2023;8 (3) 85. http://dx.doi.org/10.21931/RB/CSS/2023.08.03.85