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Epithelial-Mesenchymal Transition Axis Lin 28, ARID1A, And ELF3 As A Novel Prognostic Triad in Invasive Ductal Breast Carcinoma

Sabah Mohamed Hanafy [1], Eman Elsayed Fayad [1], Mohamed Ali Alabiad[1]
[1]Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.
Corresponding author: Mohamed Ali Alabiad**, Email:** maabyad@medicine.zu.edu.eg,
ORCID: https://orcid.org/0000-0002-6006-3711**,** Tele: 00201150509554

ABSTRACT

Background: Breast cancer is the most often identified cancer in women and considered the second principal cause of cancer death among all women**.**

Objective: The aim of the study was to evaluate the relationship between Epithelial-mesenchymal Transition markers Lin 28, ARID1A, and ELF3 and the clinicopathological parameters and outcomes in invasive ductal breast carcinoma.

Materials and Methods: This retrospective study was on 120 breast cancer cases by evaluation the immunohistochemical expression of Lin 28, ARID1A, and ELF3 and correlation their expression with the clinicopathological and the outcome parameters of the patients.

Results: Lin 28, ARID1A and ELF3 expression statistically significant correlation with tumor size, with a high KI67 index, positive ER/ PR expression and Her-2/neu, high grade lymph node metastasis, lymph vascular invasion, perineural invasion and mortality.

Conclusions: Overexpression of the mesenchymal stem cell markers (ARID1A-1 and Lin23) and a decrease in the expression of the epithelial markers (ELF3) were strongly related poor prognostic and clinicopathological parameters of invasive ductal breast cancer.

Keywords: Lin 28, ARID1A, ELF3, Invasive ductal breast carcinoma.

INTRODUCTION

Breast cancer is the most often identified cancer in women, omitting skin cancers that are not melanoma. While it is the second principal cause of cancer death among all women, after cancer lung, in 2020, there were more than 2 million new cases worldwide. Moreover, there were almost 680,000 deaths [(1)].

Although mortality is greater in Egypt, with an age-consistent rate of 20.4/100,000, matched to the US rate of 12.3/105, even though incidence is lower than the worldwide average [(2)].

Breast cancer cases are attributed to modifiable risk factors of 30%, such as being overweight, sedentary, or alcohol intake, and thus may be preventable. Once we are aware of the pathophysiology of cancer of the breast and the levels of associated molecule expression, we may be able to apply molecular therapeutics to limit metastasis. Aggressive metastatic breast tumors have a wide range of therapy responses and clinical prognoses due to a high level of heterogeneity. Bone, lung, liver, and brain are the most often affected distant metastatic organs; their 5-year overall survival rates are 22.8, 16.8, and 8.5%, respectively, and they have incredibly poor survival rates that are lower than the 5-year overall survival rate of breast cancer patients without metastasis, which is 80% [(3)].

The mesenchymal cell phenotype, that is characterized by an improved capacity for migration and invasion, a raised level of apoptosis resistance, and a markedly higher production of ECM components, is achieved by polarized epithelial cells through a biological process that involves a number of biochemical changes. These cells often connect with the basement membrane through basal plane [(4)].

Many variables, such as tissue inhibitor of metalloproteinases-2 in breast cancer, can prevent the transition of epithelial cells into mesenchymal tissue and prevent development, invasion, and metastasis. Growth Differentiation Factor 15 (GDF15), which activates ETM in colorectal cancer, is anticipated to be a unique prognostic indicator and may aid in the spread of colorectal cancer, Many distinct molecular or cellular factors, including Lin28 and its related components, have been linked to breast cancer [(5)].

An RNA-binding protein and transcription factor called Lin28 is connected to cancer, healthy development, glucose metabolism, and stem cell differentiation. The current consensus is that Lin28 primarily serves as an oncogene. A conserved RNA-binding protein called Lin28/LIN-28 can cause cancer in animals and encourages cell growth and pluripotency. Lin28 participates in a variety of malignant cancer processes through let-7 dependent and let-7 independent pathways. The members of the let-7 miRNA family reduce tumor growth by preventing the production of oncogenes and important mitogenic pathway regulators including RAS, MYC, and HMGA2 [(6)].

Downregulation of let 7 is a characteristic of breast and lung cancer. Despite being necessary used for proper development, Let-7's posttranscriptional regulation by Lin28 inhibits the diversity of embryonic stem cells

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(ESCs) that let-7 mediates, therefore promoting the pluripotent state [7].

Interactive domain rich in AT 1A (ARID1A) is a gene identified in the 1p36 region of chromosome 14 and has been linked to mutations in cancer ovaries, cancer endometrium, cancer stomach, and cancer pancreas. The gene with the greatest frequency of mutations in SWI/SNF complexes is the tumor inhibitor AT-rich interactive domain protein 1A (ARID1A). Additionally, it is known that ARID1A inhibits cell migration and proliferation by collaborating with CEBPa to treat breast cancer. ARID1A mutations are associated with decreased outcome in patients with breast cancer and enhanced immune activation in cancer GIT. ARID1A deletion encouraged cell EMT, as evidenced by an uptick in stromal markers and fusiform index, a decline in epithelial markers, and an rise in the migratory activity and drug resistance of renal cells. Wilson et al.'s findings from 2019 also suggested a direct connection between the aberrant endometrial tissue diffusion and the rise in EMT-related gene expression brought on by ARID1A deletion. So, it is important to pay attention to how ARID1A prevents EMT. Research is also being done to determine whether ARID1A affects the EMT process and contributes to breast cancer. In ARID1A mutant ovarian cancer, ATM and HDAC6 inhibitors can enhance the effectiveness of antitumor immunotherapy even more. Immune checkpoint inhibitor efficacy in metastatic breast cancer with ARID1A mutation is anticipated to be favorable [8].

