Original paper

Enhancers of cancer stem cells and epithelial–mesenchymal transition: Lin28, MUC1, and lipocalin-2 as a new prognostic axis in classical invasive lobular carcinoma of the breast

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Breast carcinoma is one of the most common causes of cancer-related mortality among women worldwide. The primary objective of the present study was to evaluate the expression of the epithelial-mesenchymal transition (EMT)-related markers Lin28, MUC1, and lipocalin-2 in invasive lobular carcinoma (ILC) and to investigate their correlation with clinicopathological characteristics and patient survival. This prospective cohort study included 120 classic ILC cases investigated for immunohistochemical expressions of Lin28, MUC1, and lipocalin-2 and followed them for five years or until death. The expression of markers in all tissue samples was assessed, analysed, and correlated with clinical-pathological parameters and outcomes. Lin28, MUC1, and lipocalin-2 were positively expressed in 55%, 75%, and 45%, respectively. The high expression of Lin28 and MUC1 and low lipocalin-2 were associated with poor clinicopathological characteristics and unfavourable overall survival. Lin28 and MUC1 were highly expressed in ILC and were associated with lower survival rates, poor outcomes, and a pessimistic prognosis in patients with ILC, while lipocalin-2 expression was associated with a positive outcome where its down-regulation was related to a poor prognosis in patients with ILC. Furthermore, we concluded that Lin28, MUC1, and lipocalin-2 could influence cancer behaviours, including proliferation, invasion, and migration, by regulating the EMT process and cancer stem cell criteria in ILC cells, making them potentially advantageous indicators and targeted treatments. Our research may have significant implications for understanding the pathophysiology and prognosis of ILC, which could help select treatment targets.

Key words: invasive lobular breast carcinoma, Lin28, MUC1, lipocalin-2, immunohistochemistry, survival.

Introduction

Approximately 2 million new breast cancer cases are identified each year, making it one of the most common cancers among women globally. About 10% of invasive breast cancers are invasive lobular carcinomas, while the majority are ductal carcinomas. Because exposure to female hormones is more strongly

related to invasive lobular cancer, its prevalence is more variable [1].

Although the prognosis for breast cancer has improved significantly in recent years, about 30% of patients with initial breast cancer may experience a recurrence in ten years. In these cases, the metastatic process is the fundamental cause of the lower patient survival. However, metastasis is still poorly understood; it is a multi-step process that begins with the detachment of cells from the primary site and the epithelial–mesenchymal transition (EMT). EMT remains the main pillar of the pathogenesis of tumour progression and metastasis. During this transition, epithelial cells down-regulate epithelial markers, losing polarity and intercellular adhesion, and up-regulate mesenchymal markers, gaining a fibroblast-like shape and improved motility [2].

EMT has long been associated with the invasiveness of breast cancer cells. Furthermore, activating EMT processes in transformed mammary epithelial cells improves their acquisition of invasive and metastatic characteristics and increases their creation of chemoresistant breast cancer stem cells (BCSC) [3].

Let-7/Lin28/TWIST pathway

The members of the let-7 family are antimetastatic microRNAs (miRNAs) significantly expressed in many cancers due to overexpression of their suppressor Lin28. When let-7 is degraded, TWIST oncogenes, transcription factor (TF) inducers, are up-regulated, increasing the production of TFs such as the zinc finger, the helix of the basic loop helix, and miRNAs involved in EMT; TFs purposely resist oncogene-induced senescence. Consequently, growth factors such as TGF-β can promote tumour invasion and apoptotic evasion in the advanced stages of cancer. EMT is the key to carcinomas reaching the metastatic stage [4].

Although EMT is most often associated with primary tumour invasion, it also affects the maintenance of CSCs. It has been demonstrated that when differentiated epithelial cells undergo EMT, they may acquire stem cell-like properties. Several research groups have confirmed this phenomenon by observing the co-expression of mesenchymal and stem cell markers in breast cancer cells [4].

Lin28, a highly conserved RNA-binding protein, is a reprogramming factor that induces pluripotency in adult human fibroblast cells. A growing body of evidence suggests that overexpression of Lin28 is associated with advanced human cancers and plays an important role in the survival of CSCs [5].

Mucin 1 (MUC1) is a transmembrane glycoprotein normally expressed in low concentrations on the epithelial apical surfaces. MUC1 is an altered oncoprotein in various cancer carcinomas. MUC1 can increase invasive and metastatic properties by reducing cell-to-cell adhesion and the cell's adhesion to the extracellular matrix. Therefore, MUC1 is a potential target for cancer therapy.

MUC1 is expressed at the apical border of normal epithelial cells. It protects against damage and inflammation, interacts with the receptor of tyrosine kinases and other molecules on cell surfaces, is usually located on the base side, and activates the intracellular signal pathway [6].

