RESEARCH ARTICLE

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Prognostic Significance of *miR-145*, NANOG, OCT4, and KLF4 in Triple-Negative Breast Cancer

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Abstract

Background: Among women, breast cancer is the leading cause of cancer-related deaths. OCT4, NANOG, and KLF4 are markers of cancer stem cells. *miR-145* is a tumor suppressor in many cancers. We aimed to estimate the expression of *miR-145* and cancer stem cell markers in triple-negative breast cancer (TNBC) and to investigate their correlations with clinicopathological characteristics and outcomes in these patients. **Methods:** The study included 90 female patients with TNBC. Grade III represented 64.4% of cases, while Grades I and II represented 11.1% and 24.4%, respectively. Seventy-two patients (80%) had lymph node involvement. Breast tissues from malignant and adjacent control tissues were used for RNA extraction and subsequent analysis of *miR-145* expression. Histopathological and immunohistochemical analyses were also performed. **Results:** It was found that lymph node (LN)-positive tumors exhibited higher OCT4 levels compared to LN-negative tumors (P = 0.039). Additionally, tumors with extensive intraductal invasion and relapse showed lower KLF4 levels (P = 0.02 and <0.001, respectively). Downregulated *miR-145* expression was associated with higher stages, relapse, and mortality (P < 0.001 for each), LN involvement (P = 0.002), and capsular invasion (P = 0.02). There were significant strong positive correlations between *miR-145* and DFS (r = 0.920, P < 0.001) and OS (r = 0.813, P < 0.001). However, significant weak negative correlations were found with KLF4 (r = -0.242, P = 0.022), NANOG (r = -0.305, P = 0.009), OCT4 (r = -0.255, P = 0.014), tumor size (r = -0.247, P = 0.019), and the number of positive LNs (r = -0.481, P < 0.001). OCT4 levels showed significant positive correlations with NANOG (r = 0.328, P = 0.002) and KLF4 expression (r = 0.344, P = 0.001). Moreover, a significant positive correlation was detected between KLF4 levels and DFS (r = 0.255, P = 0.015). The log-rank test showed a significant association of KLF4 with DFS (P = 0.005). **Conclusion:** *miR-145* and KLF4 are possible prognostic markers in TNBC. This was reflected by the positive correlations between *miR-145* and both DFS and OS, and between KLF4 levels and DFS.

Keywords: miR-145- NANOG- OCT4- KLF4- triple-negative breast cancer

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Introduction

Among women, breast cancer is the most common invasive cancer [1]. Triple-negative breast cancer (TNBC) is the most aggressive type of breast cancer. It has a high relapse and early metastasis. Metastatic poorly differentiated TNBC has few poor tumor markers [2, 3]. Thus, the role of microRNAs (miRNAs) in TNBC has been investigated. They act as either oncogenes or tumor suppressor genes [4].

miRNAs are non-coding RNAs with 20–22 nucleotides. They regulate post-transcriptional gene expression by inhibiting mRNA translation or inducing its degradation. Dysregulated miRNA expression is involved in carcinogenesis by affecting cellular growth and cell cycle [5]. Downregulated *miR-145* was detected in colorectal [6], lung [7], and laryngeal cancers [8].

The pathogenesis of breast cancer is still unclear. However, its occurrence can be explained by genetic factors or cancer stem cells (CSCs) [9]. Self-renewal in CSCs leads to making more copies of daughter cells with undifferentiated properties. Additionally, pluripotency of CSCs enables them to make several types of cells creating all diverse cell populations in cancer [10].

Octamer-binding transcription factor 4 (OCT4) is connected to the pluripotency of embryonic and cancer stem cells [11]. *NANOG* homeobox (NANOG) is another transcription factor in stem cells. It is linked to immune

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function and apoptosis. It is associated with JAK/STAT, Wnt/β-catenin, Notch, TGF-β, and Hedgehog signaling pathways [12]. NANOG expression can be detected in the nucleus and cytoplasm of many precancerous and cancerous lesions [13]. *NANOG* overexpression was detected in colorectal cancer [14]. *OCT4* and *NANOG* co-expression was detected in early stages of pancreatic cancer [15] and in lung cancer [16]. Krueppel-like factor 4 (KLF4) is a transcription factor involved in embryonic development and inflammation. Moreover, it acts as a tumor suppressor or oncogene [17].

Previous research found that *miR-145* can suppress NANOG, OCT4, and KLF4 expression. However, their combined relationship with prognosis prediction in TNBC is still under study. Thus, this study aimed to assess the correlation between *miR-145* gene expression and NANOG, OCT4, and KLF4 protein expression in TNBC and to investigate their association with survival in these patients.

Materials and Methods

This study was conducted in the Pathology, Medical Biochemistry and Molecular Biology, Clinical Pathology, Medical Microbiology and Immunology, and Medical Oncology Departments, Faculty of Medicine, Zagazig University, from 2021 to 2025. Ninety female patients from 24 to 72 years with TNBC were included in the study. The study got approval from the Institutional Review Board of the Faculty of Medicine, Zagazig University (ZU-IRB#758/26-Nov-2024). Cases with insufficient tissue for staining, incomplete data in medical records, luminal A, luminal B, and HER2 neu +ve types of breast cancer were excluded from the study.