(Specific to Erythroblast Transformation) Breast cancer has long been thought to be significantly influenced by transcription factor 3 (ELF3), which encodes an epithelial-restricted member of the ETS transcription factor family. However, ELF3 behaves differently depending on the type of cancer. Its specific function in the spread of breast cancer is yet unknown. Transcription factors control the expression of downstream genes, acting as master switches for numerous metabolic processes. Numerous studies have shown how transcription factors contribute to the initiation and spread of cancer. The exact identification of transcription factors complicated in the enhancement of cancer ovaries is still deficient. In order establish a transcription factor gene signature for progressive cancer, they used transcriptome profiling to identify E74-like factor 3 (ELF3) as one of the transcription factor-encoding genes that expressed substantially higher in long-term cancer ovaries survivors than short-term survivors. ovaries. More lately, other ETS family members are beginning to play additional important roles in PCa [9]. Small molecule inhibitors of ETS factors have been proposed for use as cancer treatments in a number of recent studies[10].

The prevalence of Lin 28, ARID1A, and ELF3 in invasive duct carcinoma, as well as their potential function as new biomarkers and the control of epithelial-mesenchymal transition (EMT), are prominently demonstrated in the current study.

MATERIALS AND METHODS

Between June 2018 and June 2022, 120 cases of breast cancer were handled in the Pathology, General Surgery, Clinical Oncology, and Medical Oncology Departments, Faculty of Medicine, Zagazig University Hospitals. They either underwent a conservative wide local removal with axillary lymph node excision or a modified radical mastectomy. The clinic-pathological data were obtained from the files of patients. Cases previously treated with chemotherapy or radiotherapy were excluded from our investigation.

Tissues sampling :

For a histological study, 4 microns sections from the paraffin-embedded tissue blocks were stained with hematoxylin and eosin. For Lin28, ARID1A and ELF3, ER, PR, KI 67 index, and HER-2/neu, immunohistochemistry (IHC) was used. According to the WHO 2003 categorization of breast tumors, tumor grade was calculated using the Elston and Ellis grading system [30], and tumor stage was determined using TNM [11]. The different types of tumors classified by the WHO/ISUP 2004 standard.

Immunohistochemistry

Four microns sections from the paraffin-embedded tissue blocks were processed for hormone receptors immunohistochemistry using monoclonal antibodies against the ki 67 index, er receptors, pr receptors, and her2 receptors (santa cruz, california, biotechnology) and 2nd antibodies. lin28, arid1a, and elf3 immunohistochemical staining was carried out using tma staining as follows:[12, 13] following a 1:50 dilution in signal stain antibody, lin28 (3978, cell signaling technology) was saved with the sections for a whole night at 4°c. after the sections had been treated with the antibodies, they were washed three times for five minutes individually with tbs-t before being exposed to an anti-rabbit biotin antibody (ls-d1, lsbio) diluted in (1:300) blocking solution for one hour at room temperature. the vectastain abc-ap reagent (ak-5000), the substrate kit (sk-5100), vector red alkaline phosphatase, and tbs-t stayed used to wash the sections three times for five minutes each before staining [14, 15].

The samples were treated with the primary antibody over night at 4 C after being diluted by 1/30 in blocking solution with (sc-81193; Santa Cruz Biotechnology, Inc.) the mouse monoclonal anti-ARID1A antibody. The binding antibody was recognized using 2 g/ml goat anti-mouse biotin-conjugated secondary antibodies (cat. no.

ab6789; 1:2,000; Abcam). (Cat. no. ab6789; 1:2,000; Abcam) were used to identify the bound antibody. Two impartial pathologists who were chosen to identify the slides in a blind manner afterwards evaluated the epithelial cells.

The addition and 48-hour incubation at 4°C using the anti-

LF3 primary antibody (1:1,000, Abcam catalog number ab133621) were performed. The specimens were then incubated with biotinylated goat anti-rabbit IgG (1:500; cat. no. sc-2004; Santa Cruz Biotechnology, Inc.) for 1 hour at 37 degrees Celsius. The sections were stained with a diaminobenzidine combination for 30 min. at 37°C (Beijing Solarbio Science & Technology Co., Ltd.), dehydrated with alcohol series with different grades, cleaned with xylene, and covered with balsam. Finally, the protein density per segment was calculated using Media Cybernetics' Image Pros Plus 5.0 program.

Evaluation of immunohistochemical staining

LIN28 scoring.

Strong expression +; temporally limited expression -, no obvious expression; not detected.

ARID1A scoring.

The grading staining intensity is as follows "0" (not detected), "1" (weak), "2" (moderate), and "3" (strong). Staining scored 0 and 1 were considered low, while 2 and 3 were considered high.

ELF3 Scoring

There were four levels of staining intensity: "0" (negative), 1 was weak, 2 were moderate, and 3 were intense. Grading systems were handled to determine the extent of staining: "0" (5%), "1" (5-25%), "2" (25-50%), "3" (50-75%), or "4" (>75%). The cytoplasmic and nuclear scores (0-12) were created by multiplying the intensity score by the extent score, and they were collective to get the immunostaining score (0-24). Cutoff values for ELF3 were based on the median of all scores. Low expressions were values ≤12 (ELF3) [16].

Ethical approval:

Approval was obtained from Zagazig University's Faculty of Medicine's Institutional Review Board (IRB), Egypt, (no. ZU-IRB#10746) to collect data and samples from relevant departments. The research was carried out in compliance with the declaration of Helsinki of the World Medical Association. Before participating in the study, all patients or their legal representatives signed informed permission forms.