MUC1 promotes EMT, lineage plasticity, resistance to DNA damage, and immune evasion. Loss of homeostasis activates MUC1, causing it to form membrane-bound homodimers with EGFR and other RTKs. Endocytic trafficking transports MUC1-C homodimers to the mitochondrial outer membrane, blocking cell death. MUC1-C homodimers are imported into the nucleus by importin and interactions with the nuclear pore complex (NPC) and TF, such as WNT/β-catenin/TCF4, NF-B, NOTCH, and MYC, to drive epithelial-mesenchymal transition, and epigenetic reprogramming of cancer stem cells, making MUC1 a dependent molecule for CSCs for tumorigenicity and self-renewal [6].

Lipocalins are low-molecular-weight glycoproteins that move small lipophilic ligands and act as iron-binding proteins. They help control cell metabolism, immune response, apoptosis, prostaglandin synthesis, stabilization of matrix metalloproteinase 9, and other processes [7].

Lipocalin expression inhibits proliferation, invasion, and metastasis in vitro and in vivo by suppressing TWIST transcription, and acts as a metastasis suppressor by reversing EMT [8].

The discovery and development of anticancer drugs, particularly cytotoxic agents, is a difficult task that differs significantly from the drug development process for other diseases. Cancer treatment today has many problems, such as terrible side effects, damage to normal tissues, and resistance to anticancer drugs. Therefore, it is always beneficial to discover a new agent that could help better control cancer. Lin28, MUC1, and lipocalin-2 represent promising druggable targets to achieve this goal.

Therefore, our objective was to evaluate the immunohistochemical expression of Lin28, MUC1, and lipocalin-2 in breast ILC and its impact on clinicopathological parameters and patient outcomes.

Material and methods

Patients and methods

This study was carried out in 120 classic cases of ILC admitted to the Department of General Surgery, Faculty of Medicine, Zagazig University. After surgery, all samples were sent to the Department of Pathology of the Faculty of Medicine, Zagazig University, where they were processed for routine

H&E staining, histological diagnosis, grading using the Bloom-Scarff-Richardson score and immunohistochemistry of Lin28, MUC1, lipocalin-2, Her2, ER, PR, and KI67 immunohistochemistry. Patients were followed for treatment, detecting disease recurrence and survival rates in the Department of Clinical Oncology of the Faculty of Medicine, Zagazig University Hospital, during the period 2018–2023. Our study included patients diagnosed with invasive lobular carcinoma who were 18 years or older, and none of the patients received neoadjuvant treatment for breast cancer before surgery. The patient data, including age, tumour size, histological type, grade, LN metastasis, and lymphovascular invasion, were extracted from oncology reports. The staging was performed according to the International Union against Cancer TNM classification [8].

Ethical approval

The Institutional Review Board (IRB) of the Faculty of Medicine of Zagazig University, Egypt, granted permission to collect data and samples (ZU-IRB# 10276). The research followed the Declaration of Helsinki of the World Medical Association. All patients or their legal representatives have signed informed consent forms.

Immunohistochemistry

We used the streptavidin-biotin method. Paraffin-embedded blocks were sectioned to a thickness of 4 μm, deparaffinization was performed in a series of xylene, and rehydration was performed in descending grades of alcohol to inhibit endogenous peroxidase activity [9–11]. During microwave antigen retrieval, the sections were incubated in 0.5% hydrogen peroxide in methanol for 10 minutes [12–15]. Primary antibodies against Lin28 (rabbit polyclonal, ab218028, Abcam), MUC1 (mouse monoclonal, ab70475, Abcam), and lipocalin-2 antibody (mouse monoclonal, ab23477, Abcam) were used. Ki67 (MM1 clone, Novocastra, New Castle, UK; MIB-1 clone, Dako, Milan, Italy), HER-2/neu (dilution 1 : 50, Dako, Denmark (16-19)), PR (clone PgR 636 (20-23) Dako, Denmark) and ER (clone 1D5, Dako, Denmark) were also used. We considered ER and PR positive if positive nuclear staining was found in more than 1% of tumour cells. The Ki67 score was given as a percentage of the total number of cells positively stained from the total number of invasive cells. HER2 was scored based on criteria established by the Food and Drug Administration. Tumours were considered negative when they received a score of 0 or 1 and positive when they received a score of +2 or +3 [24].

Evaluation of immunohistochemical markers

Lin28, MUC1, and lipocalin-2 were semi-quantitatively evaluated by estimating the proportion of positive tumour cells (0, none; 1, > 0–10%; 2, > 10–50%; 3, > 50–100%). Another score represents the staining intensity (0, none; 1, weak; 2, intermediate; and 3, strong). The final score is calculated by summation of both scores; 0 is considered negative expression, 1–2 is considered low expression, 3–4 is considered moderate expression, and 5–6 is considered high expression [25–27].

Statistical analysis

We performed statistical analyses using GraphPad software (Graph Pad version 7.0). Lin28, MUC 1, and lipocalin-2 expression levels and their association with clinical and prognostic parameters were analysed using the χ^2 test. We measured disease-free survival (DFS) and overall survival (OS) rates using the Kaplan-Meier method and examined differences in survival using the logarithmic rank test.