Full history taking, clinical examination, and imaging of the chest, abdomen, and pelvis were performed for all patients. Baseline PET-CT scans or CT scans of the chest, abdomen, and pelvis, and bone scans were included whenever indicated. Clinical follow-up visits were due for our patients every 3 months for 2 years, then every 6 months for 3 years, then annually. Mammosongraphy follow-up was requested on a yearly basis.

Breast tissues from malignant tissues and adjacent control tissues were separated into two parts. The first one was immediately frozen at -80º for subsequent RNA extraction and *miR-145* expression analysis. The other part was fixed in formalin for subsequent histopathological and immunohistochemical analysis.

About 3 µm-thick sections from the paraffin blocks were cut for histopathological evaluation using hematoxylin and eosin (H&E) staining to confirm the diagnosis. Immunohistochemical staining was performed using the streptavidin-biotin immunoperoxidase technique.

Immunohistochemical procedure

Immunohistochemical staining was done with antibodies against NANOG (ab80892, 1:400 dilution, Abcam, Cambridge, UK) and OCT4 (sc-5279, 1:250 dilution, Santa Cruz Biotechnology, Dallas, Texas, USA). Anti-KLF4 (ab34814, Abcam, Cambridge, UK) was the IgG type rabbit polyclonal antibody. The immunohistochemical staining was performed on the Dako automated platform, and antibody detection was achieved using a biotin-free EnVision™ Detection System (Dako).

For immunohistochemical validation, positive controls included seminoma, embryonal carcinoma, and placental cytotrophoblasts for NANOG. Testicular germ cells and germ cell tumors were positive controls for OCT4. Colonic mucosa, skin epidermis, and vascular endothelium were positive controls for KLF4. Skeletal muscle, adipose tissue, and liver were used as negative controls. Also, additional negative controls were obtained by omitting the primary antibody.

Scoring

All immunohistochemical slides were independently evaluated in a blinded fashion. NANOG and OCT4 nuclear and/or cytoplasmic staining was evaluated according to the percentage of tissue with positive staining (0: negative, and 1: positive in 75% of tumor cells) and the staining intensity (0: negative, 1: weakly positive, 2: moderate, and 3: high staining). IHC scores were determined by multiplying the staining intensity by the percentage of positive staining. Finally, the expression of the studied markers was graded. Score 0 represented negative, score 1-4 represented low expression, score 5-8 represented moderate expression, and score 9-12 represented high expression [18].

KLF4 nuclear and /or cytoplasmic staining was evaluated by the percentage of positive tumor cells. It was determined semi-quantitatively by assessing the entire tumor section. Each sample was assigned to one of the following categories: 0 (0–4%), 1 (5–24%), 2 (25–49%), 3 (50–74%), or 4 (75–100%). The intensity of immunostaining was determined as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong). A final immunoreactive score, ranging from 0 to 12, was calculated by multiplying the percentage of positive cells by the staining intensity score. A score of 0 was considered negative. Scores ranging from 1 to 4 indicated low expression. Scores of 5 to 8 denoted moderate expression. Scores between 9 and 12 reflected high expression [19].

Gene expression analysis

Total RNA extraction was performed using TRIzol® (Thermo Fisher Scientific). miRCURY LNA RT kit was used to synthesize cDNA for *miR-145*. Then, the Rotor-Gene Q real-time PCR system (Qiagen) was used for real-time polymerase chain reaction (RT-PCR). TOPreal SYBR Green qPCR PreMIX (Enzynomics, Korea) was used. The primers used were synthesized by Sangon Biotech (Beijing, China). The $2^{-\Delta\Delta CT}$ method was used for calculating *miR-145*-fold expression. U6 was used as a housekeeping gene. The sequence of *miR-145* primers was:

F: AACACGCGTCCAGTTTTCCCA,

R: GTCGTATCCAGTGCAGGGTCC,

RT primer: GTCGTATCCAGTGCAGGG TCCGAGGTATTCGCACT GGATACGACAGGGATT.

The sequence of U6 primers was:
F:5'CTCGCTTCGGCAGCACA3'
R: 5'AACGCTTCACGAATTTGCGT3'

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS version 21.0). Qualitative data were represented as numbers and percentages. Quantitative continuous data were represented by mean ± standard deviation (SD) when normally distributed or median and range when not normally distributed. Testing the association and differences in qualitative data was performed by the chi-squared test (X^2). Overall survival (OS) and disease-free survival (DFS) were calculated. Associations with DFS and OS were analyzed by using Kaplan-Meier curves and compared by the Log-rank test. A P value of < 0.05 was considered statistically significant.

Results

The mean age of the participants was 41.04±12.29 years. The age ranged from 24 to 72 years. About 57% of patients were >35 years. Fifty-seven patients had breast conservative surgery (BCS) while thirty-three patients had modified radical mastectomy (MRM). The mean tumor size was 46.37±14.98 mm. The size ranged from 15 to 70 mm. The majority of cases were grade III (64.4%), while grades I and II represented 11.1% and 24.4%, respectively. The mean number of dissected lymph nodes was 17.27±0.34. Their number ranged from 10 to 23. The mean number of positive lymph nodes was 7.14±5.48. Their number ranged from 0 to 20. Lymphovascular invasion (LVI) was detected in 84.4% of cases. An extensive intraductal component was detected in 24.4% of cases. Skin invasion was present in 6.7% of patients. Capsular invasion was detected in 48.9% of patients (Table 1).