Statistical analysis

Graph Pad software (version 7.0) was used to conduct statistical analysis. The $\chi 2$ test was used to assess the expression levels of Lin 28, ARID1A, and ELF3 in relation to clinicopathological and prognostic factors. We estimated the disease-free survival (DFS) pattern and overall survival (OS) frequencies using the Kaplan-Meier method, and we used the log-rank test to examine the variances in survival. The univariate and multivariate proportional hazard models were used to analyze the prognostic relevance of these parameters. There were two sides to each statistical test. At P 0.05, statistics were deemed significant.

RESULTS

One hundred and twenty cases met the requirements for selection (Diagnosed as lobular carcinoma and not exposed to chemotherapy). Table 1 provides a summary of the clinicopathologic features and staging information of patients with invasive ductal breast cancer. 52 cases (43.3%) had ages under 50, while 86 (56.7%) had ages over 50. According to histologic grading, there were 70 cases (58.3%) of grade 3 (poorly differentiated) carcinoma, 40 cases (33.3%) of moderately differentiated grade 2), and 10 cases (8.3%) of well differentiated grade 1. There were 18 cases (15%) diagnosed stage I at TNM, 54 cases (45%) at TNM stage II, and 38 cases (40%) at TNM stage III. 110 cases (91.7%) had positive lymph node metastases. Only 74 instances (61.7%) had lymph vascular invasion evidence. Regarding perineural invasion, it was present in 22 cases (18.3%). Eighty-two cases (68.3%) were ER/PR positive, while 38 (31.7%) were ER/PR negative; and finally, 18 (15%) were classified as HER-2/neu-positive, while 102 (85%) were HER-2/neu-negative. High Ki 67 index in 72 cases (60%).

Lin 28 Expression

Lin 28 was mostly expressed in the cytoplasm of cancer breast tissues. Negative Lin 28 expression was noticed in 54 out of 120 (45 %) cases, and 66 from 120 (55 %) patients showed positive Lin 28 expression. (Table 1-3) runs through the correlation of the immunohistochemical expression of Lin 28 with the clinicopathological characteristics. No statistical difference exists between age group or tumor ($p = 0.117$) or tumor grade ($p = 0.1663$) and Lin 28 expression. While a statistically significant association with tumor size was expressed in all twenty cases of tumor more than 50mm. A significant statistically correlation was found between the histological type ($p < 0.001$) and Lin 28 expression. As regards hormone receptor expression 75.2% of cases showed positive ER/ PR and 94.4% positive Her-2/neu. A significant statistical association exists with tumors with a high KI67 index ($p = 0.035$). A significant statistically correlation was found between Lin 28 expression, lymph node metastasis ($p = 0.014$) and lymph vascular invasion ($p < 0.001$). A significant statistically correlation was found among perineural invasion and Lin 28 expression ($p < 0.001$). Positive Lin 28 expression has 93.1% sensitivity and 80.6% specificity for predicting death in breast cancer. Positive Lin 28 expression statistically

significant correlation with death cases (P< 0.001) (Table 4-7) (Fig. 1).

ARID1A Expression

Table 1-3 shows an analysis of clinicopathologic features and ARID1A expression. The majority of the ARID1A appearance was found in the nucleus of breast cancer tissue. Negative ARIDIA expression was detected in sixty-six cases (55%), while positive in 54 cases (45%). The correlation of ARID1A expression with the clinicopathological characteristics was presented in Table….. As regard age groups not correlated with ARID1A expression and age group (p = 0.796), the difference in tumour stage (p = 0.519), lymph node metastasis (p=0.367) and all tumour size (p=0.252). A statistically significant relationship was found with ARID1A expression tumour grade (p < 0.001), which high expressed in grade III (71.4%). There is no statistical difference between ARID1A expression and lymph vascular invasion (p=0.189) and perineural invasion (p=0.315). A significant statistically association was located between the histological type (p < 0.001) and ARID1A expression. 56.1% and 83.3% of cases confirmed positive ER/ PR expression and Her-2/neu, respectively. There is A significant statistically association between ARIDIA expression and tumors with positive expression of ER/ PR (p = 0.013) with breast cancer positive for Her-2/neu (p < 0.001). There is a statistically significant association with tumors having a high KI67 index (p = 0.035). Negative ARID1A expression has 72.4% sensitivity and 80.6 % specificity for predicting death in breast cancer. Negative ARID1A expression statistically significant correlation with death cases (P= 0.002) (Table 4-7) (Fig. 2).

ELF3 Expression

Table 1-3 provided an analysis of ELF3 expression with clinicopathologic traits. ELF3 expression was positive in 30 cases (25%), while 66 cases had negative expression (75%). There is statistically no substantial relationship between ELF3 and tumor grade (p< 0.109) and tumor stage (P=0.751). There is a statistically significant negative correlation between the ELF3 expression and lymph node expression (p < 0.001) and also with lymph vascular invasion (p=0.066), while non-significant correlation with perineural invasion (p=0.315). A significant relationship between ELF3 expression and tumors positive for ER/ PR (p < 0.001) or tumors positive for Her-2/neu (p = 0.031) and also tumors with high KI67 index (p = 0.054). Negative ELF3 expression has 93.1% sensitivity and 60. % specificity for predicting death in breast cancer. Negative ELF3 expression statistically significant correlation with death cases (P= 0.002) (Table 4-7) (Fig. 3).

Correlation between Lin 28, ARID1A, and ELF3 expression in breast cancer

A considerable association exists between Lin 28 expression and ELF3 (p 0.001), as well as between Lin 28 expression and ARID1A expression (p 0.001). The ARID1A expression and ELF3 expression have a substantial positive correlation (p 0.001).