Predictive values of these ten variables were tested using univariate and multivariate Cox proportional hazard models. All statistical tests were on both sides. $P < 0.05$ was considered statistically significant.

Results

The clinicopathological data of the 120 studied cases are summarized in Table I. The study included 120 ILC cases, 56.7% were above 50 years, 70 out of 120 patients (58.3%) were grade III, 40 cases (33.3%) were grade II, and 10 cases (8.3%) were grade I. 15% were stage I, 45% were stage II and 40% were stage III. 23.3% of the tumour samples were below 20 mm in size, 60% were between 20 and 50 mm, and 16.7% were above 50 mm. 68.3% of patients were positive for ER/PR while 15% were positive for Her2-neu. Regarding the Ki67 index, 60% of patients showed high expression. 91.7% (110/120) of patients had lymph node metastasis, and 61.7% showed lymphovascular invasion, while 18.3% showed perineural invasion.

The immunohistochemical expression of Lin28, MUC1, and lipocalin-2 in the studied patients is summarized in Table II. In the current study, cytoplasmic Lin28 expression was found in 66/120 (55%) cases (Fig. 1), membranous expression of MUC1 was found in 90/120 (75%) cases (Fig. 2), and nuclear expression of lipocalin-2 was found in 54/120 (45%) cases (Fig. 3).

The relationship between Lin28, MUC1, and lipocalin-2 expression levels in the studied patients with clinicopathological parameters and outcome is presented in Table III. There is a statistically significant relationship between Lin28 expression and tumour stage, LN metastasis, Her2, ER/PR, lymphovascular invasion, Ki67 index, and perineural invasion, while expression of MUC1 has a statistically significant relationship with lymph node metastasis and ER/PR and HER2 expression. There is a statistically signifi-

Table I. Clinicopathological data of the studied patients ($N = 120$)

Parameters	n (%)
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)
Tumour size	
≤ 20 mm	28 (23.3)
> 20–50 mm	72 (60.0)
> 50 mm	20 (16.7)
ER/PR status	
Negative	38 (31.7)
Positive	82 (68.3)
Her2-neu	
Negative	102 (85)
Positive	18 (15)
Ki67 index	
Low	48 (40)
High	72 (60)
Lymphovascular invasion	
Negative	46 (38.3)
Positive	74 (1.7)
Perineural invasion	
Negative	98 (81.7)
Positive	22 (18.3)

Table II. Expression of the three markers by immunohistochemistry ($N = 120$)

Expressed marker	n (%)
Lin28	
Negative	54 (45)
Positive	66 (55)
MUC1	
Negative	30 (25)
Positive	90 (75)
Lipocalin-2	
Low	54 (45)
High	66 (55)

cant correlation between lipocalin-2 expression, with the ER/PR Her2-neu receptor, and the histological grade. Moreover, a statistically significant relationship exists between Lin28, MUC1, lipocalin-2, and patient mortality.

The performance of the investigated markers in predicting death in the studied patients is illustrated in Table IV. Positive Lin28 expression has a positive predictive value of 81.8%, a negative predictive value of 92.6%, an accuracy of 86.7%, a sensitivity of 93.1%, and a specificity of 80.6% to predict mortality among study patients. At the same time, positive MUC1 expression can predict death in the studied patients with a sensitivity of 93.1%, a specificity of 41.9%, a positive predictive value of 60%, a negative predictive value of 86.7%, and an accuracy of 66.7%. Low lipocalin-2 can predict patient mortality with a sensitivity of 72.4%, a specificity of 80.6%, a positive predictive value of 77.8%, a negative predictive value of 75.8%, and an accuracy of 76.74%.

The degree of correlation of Lin28, MUC1, and lipocalin-2 with each other is summarized in Table V. There is a significant positive correlation between Lin28 and MUC1 levels, while there is a significant negative correlation between lipocalin-2 and both the Lin28 and MUC1 expression levels.

Survival analysis is presented in Table VI. The median survival rate of Lin28-positive patients was 34.2 months and 57.8 months for Lin28-negative patients, $p = 0.001$; moreover, 40.9 months was the median survival in positive MUC1 versus 52.8-month median survival in MUC1 negative patients, $p = 0.003$. In lipocalin-2 expression, the median survival in the low lipocalin group was 34.6 months compared to 52.8 months in the high lipocalin group, $p = 0.001$) (Fig. 4).