Concerning immunohistochemical expression, 64.4% of patients were negative for NANOG staining. Positive NANOG expression was classified into low, moderate, and high (12.2%, 14.4%, and 8.9%, respectively). Negative OCT4 expression was detected in 54.4% of patients. Positive OCT4 expression was classified into low, moderate, and high (15.6%, 12.2%, and 17.8%, respectively). Negative KLF4 expression was detected in 46.7% of patients. Positive KLF4 expression was classified into low, moderate, and high (18.9%, 15.6%, and 18.9%, respectively) (Table 1, Figures 1 and 2).

A total of eighty-one patients received adjuvant chemotherapy. Neoadjuvant chemotherapy was administered in twenty patients. The most often used chemotherapy regimen was an anthracycline/Taxane-based regimen. Adjuvant capecitabine was added for patients with residual disease after neoadjuvant chemotherapy. Ninety-two percent of patients received adjuvant radiation. Disease recurrence was evident in 66.7% of patients. By the end of our study, 36.7% of patients had died (Table 2).

Regarding the association of CSC markers with patient parameters, all CSC markers had no association with age group (P=0.673, 0.940, and 0.914, respectively), stage (P=0.524, 0.185, and 0.169, respectively), grade (P=0.413, 0.150, and 0.081, respectively), N (P=0.744, 0.222, and 0.735, respectively), LVI (P=0.675, 0.213, and 0.163, respectively), skin invasion (P=0.873, 0.548, 0.190, respectively), or capsular invasion (P=0.524, 0.397, and 0.842, respectively). OCT4 expression had a significant association with LN involvement (P=0.039). However, lower KLF4 expression showed a significant association with extensive intraductal invasion (P=0.02) (Supplementary Table 1). Additionally, negative KLF4

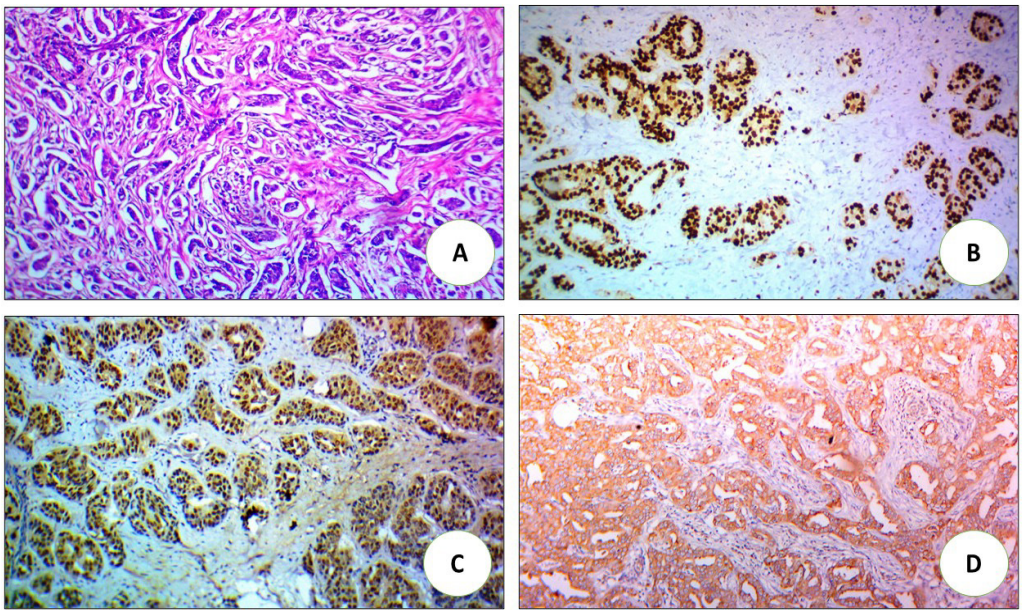

Figure 1. A: Low-grade breast carcinoma with tubule formation, low mitotic activity, and minimal nuclear atypia (x100 HPF), B: Low-grade breast carcinoma with strong NANOG nuclear expression (x100 HPF), C: Low-grade breast carcinoma with strong OCT4 nuclear expression (x100 HPF), D: Low-grade breast carcinoma with moderate KLF4 cytoplasmic expression (x100 HPF).

Table 1. Clinicopathologic Characteristics of Studied Groups

Parameter	Number (N = 90)	%
Age (years)		
< 35	39	43.3
≥35	51	56.7
Family history of BC		
Absent	77	85.6
Present	13	14.4
Site		
Right	38	42.2
Left	49	54.4
Bilateral	3	3.3
Stage		
Stage I	12	13.3
Stage II	22	24.4
Stage III	56	62.2
Grade		
I	10	11.1
II	22	24.4
III	58	64.4
T		
T1	18	20
T2	49	54.4
T3	17	18.9
T4	6	6.7
N		
N0	18	20
N1	22	24.4
N2	34	37.8
N3	16	17.8
LN		
Absent	18	20
Present	72	80
LVI		
Absent	14	15.6
Present	76	84.4
Extensive intraductal component		
Absent	68	75.6
Present	22	24.4
Skin invasion		
Absent	84	93.3
Present	6	6.7
Capsular invasion		
Absent	46	51.1
Present	44	48.9
NANOG IHC staining		
Negative	58	64.4
Low	11	12.2
Moderate	13	14.4
High	8	8.9
OCT 4 IHC staining		
Negative	49	54.4
Low	14	15.6
Moderate	11	12.2
High	16	17.8
KLF4 IHC staining		
Negative	42	46.7
Low	17	18.9
Moderate	14	15.6
High	17	18.9
miR-145		
Median	0.13	
range	(0.07-0.31)	
Age		
Mean± SD	41.04±12.29	
range	(24-72)	
Tumor size (mm)		
Mean± SD	46.37±14.98	
range	(15-70)	
Total number of dissected LNs		
Mean± SD	17.27±0.34	
range	(10-23)	
Number of positive LNs		
Mean± SD	7.14±5.48	
range	(0-20)	