Table [1] Clinicopathological data of the studied patients (N=120)

Parameters	**N=120**	Percent
Age group:		
≤50 years	52	43.3
>50 years	86	56.7
Grade:		
I	10	8.3
II	40	33.3
III	70	58.3
Stage:		
I	18	15
II	54	45
III	48	40
LN metastasis:		
Negative	10	8.3
Positive	110	91.7
Tumor size:		
≤20 mm	28	23.3
>20 – 50 mm	72	60
>50 mm	20	16.7
ER/PR status:		
Negative	38	31.7
Positive	82	68.3
Her2-neu:		
Negative	102	85
Positive	18	15
Ki 67 index:		
Low	48	40
High	72	60
Lymphovascular invasion:		
Negative	46	38.3
Positive	74	61.7
Perineural invasion:		
Negative	98	81.7
Positive	22	18.3

Table [2]: Expression of the three markers by immunohistochemistry.

Expressed Marker		N=120	Percentage
Lin 28	Negative	54	45%
	Positive	66	55%
ELF3	Negative	90	75
	Positive	30	25
ARID1A	Negative	66	55
	Positive	54	45

Table [3]: Relationship between the expression levels of the three markers, clinicopathological parameters, and outcome:

	Total	Lin 28		P	ELF3		P	ARID1A		P
		Negative	Positive		Negative	Positive		Negative	Positive	
		N=54 (%)	N=66(%)		N=90(%)	N=30(%)		N=66(%)	N=54(%)	
Age group:										
≤50 years	52	15(57.7)	22 (42.3)	**0.117**	32(61.5)	20 (38.5)	**0.069**	30(57.7)	22(42.3)	**0.796**
>50 years	68	12(35.3)	44 (64.7)		58 (85.3)	10(14.7)		36(52.9)	32(47.1)	
Grade:										
I	10	0 (0)	10 (100)		10(100)	0 (0)		10 (100)	0 (0)	
II	40	20 (50)	20 (50)	**0.1663**	32(80)	8(20)	**0.109**	36 (90)	4(10)	**<0.001**
III	70	34 (48.6)	36(51.4)		48(68.6)	22(31.4)		20 (28.6)	50(71.4)	
Stage:										
I	18	14 (77.8)	4 (22.2)		16 (88.9)	2 (11.1)		8(44.4)	10(55.6)	**0.519**
II	54	34 (63)	20 (37)	**<0.001**	34(63)	20 (37)	**0.751**	30(55.6)	24(44.4)	
III	48	6 (12.5)	42 (87.5)		40 (83.3)	8 (16.7)		28(58.3)	20(41.7)	
LN metastasis:										
Negative	10	10 (100)	0 (0)	**0.014**	0 (0)	10(100)	**<0.001**	8(80)	2(20)	**0.367**
Positive	110	44 (40)	66 (60)		90 (81.8)	20(18.2)		58(52.7)	52(47.3)	
Tumor size:										
≤20 mm	28	26 (92.9)	2 (7.1)		24(85.7)	4(14.3)		18(64.3)	10 (35.7)	
>20 – 50	72	28 (38.9)	44 (61.1)	**<0.001**	52(72.2)	20(27.8)	**0.347**	40(55.6)	32(44.4)	**0.252**
>50 mm	20	0 (0)	20 (100)		14(70)	6(30)		8(40)	12(60)	
ER/PR status:										
Negative	38	32 (84.2)	6 (15.8)	**<0.001**	12(31.2)	26(68.4)	**<0.001**	30(78.9)	8(21.1)	**0.013**
Positive	82	22 (26.8)	60 (75.2)		78(95.1)	4(4.9)		36(43.9)	46(56.1)	
Her2-neu expression:										
Negative	102	53 (51.9)	49 (48)	**<0.001**	73(71.5)	29 (28.4)	**0.031**	63 (61.7)	39 (38.2)	**<0.001**
Positive	18	1 (5.6)	17 (94.4)		17 (94.4)	1 (5.6)		3 (16.6)	15 (83.3)	
KI 67 index:										
Low	48	30 (62.5)	18 (37.5)	**0.035**	26(54.2)	22(45.8)	**0.054**	32(66.7)	16(33.3)	**0.008**
High	72	24 (33.3)	48 (66.7)		64(77.8)	8(22.2)		22(30.6)	50(69.4)	
Lymphovascular invasion:										
Negative	26	38 (82.6)	8 (17.4)	**<0.001**	24(60.9)	22(39.1)	**0.066**	20(43.5)	26(56.5)	**0.189**
Positive	74	16 (21.6)	58 (78.4)		66(83.8)	8 (16.2)		46(62.2)	28(37.8)	
Perineural invasion										
Negative	98	54 (55.1)	44 (44.9)	**<0.001**	70(71.4)	28 (28.6)	**0.262**	50(51)	48(49)	**0.315**
Positive	22	0 (0)	22 (100)		20(90.9)	2 (9.1)		16(72.7)	6(27.3)	
Death:										
Yes	**58**	4 (6.9)	54 (93.1)	**<0.001**	54(93.1)	4 (6.9)	**0.002**	16(27.6)	42(45.5)	**<0.001**
No	**62**	50 (80.6)	12 (19.4)		36(58.1)	26(41.9)		50(80.6)	12(29.4)	

Table [4]: performance of the investigated markers in predicting death in the patients under study:

Marker	Sensitivity	Specificity	PPV	NPV	Accuracy
+ve Lin28	93.1%	80.6%	81.8%	92.6%	86.7%
-ve ELF3	93.1%	41.9%	60%	86.7%	66.7%
-ve ARID1A	72.4%	80.6%	77.8%	75.8%	76.7%

Table [5]: Correlation between Lin 28, Muc 1 and Lipocalin 2

	Lin 28		Muc 1		Lipocalin 2	
	Phi	**p**	**Phi**	**p**	**Phi**	**p**
Lin 28			+0.638	<0.001	-0.818	<0.001
ELF3	+0.638	<0.001			-0.522	<0.001
ARID1A	-0.818	<0.001	-0.522	<0.001		

Table [6]: Kaplan– Meier survival curves illustrating survival time differences in patients as regard markers expressions.