Univariate and multivariate analyses for overall survival (OS) are presented in Table VII. Cox regression was performed to ascertain the effects of age, grade, stage, LN metastasis tumour size, ER/PR status, Her2-neu expression, Ki67 index index, lymphovascular invasion, perineural invasion, Lin28 expression, MUC1 expression and lipocalin-2 expression on the participants' survival. The univariate analysis showed that grade, size and Lin28 expression were significantly associated with OS. A multivariate analysis that included all the significant parameters in univariate analysis revealed that only grade and cyto-

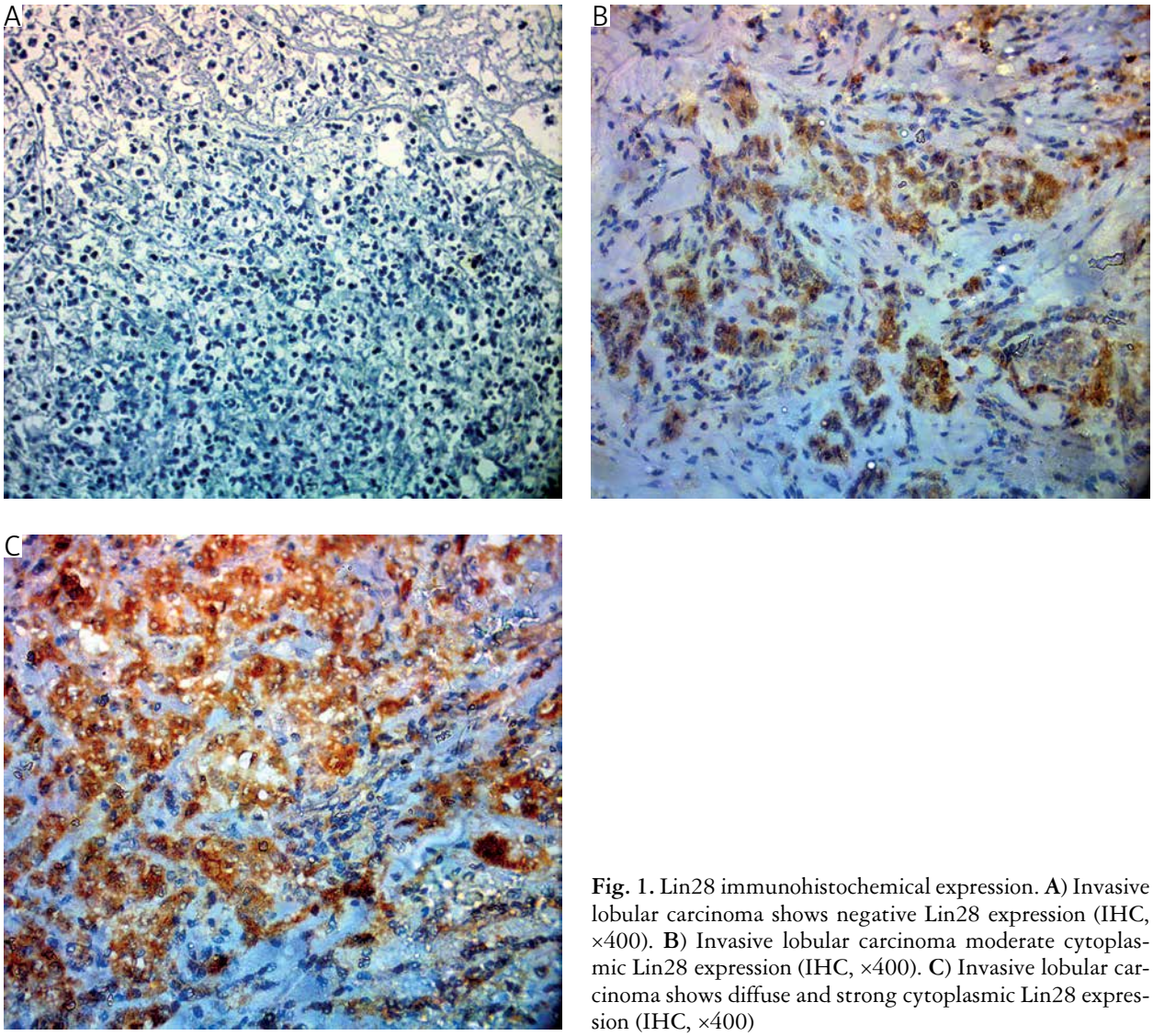

Fig. 1. Lin28 immunohistochemical expression. **A**) Invasive lobular carcinoma shows negative Lin28 expression (IHC, ×400). **B**) Invasive lobular carcinoma moderate cytoplasmic Lin28 expression (IHC, ×400). **C**) Invasive lobular carcinoma shows diffuse and strong cytoplasmic Lin28 expression (IHC, ×400)