expression showed a significant association with relapse (P < 0.001) (Table 3).

Regarding *miR-145* expression, downregulated expression was associated with higher stages (P<0.001), N (P<0.001), LN involvement (P=0.002), capsular invasion (P=0.02), and relapse and mortality (P<0.001 for both) (Table 4).

Correlation analyses showed significant negative correlations between *miR-145* expression and each of NANOG IHC expression (r=-0.305, P=0.009), OCT4 IHC expression (r=-0.255, P=0.014), and KLF4 IHC expression (r=-0.242, P=0.022). Additionally, *miR-145* showed significant negative correlations with tumor size (r=-0.247, P=0.019) and number of positive LNs (r=-0.481, P<0.001). However, *miR-145* expression showed significant strong positive correlations with DFS (r=0.920, P<0.001), and OS (r=0.813, P<0.001). OCT4 expression showed significant positive correlations with NANOG expression (r=0.328, P=0.002) and KLF expression (r=0.344, P=0.001). Moreover, a significant positive correlation was detected between *KLF* expression and DFS (r=0.255, P=0.015) (Table 5, Figure 3).

The log-rank test showed a significant association between higher KLF4 expression and DFS where patients with moderate KLF4 expression had a median DFS of 45 months (95% confidence interval (CI):17.07-72.93). Additionally, patients with high KLF4 expression had

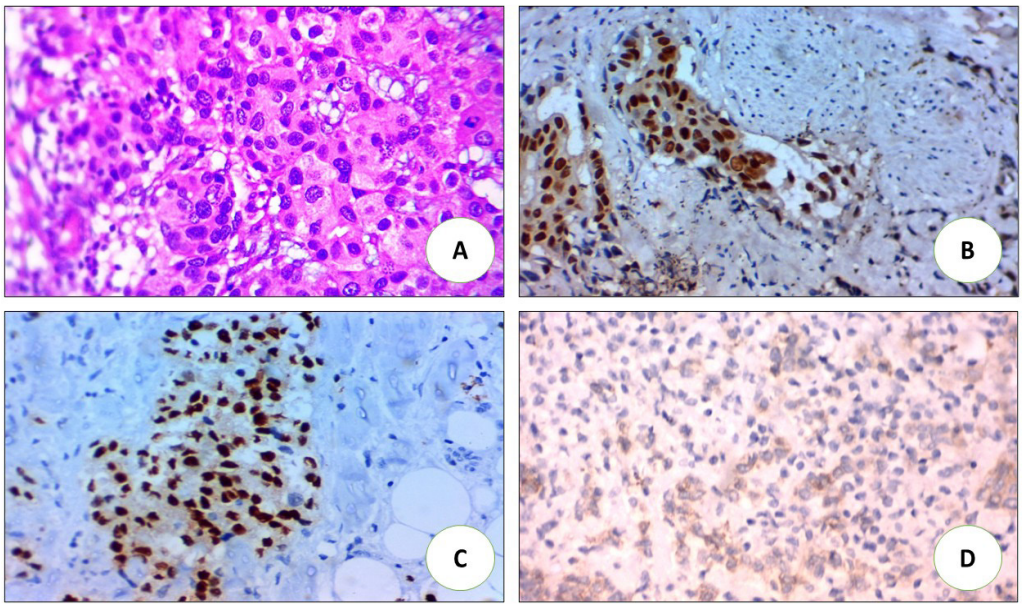

Figure 2. A: High-grade breast carcinoma with sheet formation, high mitotic activity, and marked nuclear atypia (x400 HPF), B: High-grade breast carcinoma with strong NANOG nuclear expression (x400 HPF), C: High-grade breast carcinoma with strong OCT4 nuclear expression (x400 HPF), D: High-grade breast carcinoma with weak KLF4 cytoplasmic expression (x400 HPF).

a median DFS of 44 months (95% CI:10.34-67.66). However, patients with negative KLF4 expression had a median DFS of 18 months (95% CI:15.57-20.43). However, no significant associations were detected between OCT4 or NANOG and DFS or OS (Table 6, Figures 4 and 5).