	Total N	N of Event	Censored		Survival time, Months				OS Rate%	P
					Mean		Median			
			N	%	Estimate ±SE	95% CI	Estimate ±SE	95% CI		
Lin 28										
Positive	66	54	12	18.2%	34.2±2.2	29.9-38.5	34.0±0.9	32.2-35.8	11.3%	<0.001
Negative	54	4	50	92.6%	57.8±1.5	55.0-60.7	NR		92.3%	
ELF3										
Negative	90	54	36	40.0%	41.0±2.4	36.3-45.6	35.0±1.1	32.9-37.1	34.7%	0.003
Positive	30	4	26	86.7%	52.8±2.1	48.6-57.0	NR		86.4%	
ARID1A										
Negative	54	42	12	22.2%	34.6±2.7	29.4-39.9	34.0±0.6	32.8-35.2	14.0%	<0.001
Positive	66	16	50	75.8%	53.1±2.1	48.9-57.3	NR		74.7%	
Overall	120	58	62	51.7%	44.9±2.1	40.8-48.9	49.0		47.6%	

Table [7]: Analysis for overall survival using single and multiple variables.

Co-variates	Univariate Analysis			Multivariate Analysis		
	Sig.	HR	95.0% CI for HR	Sig.	HR	95.0% CI for HR
Age <50 vs =>50ys	0.827	1.1	0.551-2.108			
Grade	Ref					
Grade (2 vs 1)	0.394	0.4	0.056-3.103	0.882	0.9	0.11-6.65
Grade (3 vs 1)	0.001	0.2	0.09-0.522	<0.001	0.2	0.065-0.426
Stage	Ref					
Stage (2 vs 1)	0.514	0.7	0.264-1.947			
Stage (3 vs 1)	0.207	0.6	0.298-1.3			
LN. META Yes vs No	0.999	1.0	0.306-3.279			
Size	Ref			0.131		
20-50 Vs. <20	0.652	0.7	0.139-3.44	0.564	1.9	0.217-16.399
>50 Vs. <20	0.023	4.0	1.209-13.152	0.050	3.4	0.998-11.84
ER.PR status N vs P	0.379	1.4	0.674-2.824			
Her2-neu expression N vs P	0.157	0.6	0.313-1.206			
KI.67. index H vs L	0.274	0.7	0.335-1.363			
Lympho-vascular Invasion yes vs no	0.121	0.6	0.266-1.167			
Perineural Invasion yes vs no	0.879	0.9	0.427-2.074			
Cytoplasmic Lin 28 expression N vs P	0.013	0.3	0.114-0.772	**0.003**	0.2	0.081-0.602
ELF3 expression N vs P	0.934	1.0	0.494-2.155			
ARID1A expression N vs P	0.067	1.9	0.956-3.697			

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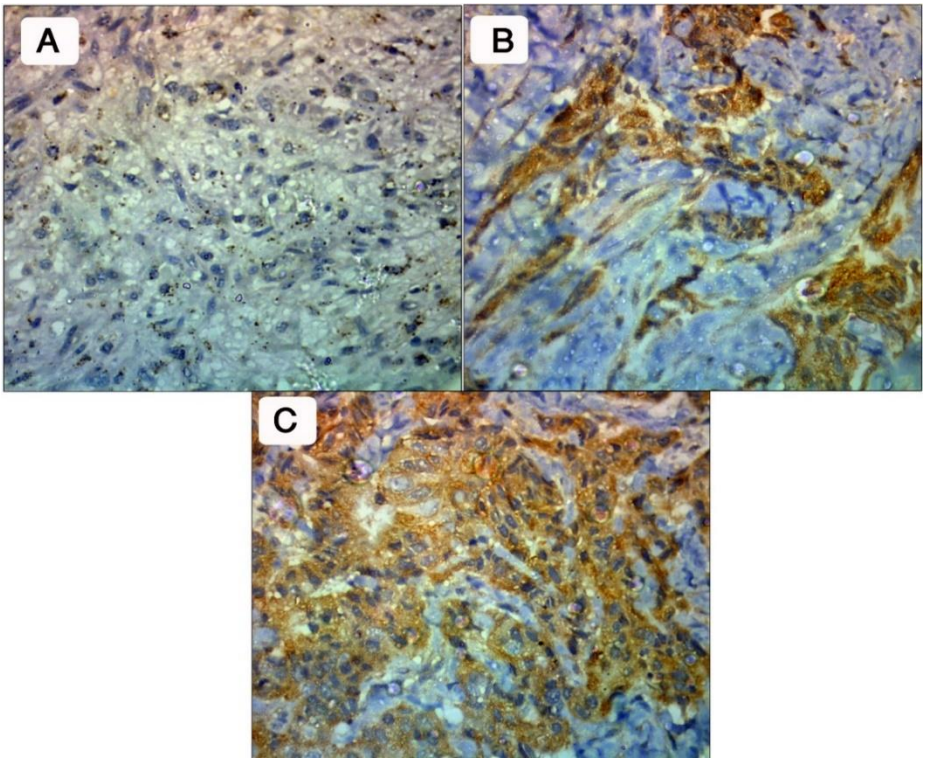

Figure 1: Photomicrograph of lobular carcinoma of the breast showing Lin 28 immunohistochemical expression: (a) Invasive ductal carcinoma shows negative Lin 28 expression (IHC, X :400) (b) Invasive ductal carcinoma showing moderate cytoplasmic Lin28 expression (IHC, X :400) (c) Invasive lobular carcinoma shows diffuse and strong cytoplasmic Lin 28 expression (IHC, X :400).