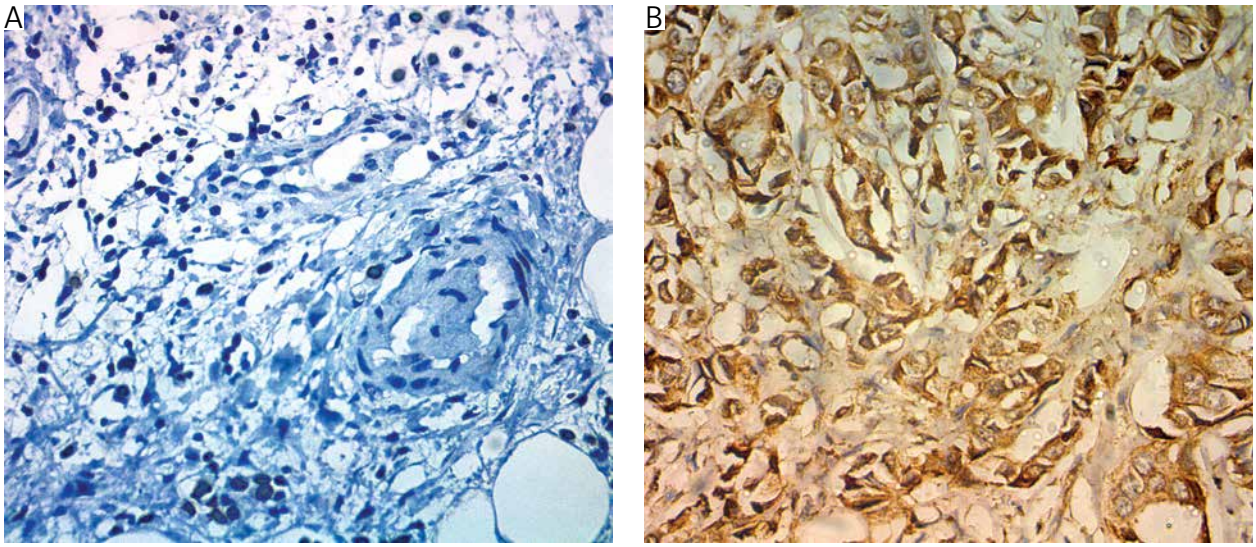

Fig. 2. MUC1 immunohistochemical expression. **A**) Invasive lobular carcinoma shows negative MUC1 expression (IHC, ×400). **B**) Invasive lobular carcinoma shows positive MUC1 expression (IHC, ×400)