Table 2. Mode of Treatment and Clinical Outcome of Patients with Triple-Negative Breast Cancer

Parameter	Number (N = 90)	%
Chemotherapy		
No	2	2.2
Neoadjuvant AC- Paclitaxel only	7	7.8
Neoadjuvant AC-Paclitaxel+ adjuvant capecitabine	13	13.9
Adjuvant AC-Paclitaxel	55	61.1
Adjuvant AC-Docetaxel	3	3.3
Adjuvant TC	6	6.7
Adjuvant AC	4	4.4
Surgery		
BCS	57	63.3
MRM	33	36.7
Radiotherapy		
No	7	7.9
Yes	83	92.2
Relapse		
Absent	30	33.3
Present	60	66.7
Mortality		
Alive	57	63.3
Died	33	36.7

Discussion

CSCs have a tumorigenic potential and correlations with cancer recurrence, metastasis, and chemo- or radio-resistance [20]. It was observed that *miR-145*-5p was the common miRNA targeting stemness markers [21]. Thus, the current study investigated the prognostic significance of *miR-145* and CSC markers (OCT4, NANOG, and KLF4) in TNBC.

Regarding *miR-145* expression in our study, it was significantly downregulated in breast cancer tissues compared to adjacent control tissues. Previous studies found similar results [21-23].

Downregulated *miR-145* expression was associated with higher stages, N, LN involvement, capsular invasion, relapse, and mortality. There were significant positive correlations between *miR-145* and DFS and OS. Additionally, significant negative correlations were found between *miR-145* and tumor size and the number of involved LNs. Similarly, patients with low *miR-145* expression exhibited larger tumor size and LN metastasis [23]. Additionally, downregulated *miR-145*-5p was significantly related to larger tumor size, distant metastasis, higher Ki67 expression, and shorter OS [24]. Moreover, the survival rate in the high *miR-145* expression group was higher than that of the low *miR-145* expression group [19].

Regarding the role of *miR-145* in breast cancer suppression, it was an unfavorable prognostic factor [24]. Additionally, overexpression of *miR-145* significantly decreased invasion and migration by direct or indirect regulation of transforming growth factor-β1 (TGF-β1) [22]. TGF-β1 is responsible for cancer growth and

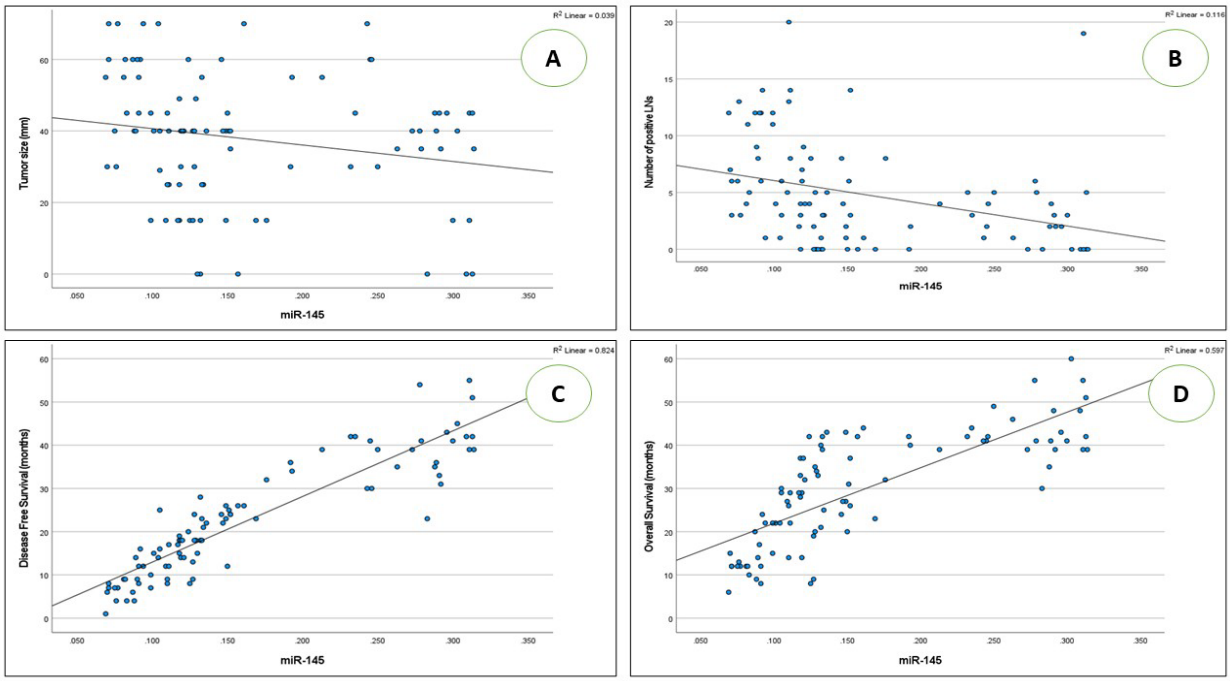

Figure 3. Correlation Results of *miR-145* Showing A: a significant negative correlation with tumor size, B: a significant negative correlation with the number of positive lymph nodes, C: a significant positive correlation with disease-free survival, D: a significant positive correlation with overall survival.

metastasis [25]. Moreover, the tumor suppressor effect of *miR-145* on breast cancer didn't differ between ER-positive and TNBC [24, 26].