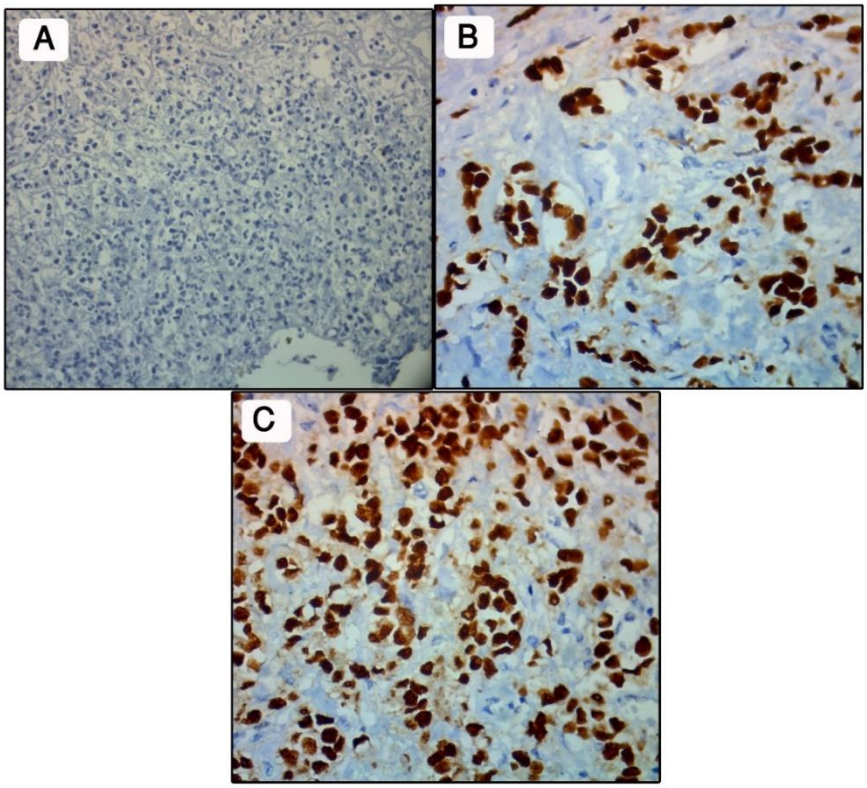

Figure 2: Photomicrograph of lobular carcinoma of the breast showing ARID1A immunohistochemical expression: (a) Invasive ductal carcinoma shows negative ARID1A expression (IHC, X :400) (b) Invasive ductal carcinoma showing low nuclear ARID1A expression (IHC, X :400) (c) Invasive lobular carcinoma shows diffuse and strong nuclear ARID1A expression (IHC, X :400).

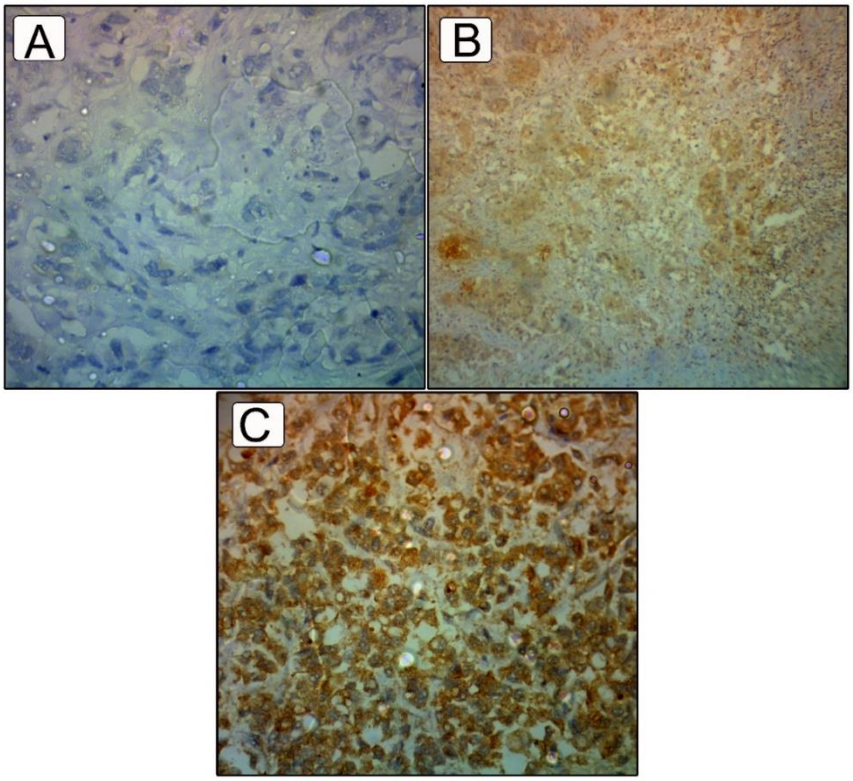

Figure 3: Photomicrograph of lobular carcinoma of the breast showing ELF3 immunohistochemical expression: (a) Invasive ductal carcinoma shows negative ELF3 expression (IHC, X:400) (b) Invasive ductal carcinoma showing low ELF3 expression (IHC, X:400) (c) Invasive lobular carcinoma shows diffuse and strong nuclear ELF3 expression (IHC, X :400).

DISCUSSION

Epithelial cells undergo a process called EMT, which is characterized by increased vimentin expression and decreased E-cadherin, to become mesenchymal cells. This occurred with the spread of many cancer types. In breast cancer, the development of a mesenchymal-like phenotype known Since oncogenic EMT is associated with pro-metastatic characteristics such heightened motility, invasion, cancer resistance, immunosuppression, and traits of cancer stem cells like self-renewal, multipotency, and treatment resistance. As a result, there is a significant amount of interaction between the fields of EMT and cancer stem cells [(17)].