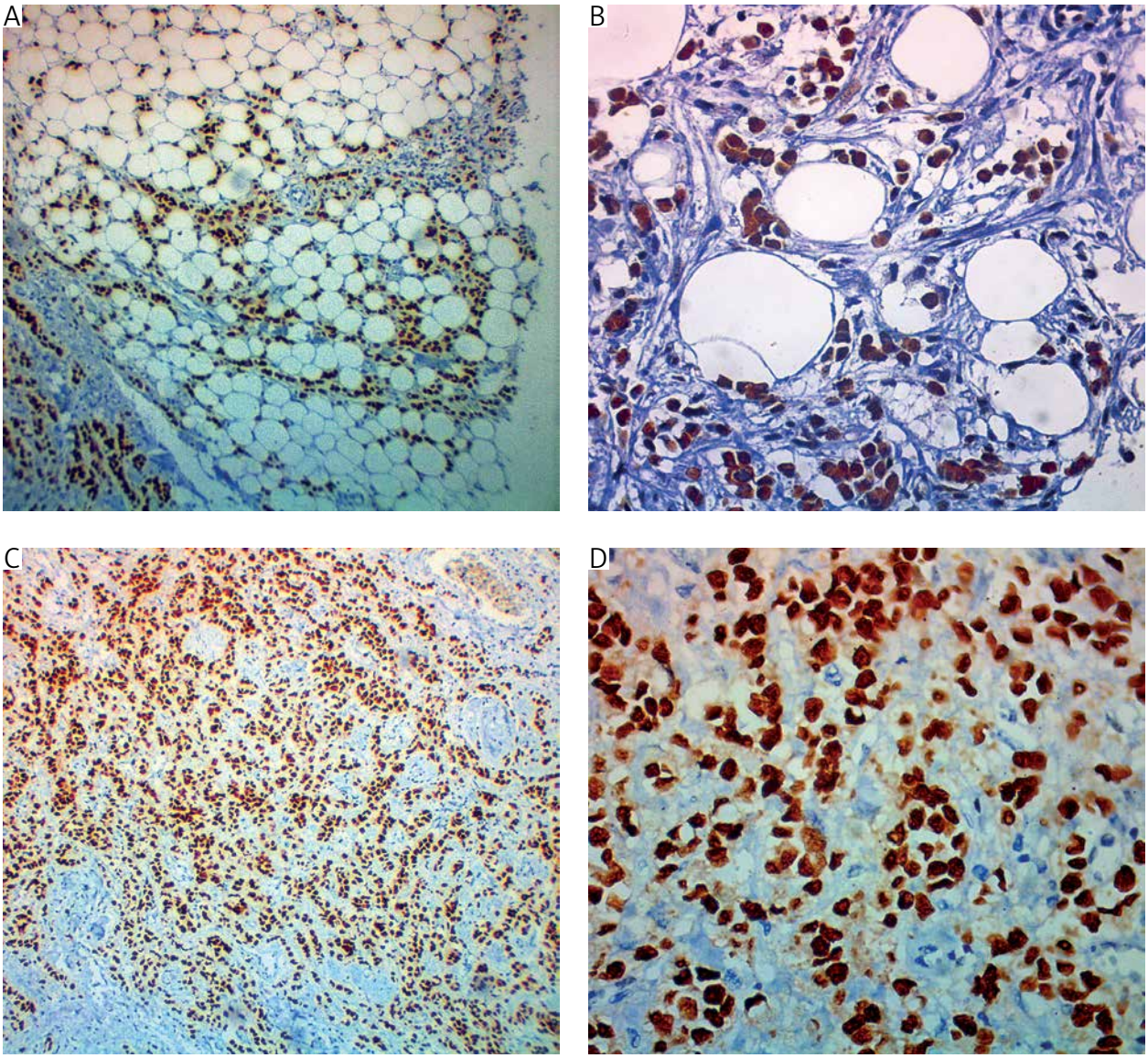

Fig. 3. Lipocalin-2 immunohistochemical expression. **A**) Invasive lobular carcinoma shows moderate nuclear lipocalin-2 expression, low power (IHC, ×100). **B**) Invasive lobular carcinoma shows moderate nuclear lipocalin-2 expression, high power (IHC, ×400). **C**) Invasive lobular carcinoma shows high nuclear lipocalin-2 expression, low power (IHC, ×100). **D**) Invasive lobular carcinoma shows high nuclear lipocalin-2 expression, high power (IHC, ×400)

plasmic Lin28 expression were independently associated with shorter OS.

Discussion

In our study we tried to understand the pathophysiology and prognosis of ILC, which could help in selecting target therapy. We found that Lin28 and MUC1 were highly associated with poor clinicopathological parameters and negative outcomes in ILC while lipocalin-2 expression was associated with good clinicopathological parameters and positive outcomes.

Classic invasive lobular carcinoma is the most common variant of ILC, consisting of uniform cells with round nuclei and small nucleoli. They also have a discohesive architecture with a linear growth pattern; moreover, a variable number of ILC cells release intracytoplasmic mucin; most ILCs express ER and PR receptors but lack Her2 neu amplification [28].

However, a variety of factors affect the development of metastases, which determine tumour growth, angiogenesis, survival, and invasion. Concerning epithelial neoplasms, EMT and the presence of cancer stem cell populations are considered crucial events in the metastatic process; the overexpression of CSC and EMT markers in tumour cells of metastatic breast cancer supported that [29].

The term EMT refers to a dynamic process marked by variations in the cell phenotype between the epithelial and mesenchymal state. EMT plays a vital role in the invasion process, in which epithelial cells lose

Table III. Relationship between expression levels of the three markers, clinicopathological parameters, and outcome

	Total	Lin28		*p*	MUC1		*p*	Lipocalin-2		*p*
		Negative	Positive		Negative	Positive		High	Low	
		n = 54 (%)	*n* = 66 (%)		*n* = 30 (%)	*n* = 90 (%)		*n* = 66 (%)	*n* = 54 (%)	
Age group										
≤ 50 years	52	15 (57.7)	22 (42.3)	0.117	20 (38.5)	32 (61.5)	0.069	30 (57.7)	22 (42.3)	0.796
> 50 years	68	12 (35.3)	44 (64.7)		10 (14.7)	58 (85.3)		36 (52.9)	32 (47.1)	
Grade										
I	10	0 (0)	10 (100)	0.1663	0 (0)	10 (100)	0.109	10 (100)	0 (0)	< 0.001
II	40	20 (50)	20 (50)		8 (20)	32 (80)		36 (90)	4 (10)	
III	70	34 (48.6)	36 (51.4)		22 (31.4)	48 (68.6)		20 (28.6)	50 (71.4)	
Stage										
I	18	14 (77.8)	4 (22.2)	< 0.001	2 (11.1)	16 (88.9)	0.751	8 (44.4)	10 (55.6)	0.519
II	54	34 (63)	20 (37)		20 (37)	34 (63)		30 (55.6)	24 (44.4)	
III	48	6 (12.5)	42 (87.5)		8 (16.7)	40 (83.3)		28 (58.3)	20 (41.7)	
LN metastasis										
Negative	10	10 (100)	0 (0)	0.014	10 (100)	0 (0)	< 0.001	8 (80)	2 (20)	0.367
Positive	110	44 (40)	66 (60)		20 (18.2)	90 (81.8)		58 (52.7)	52 (47.3)	
Tumour size (mm)										
≤ 20	28	26 (92.9)	2 (7.1)	< 0.001	4 (14.3)	24 (85.7)	0.347	18 (64.3)	10 (35.7)	0.252
> 20–50	72	28 (38.9)	44 (61.1)		20 (27.8)	52 (72.2)		40 (55.6)	32 (44.4)	
> 50	20	0 (0)	20 (100)		6 (30)	14 (70)		8 (40)	12 (60)	
ER/PR status										
Negative	38	32 (84.2)	6 (15.8)	< 0.001	26 (68.4)	12 (31.2)	< 0.001	30 (78.9)	8 (21.1)	0.013
Positive	82	22 (26.8)	60 (75.2)		4 (4.9)	78 (95.1)		36 (43.9)	46 (56.1)	
Her2-neu expression										
Negative	102	53 (51.9)	49 (48)	< 0.001	29 (28.4)	73 (71.5)	0.031	63 (61.7)	39 (38.2)	< 0.001
Positive	18	1 (5.6)	17 (94.4)		1 (5.6)	17 (94.4)		3 (16.6)	15 (83.3)	