Previous research investigated the role of *miR-145* in other cancers. In colorectal cancer, it decreased cell migration and invasion by targeting paxillin, a protein involved in cell adhesion to the extracellular matrix [6]. In lung cancer, it inhibited the migration and invasion by decreasing fascin actin-bundling protein 1 (FSCN1), a protein involved in cell migration, motility, adhesion, and cellular interactions by bundling actin filaments [7]. In ovarian cancer, it targeted tripartite motif-containing

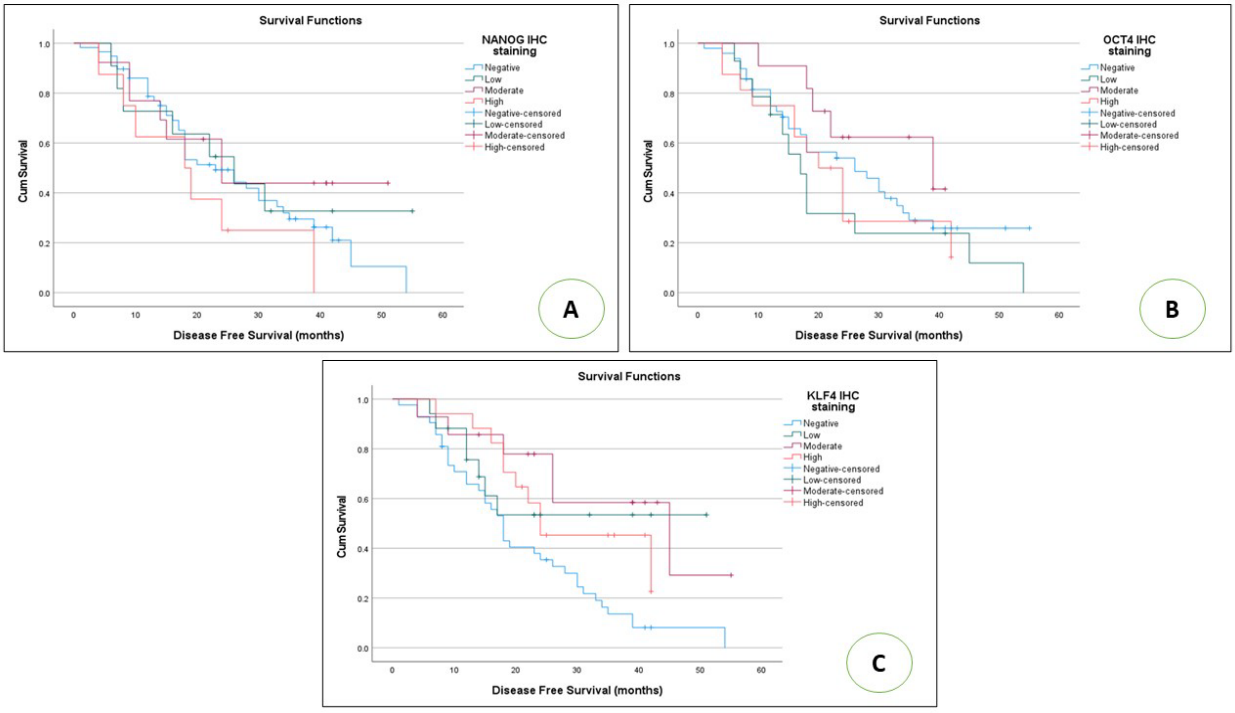

Figure 4. A: The Kaplan-Meier curve of NANOG IHC expression according to the disease-free survival rate of patients with triple-negative breast cancer, B: The Kaplan-Meier curve of OCT4 IHC expression according to the disease-free survival rate of patients with triple-negative breast cancer, C: The Kaplan-Meier curve of KLF4 IHC expression according to the disease-free survival rate of patients with triple-negative breast cancer.

protein 2 (TRIM2) and exerted tumor-suppressing functions [27].

Concerning CSC markers, NANOG and OCT4 are exclusively expressed in pluripotent cells, including germline stem cells. They are not expressed in most somatic adult cells [28]. Our results showed that NANOG and OCT4 positivity were detected in 35.6 and 45.6% of patients, respectively. Nagata et al. [29] and Liu et al. [30] detected nuclear and cytoplasmic NANOG and OCT4 staining in breast cancer cells, respectively. Additionally, strong NANOG expression was related to chemoresistance and poor prognosis in breast cancer.

Our results showed that OCT4 was significantly higher in LN involvement. This finding was similar to that found by Wang et al. [20]. Additionally, no significant differences between OCT4 and NANOG expressions and patient age, T, N, tumor size, grade, or clinical stage were detected. Almási et al. [31] found similar results. Additionally, Wang et al. [20] detected that OCT4 and NANOG expressions were not related to patient age or tumor stage. Moreover, Nagata et al. [32] found no correlation between NANOG expression and TNM stage.

Our findings found no significant differences between OCT4 and NANOG and DFS or OS. Almási et al. [31] found similar results. However, Nagata et al. [28] found that NANOG overexpression was significantly correlated with worse OS and DFS, particularly in ER-positive breast cancer. However, no significant differences between OCT4 expression and DFS or OS were detected. Additionally, Naderi et al. [33] identified that NANOG gene expression was correlated with the metastasis and proliferation of breast cancer, and it was correlated with the prognosis. This discrepancy in results can be explained by differences between protein and gene expression due to post-translational modification.

Our results showed KLF4 positivity in 53.3% of cases. Lower KLF4 expression was associated with intraductal invasion and tumor relapse. Upregulated KLF4 was significantly correlated with better DFS. Similar results were found by Nagata et al. [29]. They found that KLF4 inhibited the growth and metastasis of breast cancer. Akaogi et al. [34] reported downregulated KLF4 expression in cultured breast cancer cells compared to the normal mammary gland.