Multiple cancers, including gastric cancer stomach, neuroblastoma, and cancer liver, are affected by ARID1A's effects on the EMT process. Vimentin and N-cadherin expression are increased in cancer stomach cell lines where ARID1A is silenced, which encourages both local metastasis to lymph node and distant metastasis. Recent research has shown that the JEG-3 choriocarcinoma cell line's enhanced MMP-9 protein stability is the cause of the overexpression of MMP-9 upon inhibition of ARID1A. Meanwhile, it has been discovered that reduced ARID1A increases the expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) and declines the expression of E-selectin in neuroblastoma SK-N-SH cells. However, when ARID1A is changed and EMT-related gene expression is elevated, this restriction is lifted [(18)].

As transcription regulators, the ARID family controls cellular formation, differentiation, and growth in a variety of malignancies. Inhibiting UCA1 transcription was one of ARID1A's functions like a breast cancer probable tumor-suppressor gene that worked in conjunction with CEBP; by slowing cell growth, ARID1A also improved the sensitivity of medication therapy. Breast cancer has a significant pathobiological significance for ARID1A, and partial reduction of its expression is linked to poor outcomes for patients. Breast cancer cells are made more sensible to the anticancer drug 5-fluorouracil (5-FU) through ARID1A, which also inhibits cancer cell invasion and migration [(18)].

In several female malignancies, including breast cancer, triggered estrogen receptor (ER+) functions as an oncogenic indicator by limiting tumor cell growth and regulating carcinogenesis by attracting a variety of cofactors for the estrogen response elements (EREs) that

control the transcription of genes. ARID1A controls ER-dependent transcription by attaching to ER-bound enhancers. In women with positive ER breast cancer, postulated that ARID1A wild-type expression is associated with an improved clinical consequence [19].

The loss of BAF250a in breast cancer appears to be an early event in the formation of breast malignancies, according to Zhao et al.'s 2014 finding that ARID1A encodes a big nuclear protein (BAF250a) whose expression may be downregulated throughout the growth of breast tumors [20].

The results of Mamo et al., who reported that negative of ARID1A large nuclear protein (BAF250a) is related to the onset of breast cancer by analysis on 236 cancer breast cases and corresponding normal breast tissue and adjacent preinvasive areas from cancer patients. Compared to invasive duct carcinoma (64%) and lymph nodes metastasis (80%), 37% of normal epithelial cells displayed modest nuclear staining ARID1A large nuclear protein (BAF250a). It was statistically significant that (BAF250a) nuclear expression decreased over time in different stages of breast cancer progression [21]. In our study, ARIDIA was a negative expression in 66% of invasive breast carcinoma, negative in 52% of metastatic lymph nodes, 62.2% in lymphovascular invasion and negative in 51% of perineural invasion.

Our study's According to clinicopathological analysis, histological grade II was related with negative ARIDIA expression in breast cancer (90%), 58.3% stage III, and lymph node metastasis (52.7%). While its positive, 50.1% in ER/PR (+ve), 38.2% in Her2neu (-ve) and 69.4% in high KI 67. But was not associated with tumour size.

Zhao *et al*.[20] discovered that tumor size was unrelated to low ARID1A encodes Breast cancer BAF250a expression was associated with tumor grade, metastatic lymph node, TNM stage, ER(-ve), PR(-ve), c-erbB-2(+ve), and p53(+ve)%, and not correlated with other factors as lymph node metastasis or lymph node metastasis. The low BAF250a expression, at the same time, increased with the severity of the clinical tumor stage and histological grade.

Patients who have low BAF250a expression have a worse prognosis than those with higher BAF250a expression. Reduced BAF250a expression did not constitute a distinct prognostic indicator for overall survival in the Cox proportional hazard regression model [20].

Decrease ARID1A expression was linked to lymph node metastases, mastectomy, a Ki-67 low labeling index and (-ve) p53, according to Hyun et al.'s 2015 research [22].

In contrast to some publications, some studies found no correlation between negative ARID1A expression and the prognostic variables low Ki-67 labeling index, p53 (-ve) expression and low histologic grade. According to several research, colorectal and gastric cancer may exhibit less aggressive clinicopathologic traits when ARID1A expression is lost. However, Zhang et al. discovered that in breast cancer, cases with reduced ARID1A expression were linked to ER (-ve), a larger number of p53(+) cells, Ki-67 high labelling index, and Triple-negative breast cancer[23].

DFS and OS were poorer in cases with low ARID1A expression than high ARID1A expression cases, which is consistent with a prior study [24]. Low ARID1A expression was also identified by the multivariate analysis shorter DFS and OS in breast cancer cases, as a major independent prognostic component. As a result, in individuals with breast cancer, reduced ARID1A expression may serve as a useful prognostic indicator for relapse and disease-related mortality.

Lin28 is low expressed in healthy tissues and is largely limited to embryonic stem cells. Lin28 was increased in human malignancies and worked as an oncogene to encourage malignant alteration and tumor growth [25].

According to the majority of research, cases with lymph node metastases or a proliferation index higher compared to normal tissues had down-regulated levels of the majority of let-7 family members [26].

The mesenchymal marker like vimentin was upregulated however the epithelial marker as E-cadherin was downregulated through let-7a suppression in pc-Lin28-1 and pcLin28-2 cells, indicating that Lin28 caused the EMT in breast cancer cells. Liu et al. also found that cells with Lin28 positive expression revealed a distinct spindle structure and were disconnected from one another [27].

We investigated Lin28 immunohistochemically in 120 invasive breast cancer tissue sections due to the fact that Lin28 can promote EMT, which is frequently regarded as a requirement for tumor invasion and metastasis. We discovered that, while Lin28 expression was positive (greater) in breast tumors that had spread to lymph nodes (60%), it was negative (low) in cancers of the breast that had not yet done so. Additionally, those who had invasion of lymph vessels had significantly higher Lin28 expression. metastases (78.4%) and perineural invasion (100%). This results in agreement with **Liu *et al*.**[27].