Table III. Cont.

	Total	Lin28		p	MUC1		p	Lipocalin-2		p
		Negative	Positive		Negative	Positive		High	Low	
		N = 54 (%)	N = 66 (%)		N = 30 (%)	N = 90 (%)		N = 66 (%)	N = 54 (%)	
Ki67 index										
Low	48	30 (62.5)	18 (37.5)	0.035	22 (45.8)	26 (54.2)	0.054	32 (66.7)	16 (33.3)	0.008
High	72	24 (33.3)	48 (66.7)		8 (22.2)	64 (77.8)		22 (30.6)	50 (69.4)	
Lymphovascular invasion										
Negative	26	38 (82.6)	8 (17.4)	< 0.001	22 (39.1)	24 (60.9)	0.066	20 (43.5)	26 (56.5)	0.189
Positive	74	16 (21.6)	58 (78.4)		8 (16.2)	66 (83.8)		46 (62.2)	28 (37.8)	
Perineural invasion:										
Negative	98	54 (55.1)	44 (44.9)	< 0.001	28 (28.6)	70 (71.4)	0.262	50 (51)	48 (49)	0.315
Positive	22	0 (0)	22 (100)		2 (9.1)	20 (90.9)		16 (72.7)	6 (27.3)	
Death										
Yes	58	4 (6.9)	54 (93.1)	< 0.001	4 (6.9)	54 (93.1)	0.002	16 (27.6)	42 (45.5)	< 0.001
No	62	50 (80.6)	12 (19.4)		26 (41.9)	36 (58.1)		50 (80.6)	12 (29.4)	

their apicobasal polarity, reconstruct cytoskeletal elements, and develop the ability to move and invade the extracellular matrix through a complicated molecular and cellular process. The epithelial-mesenchymal transition has been found to support therapeutic resistance and the metastatic cascade [30].

Cancer stem cells (CSCs) have been demonstrated to be cells inside the tumour responsible for tumour relapse as they have self-renewal and drug resistance characteristics; increasing knowledge of CSCs and the tumour microenvironment improves understanding of cancer immunity mechanisms [31].

Lin28 is up-regulated in breast cancers through canonical and non-canonical pathways; Lin28 overexpression can enhance cancer cell growth, cancer cell metastasis, metabolism reprogramming, cancer stem cell proliferation, and resistance to radiation and chemotherapy in different breast cancers [32]. A better understanding of the Lin28-related regulatory network is needed; doing so would open up a fascinating new area of research and provide a valuable target for future breast cancer therapeutic strategies.

The present study showed that Lin28 overexpression was significantly correlated with poor clinicopathological characteristics and all prognostic parameters, including tumour size, advanced stage, LN metastasis, negative expression of ER and PR, high Ki67 index, and Her2-neu expression. Meanwhile, high Lin28 was not related to age, perineural invasion, or tumour grade. As it was the first time for Lin28 expression to be examined in ILC, we discussed Lin28 expression results with its expression in the most closely related tumour: infiltrating duct carcinoma.

Xie *et al*. [33] found that Lin28 expression correlates with aggressive clinicopathological characteristics in breast invasive ductal carcinoma. Xu *et al*. [34] found that Lin28 was positive in 37.1% (108 out of 291) and was related to LN metastases ($p = 0.001$), HER-2neu ($p = 0.024$), ER ($p = 0.039$) and PR ($p = 0.027$). In agreement with our results, Xie *et al*. [35], when studying Lin28 expression in 190 breast cancer cases, found that 43.7% of the cases were positive, associated with high grade, stage and Ki67, as was also confirmed by Shen and his co-workers [36]. Furthermore, patients with positive expression of Lin28 showed lower OS rates than patients with negative patients; similarly, Xu *et al*. [34] found that Lin28 expression was correlated with lower OS rates.