Correlation analysis detected a significant positive correlation between OCT4 and NANOG expressions. Rodda et al. [35] found that *OCT4* and *SOX2* synergistically regulate other stem cell marker expression, including NANOG. Additionally, Olariu et al. [36] stated that *NANOG* and *OCT4* induce the expression of each other.

Regarding the role of NANOG and OCT4 in oncogenesis, their co-expression promoted the epithelial-mesenchymal transition (EMT) and enhanced cancer stem-like properties in the lung adenocarcinoma cells [15]. In ovarian cancer, NANOG regulated cell migration and invasion via dysregulation of E-cadherin and FoxJ1 [37].

We revealed significant negative correlations between *miR-145* and the studied CSC markers. Xu et al. [38] and Sawant et al. [39] found that *miR-145* binds to the 3′-UTRs of *OCT4*, *SOX2*, *KLF4*, and *NANOG*, leading to their

Table 3. The Association between NANOG, OCT4, and KLF4 Expressions and the Clinical Outcome of Patients with Triple-Negative Breast Cancer

Variable		NANOG expression			OCT4 expression			KLF4 expression		
		-ve	Low	Moderate	High	-ve	Low	Moderate	High	-ve	Low	Moderate	High
Relapse	No	19	4	6	1	18	2	6	4	5	10	8	7
		32.80%	36.40%	46.20%	12.50%	36.70%	14.30%	54.50%	25%	11.90%	58.80%	57.10%	41.20%
	Yes	39	7	7	7	31	12	5	12	37	7	6	10
		67.20%	63.60%	53.80%	87.50%	63.30%	85.70%	45.50%	75%	88.10%	41.20%	42.90%	58.80%
$\chi 2$		2.578				5.268				17.691			
P		0.461				0.153				<0.001**			
Mortality	Alive	39	8	8	2	33	6	9	9	22	10	10	15
		67.20%	72.70%	61.50%	25%	67.40%	42.90%	81.80%	56.30%	52.40%	58.80%	71.40%	88.20%
	Dead	19	3	5	6	16	8	2	7	20	7	4	2
		32.80%	27.30%	38.50%	75%	32.7%	57.10%	18.20%	43.80%	47.60%	41.20%	28.60%	11.80%
$\chi 2$		5.88				4.832				7.253			
P		0.118				0.185				0.064			

Table 4. The Association between *miR-145* Expression and Demographical, Clinical, Histopathological Parameters, and Outcome of Patients with Triple-Negative Breast Cancer

Parameter		(N)	Median	Range	Test	P
Age	< 35	39	0.136	0.071-0.314	0.93^	0.35
	≥35	51	0.129	0.069-0.311		
Stage	I	12	0.163	0.118-0.313	18.55$	<0.001**
	II	22	0.149	0.105-0.314		
	III	56	0.115	0.069-0.313		
Grade	I	10	0.151	0.083-0.313	0.24$	0.89
	II	22	0.131	0.070-0.313		
	II	58	0.129	0.069-0.314		
T	T1	18	0.141	0.099-0.313		
	T2	49	0.129	0..070-0.314	6.57$	0.09
	T3	17	0.124	0.069-0.279		
	T4	6	0.099	0.071-0.243		
N	N0	18	0.163	0.118-0.314		
	N1	22	0.151	0.077-0.300	20.28$	<0.001**
	N2	34	0.123	0.070-0.313		
	N3	16	0.096	0.069-0.311		
LN	Absent	18	0.163	0.118-0.314	3.10^	0.002*
	Present	72	0.123	0.069-0.313		
LVI	Absent	14	0.131	0.088-0.296	0.11^	0.92
	Present	76	0.131	0.069-0.314		
Extensive intraductal component	Absent	68	0.131	0.069-0.314	0.49^	0.62
	Present	22	0.13	0.076-0.313		
Skin invasion	Absent	84	0.132	0.069-0.314	1.5^	0.14
	Present	6	0.099	0.071-0.243		
Capsular invasion	Absent	46	0.143	0.070-0.314	2.31^	0.02*
	Present	44	0.118	0.069-0.313		
Relapse	Absent	30	0.203	0.089-0.314	4.07^	<0.001**
	Present	60	0.12	0.069-0.303		
Mortality	Alive	57	0.152	0.088-0.314	4.96^	<0.001**
	Died	33	0.11	0.069-0.291		

^, Mann Whitney test, $, Kruskal Wallis test, *, difference Significant (P<0.05), **, Highly difference significant (P<0.001)