In our cases of the relation between Lin28-positive breast cancer cases and the clinicopathological status, we found that Lin28-positive breast cancer cases distinctly increased as tumor size enlarged 100% for tumors more than 50mm and staging ($p < 0.01$), similar to **Sakurai *et al.*** [28].

Lin28 is associated with ER/PR positive breast tumors or Her-2 expression and high KI 67 index regarding hormone receptors, but not associated with a high grade as well; these results have been similar to Liu et al., 2013 but with some differences regarding the grade, which was significant with Lin 28 expression but no correlation between ER or Her-2 expression and patient prognoses.

Sakurai *et al.,* and **Xie *et al.*** found that Lin28 expression was positively correlated with ER and PR status but inversely correlated with HER2 status [29].

A 57-month follow-up period revealed, according to Liu et al. (2013), that cases with low Lin28 expression levels 35 cases lived noticeably prolonged than those with high Lin28 expression levels 51 cases, with a p value of 0.047. These findings showed that Lin28 was connected to poor clinical outcomes and progressed illness in breast cancer.

Targeting Lin28 as a therapeutic method can be utilized to get rid of metastatic cells to avoid relapse and increase the patient's survival. Lin28 plays a significant role in inhibiting let-7a, EMT and origins are induced. The characteristics of several EMT-inducing transcription factors (EMT-TFs), including ZEB1/2, SNAI1/2, and TWIST, are well known. Among them the transcription factor E74-like factor 3 (ELF3) is a member of the E26 transformation-specific (ETS) family of transcription factors and is one of the prospective candidate transcription factors that may induce MET.

It is highly expressed in epithelial tissues, including the lungs, bladder, and digestive tract, which are important for differentiation and homeostasis. Additionally, it has been demonstrated to prevent EMT in several cancer types. For instance, overexpression of ELF3 in bladder cancer cells decreased invasion and mesenchymal marker expression. Similar to this, ELF3 was associated with an epithelial phenotype in ovarian cancer cells. When it was overexpressed in SKOV3 cells, invasion was inhibited, mesenchymal markers were downregulated, and epithelial markers were upregulated [30].

ELF3 levels reduced as EMT progressed and then increased when MET was induced. EMT produced by SNAIL and TGF in MCF10A breast epithelial cells. According to Subbalakshmi et al. (2013), just 12% of mesenchymal cells showed elevated ELF3 expression, compared to roughly 71% of epithelial cells. This proves that an epithelial phenotype is primarily related with increased ELF3 expression [31].

High ELF3 levels were associated with worse patient outcomes in terms of overall survival, relapse-free survival, and metastasis-free survival in breast cancer, according to Subbalakshmi et al., 2023; this finding is consistent with previous findings that ELF3 can serve as an independent prognostic marker for poor survival in hormone receptor-positive (ER+, PR+) HER2+breast cancer patients. **Yeung *et al*.** found that ELF3 expression strongly expressed in epithelium of cancer ovarian. Collectively with the statement that ELF3 expression has been associated with differentiation of epithelial cell [32].

ELF3 may also be implicated in EMT, according to Li X et al.,2016 findings, which demonstrated substantial correlations between EMT and ELF3 marker appearance. A high-grade ovarian tumor's downregulation of ELF3 expression enhances EMT, which may result in the emergence of cancer ovary with a more destructive phenotype and, consequently, a bad prognosis [33].

In our study, 90% of breast cancer cases had ELF3 negative expression, which was associated with negative expression in 81.8% of cases with lymph node metastasis, negative in 95.1 positive (ER/PR status), negative expression in 71.5% of Her2neu negative cases, high KI index, but not with tumor grade, stage, or perineural invasion.

Additionally, greater ELF3 nuclear expression was connected to better survival, according to Subbalakshmi et al., 2023 study (p 0.001). According to the molecular subtype of the disease, low ELF3 expression had a median survival time of 32 months in 52 cases, while high ELF3 expression had 69 months [32]. This suggests that depending on the molecular subtype of the disease, ELF3 may either function as a tumor promoter or suppressor.

ELF3 directly binds to and represses the transcriptional activity of ER in breast cancer that is ER-positive, indicating that it may have a tumor-suppressive function. Ectopic production of ELF3 significantly lowered the expression of ER target genes and oestrogen dependent MCF7 cell growth.

Similar to this, ELF3 mRNA expression is decreased in distant metastases and primary tumors of triple-negative breast cancer evaluated to normal breast epithelium. It's interesting to note that the stem-like or more differentiated populations of TNBC organoids showed increased ELF3 mRNA expression. Following pharmacological stimulation of TNBC cell differentiation, ELF3 mRNA was also produced, and investigations using ELF3 knockdown and overexpression showed that ELF3 was necessary for this drug's ability to promote differentiation.ELF3 was shown to be necessary for the pharmacological treatment's ability to induce differentiation by knockdown and overexpression tests, respectively [34].

On the other hand, ELF3 may have a pro-tumorigenic function in HER2 +ve malignancies while it is elevated as a result of the motivation of the ELF3 promoter by HER2-signaling. High ELF3 mRNA

expression in HER2+ breast malignancies is correlated with a poorer prediction, while ELF3 knockdown in HER2+ breast cancer cell lines inhibited tumor development by blocking AKT signaling. Additionally, HER2+ trastuzumab-resistant breast cancer cell lines showed growth inhibition after ELF3 knockdown [(35)].

CONCLUSION

In breast cancer tissues, there was an overexpression of the mesenchymal markers (ARID1A-1 and Lin23) and a decrease in the expression of the epithelial markers (ELF3). These alterations were strongly related to lymph node involvement and an advanced tumor stage. Based on molecular grounds, a targeted therapy for breast cancer can be designed to prevent tumor development and spread.

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