In this study, MUC1 was expressed in 75% of patients with classic ILC and was associated with lymph node metastases, a lack of ER/R, and high Her2 neu expression. Similarly, Croce *et al*. [37] found that 80% of patients with ILC showed positive expression of MUC1; our results were also consistent with Jing *et al*., McGuckin *et al*., Atta Manu *et al*. and Stergiou *et al*. [38-41], reporting that MUC1 was overexpressed in invasive lobular carcinoma and was asso-

Table IV. 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 MUC1	93.1%	41.9%	60%	86.7%	66.7%
Low lipocalin-2	72.4%	80.6%	77.8%	75.8%	76.7%

Table V. Correlation between Lin28, MUC1 and lipocalin-2

	Lin28		MUC1		Lipocalin-2	
	Phi	*p*	Phi	*p*	Phi	*p*
Lin28			+0.638	< 0.001	–0.818	< 0.001
MUC1	+0.638	< 0.001			–0.522	< 0.001
Lipocalin-2	–0.818	< 0.001	–0.522	< 0.001		

ciated with a poor prognosis and decreased survival, as it was significantly related to the presence of LN metastases, the presence of ER/PR receptors, reduced overall survival, low disease-specific survival, and low relapse-free survival time. In accordance with our results, Chang *et al*. [42] and Atta Manu *et al*. [40] found that MUC1 was significantly correlated with Her2 neu expression. Some studies reported different expression of MUC1 in ILC patients; Chu *et al*. [43] found that 66.6% (4/6) of cases of ILC were positive for MUC1, while Chang *et al.* [42] reported 100% (8/8) cases of ILC, which may be due to the small number of ILC cases studied. Rakha *et al*. [44] has clarified that the prognostic effect of MUC1 depends on the subcellular localization of its expression, and the cytoplasmic/membranous localization of MUC1 was associated with poor outcomes compared to the apical localization, which is the normal physiological site of MUC1, which was associated with favourable clinicopathological outcomes. This makes MUC1 attractive as a potential target for immunotherapy [45].

Lipocalin-2 is a glycoprotein released by various types of cancer, and recently, the oncological role of lipocalin-2 in pathogenesis and metastasis has been studied in several types of malignancies; it was identified as negatively related to EMT signalling in the development of cancer. Lipocalin-2 inhibits proliferation, invasion, and metastasis through transcriptional suppression of Twist1 and matrix metalloprotease-2. Therefore, lipocalin-2 is considered a potential metastasis suppressor because of its ability to reverse the EMT; therefore, lipocalin-2 could be a potential therapeutic target [7, 27, 46, 47]. Lipocalin-2, which was dependent on activation of the AKT and c-Jun pathway [35, 36], also caused a significant reduction in the percentage of cancer stem cell populations.

The results of our study showed that 45% of the cases showed low lipocalin-2 nuclear expression. The nuclear immunoreactivity of lipocalin-2 from invasive lobular carcinoma cells was lower than that of adjacent normal epithelial cells. Lipocalin-2's low nuclear expression is closely associated with high tumour proliferation, negative expression of hormonal receptors, positive HER2, high histological grade, short OS, and poor prognostic outcome.

Similarly, Kurozumi *et al*. [48], Villodre *et al*. [49] Bauer *et al*. [50] proved that low LCN2 expression was correlated with negative ER/PR, high Ki67 index, HER2neu positivity, high histological grade and tumour size, poor clinicopathological parameters and poor outcome in invasive breast cancer. LCN2 can be used as a potential therapeutic target for breast cancer metastases since it negatively regulates cell proliferation and EMT by controlling the expression of metabolic genes, angiogenesis, cell migration and invasion.

Conclusions

Lin28 and MUC1 were highly expressed in ILC and were associated with lower survival rates, poor outcomes, and a pessimistic prognosis in patients with ILC, while lipocalin-2 expression was associated with a positive outcome where its down-regulation was related to a poor prognosis in patients with ILC. Furthermore, we concluded that Lin28, MUC1, and lipocalin-2 could influence cancer behaviours, including proliferation, invasion, and migration, by regulating the EMT process and CSC criteria in ILC cells, making them potentially advantageous indicators and targeted treatments. Our research may have significant implications for understanding the pathophysiology and prognosis of ILC, which could help select treatment targets.

Table VI. Kaplan-Meier survival curves illustrating survival time differences in patients as regards marker expression

	Total *N*	*N* of Events	Censored		Survival time, months				OS rate, %	*p*
					Mean		Median			
			N	%	Estimate ± SE	95% CI	Estimate ± SE	95% CI		
Lin28										
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	
MUC1										
Positive	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
Negative	30	4	26	86.7	52.8 ± 2.1	48.6-57.0	NR		86.4	
Lipocalin-2										
Low	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
High	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	

Fig. 4. Kaplan-Meier plot for overall survival concerning different marker expression. **A**) Lin28 expression, **B**) MUC1 expression and **C**) lipocalin-2 expression

Table VII. 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. ≥ 50 years	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			
Ki67 index, H vs. L	0.274	0.7	0.335-1.363			
Lymphovascular 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
Membranous MUC 1 expression, N vs. P	0.934	1.0	0.494-2.155			
Lipocalin-2 expression, N vs. P	0.067	1.9	0.956-3.697			

Disclosures

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