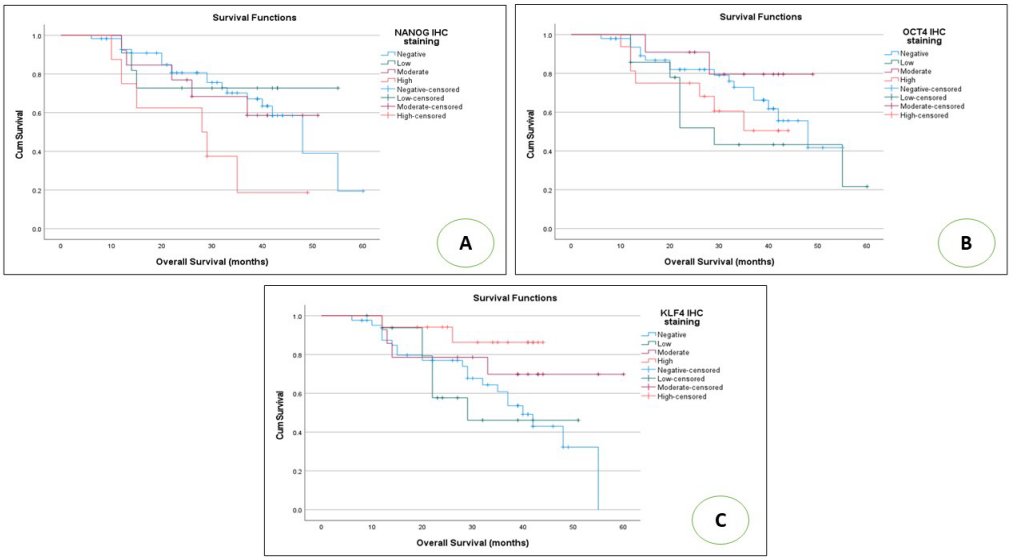

Figure 5. A: The Kaplan-Meier curve of NANOG IHC expression according to the overall survival rate of patients with triple-negative breast cancer, B: The Kaplan-Meier curve of OCT4 IHC expression according to the overall survival rate of patients with triple-negative breast cancer, C: The Kaplan-Meier curve of KLF4 IHC expression according to the overall survival rate of patients with triple-negative breast cancer.

Table 5. Correlation Matrix between NANOG, OCT4, KLF4, and *miR-145* Expressions and some Demographical, Clinical, Histopathological Parameters, and Outcome in Patients with Triple-Negative Breast Cancer

		miR-145	NANOG IHC staining	OCT4 IHC staining	KLF4 IHC staining
NANOG IHC staining	r	-0.305			
	P	0.009*			
OCT4 IHC staining	r	-0.255	0.328*		
	P	0.014*	0.002		
KLF4 IHC staining	r	-0.242	-0.064	0.344*	
	P	0.022*	0.548	0.001	
Age	r	-0.074	0.046	0.006	0.063
	P	0.489	0.666	0.953	0.556
Tumor size (mm)	r	-0.247*	-0.019	0.206	0.038
	P	0.019*	0.862	0.051	0.719
Total number of dissected LN	r	-0.129	-0.021	-0.007	-0.057
	P	0.224	0.845	0.949	0.594
Number of positive LNs	r	-0.481	0.19	0.189	-0.126
	P	<0.001**	0.074	0.075	0.235
Disease Free Survival (months)	r	0.92	0.025	0.028	0.255*
	P	<0.001**	0.814	0.79	0.015
Overall Survival (months)	r	0.813	0.004	0.002	0.134
	P	<0.001**	0.973	0.983	0.207

r, Spearman's correlation coefficient; *, difference Significant (P<0.05);**, Highly difference significant (P<0.001)

Table 6. Disease-Free Survival and Overall Survival According NANOG, OCT4, KLF4 Expressions among Patients with Triple-Negative Breast Cancer.

Variable		DFS			Log-rank	OS			Log-rank
		Median	CI (95%)		P	Median	CI (95%)		P
NANOG IHC expression	Negative	23	17.12	28.88		48	37.81	58.19	6.4
	Low	26	10.84	41.17	2.99	44	32.84	54.61	0.09
	Moderate	24	9.7	38.31	0.39	39	30.95	47.73	
	High	18	5.53	30.47		28	8.6	47.4	
OCT4 IHC expression	Negative	26	14.68	37.32		48	36.12	59.88	3.66
	Low	17	13.59	20.41	3.57	29	21.69	36.31	0.3
	Moderate	39	7.42	70.58	0.31	44	36.63	50.42	
	High	20	14.69	25.31		33	25.96	39.37	
KLF4 IHC expression	Negative	18	15.57	20.43		40	32.95	47.05	7.41
	Low	33	5.08	23	12.83	35	27.01	43.54	0.06
	Moderate	45	17.07	72.93	0.005*	47	37.16	57.99	
	High	44	10.34	67.66		41	36.39	45.03	

*, difference Significant (P<0.05)

downregulation. This reduces stem-like properties and EMT [21]. Polytarchou et al. [40] found that KLF4 was inhibited by *miR-145*, whose expression was inhibited by the Zeb repressors in CSCs.

In conclusion, *miR-145* and KLF4 may serve as important predictors for prognosis in patients with triple-negative breast cancer. *miR-145* showed significant positive correlations with disease-free survival (DFS) and overall survival (OS). KLF4 expression showed a significant positive correlation with DFS. Log-rank test showed a significant relation of KLF4 with DFS. Thus, *miR-145* can be considered a potential therapeutic molecule for targeting CSC-mediated chemoresistance and metastasis.

Author Contribution Statement

SH, RS, and HMA contributed to the study conception and design. All authors were responsible for the methodology and statistical analysis of data. RS, ABW, HMA, and SH wrote the manuscript draft. All authors read and approved the final manuscript.

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Ethical approval and consent to participate

This study got approval (ZU-IRB#758/26-Nov-2024) and written informed consent was obtained from all participants.

Availability of data and material

Available upon a reasonable request from the author.

Competing interests

The authors declare that there is no conflict of interest.

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