ALDH1 expression in inflammatory breast cancer tumor using Real-time RT-PCR gene expression quantifications: Moroccan prospective study

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Abstract

Inflammatory breast cancer (IBC) is a rare but aggressive type of breast carcinoma. There are few diagnostic and prognostic biomarkers in IBC. Aldehyde dehydrogenase 1 (*ALDH1*) is a putative stem cell marker in a variety of cancers. Our study aims to investigate the expressions of the *ALDH1* in a cohort of IBC tumors and to assess the relationship of their expression with *Notch1* and some clinic-pathological characteristics.

Methods: We investigated the status of *ALDH1* and *Notch1* in 81 female IBC cases. Paraffin-embedded tumor tissues (FFPE: formalin-fixed paraffin embedded) were obtained from all cases and to mRNA was extracted from FFPE tissue. Pure RNA was used for the synthesis of cDNA, which was used for quantitative real-time RT-PCR (qRT-PCR) analysis of relative expression levels of target genes.

Results: Positive *ALDH1* (*ALDH1*+) and *Notch1* (*Notch1*+) expression was observed in 69 (85%) of 81 IBC cases. Of the *ALDH1*+ tumours, 29 (34.9%) were of the luminal subtype, 21 (25.3%) were in the HER2 subtype and one (1.5%) was in the unclassified category. In addition, 32 (38.2%) were triple-negative tumours. The majority 50 (60.2%) of *ALDH1*+ cases were high-grade ductal carcinomas. *ALDH1* expression is significantly correlated with age, response treatment and *Notch1* expression ($p < 0.05$). Interestingly, statistic analysis using *Khi2* test shows that the positively *ALDH1* and *Notch1* have an interactive effect IBC cases ($p < 0.05$).

Conclusion: This finding reflects that the assessment of *ALDH1* expression may have a potential role in incidence of IBC and be helpful tool in the future anticancer strategies.

Keywords: Inflammatory breast cancer, *ALDH1*, *Notch1*, qRT-PCR, North Africa

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Introduction

Breast cancer is the most common malignancy among women both in Morocco and the North Africa (including Morocco, Algeria, Tunisia, Libya, Tunisia, and Egypt), representing 25% to 35% of all female cancers¹⁾. Inflammatory breast cancer (IBC) is the most aggressive form of locally-advanced breast cancer. IBC constitutes a cluster with high incidence in Morocco, Algeria and Tunisia compared to occidental countries, accounting 6–10% of all invasive breast tumors in North Africa²⁻⁴⁾. In the United States, IBC accounts for a disproportionate number of breast cancer deaths (7%) given that it constitutes only approximately 2% of breast cancer cases⁵⁾. IBC

is characterized by rapid progression, local and distant metastases, younger age of onset, and lower overall survival compared with other breast cancers⁶⁾. Historically, IBC is a lethal disease with less than a 5% survival rate beyond 5 years when treated with surgery or radiation therapy. Because of its rarity, IBC is often misdiagnosed as mastitis or generalized dermatitis^{6,7)}. Although primary IBC is less commonly diagnosed than other types of breast cancer, IBC is responsible for a disproportionate number of breast cancer-related deaths that occur each year world-wide⁵⁾. Due to tumor infiltration of the dermal lymphatics, which contributes to rapid engorgement of the breast and clinical hallmarks of inflammation, the patients develop rapid onset of characteristic skin changes from the time of confirmed diagnosis. Thus, it is crucial to make an accurate diagnosis and start rapid approach to treatment for this fatal malignancy^{8,9)}.

A number of molecular changes have been described

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in IBC, including aldehyde dehydrogenase 1 (*ALDH1*) positivity, CD44 positivity, CD24 negativity, *RhoC* over-expression, hypomethylation of caveolin promoters, and deletion of some tumor suppressors¹⁰⁻¹⁴. A number of studies have reported that patients with higher expression of *ALDH1* have poor prognosis in several cancers including IBC¹⁵. *ALDH1* is an aldehyde dehydrogenase, responsible for oxidation and metabolism of intracellular aldehydes into their corresponding carboxylic acids, thereby protecting stem cells against oxidative damage¹⁶. *ALDH1* is produced in both the central nervous system and other tissues; in addition to participating in acetaldehyde metabolism, this enzyme is involved in the synthesis of retinoic acid, a precursor of vitamin A. While there are three different isoforms for *ALDH1* (*ALDH1A1*, *ALDH1A2*, and *ALDH1A3*), *ALDH1* activity is predominantly attributed to *ALDH1A1* isotype. Previous study suggested that the behavior of IBC could be mediated by the expression of *ALDH1* in Cancer stem cells (CSCs). *ALDH1* expression was the first independent prognostic marker that predicted metastasis and poor patient outcome in IBC¹⁵.

The *Notch* signaling pathway is one of the most commonly activated signaling pathways in cancer. Alterations include activating mutations and amplification of the *Notch* pathway, which play key roles in the progression of cancer¹⁷⁻¹⁹. Among *Notch* receptor with potential roles in breast carcinogenesis, *Notch1* is relatively the best studied. Studies have reported that mutations in *Notch1* prevent functioning of domains that are essential to prevent spontaneous activation of *Notch*. These mutations lead to increase in *Notch* signaling which in turn leads to deregulation of the signaling pathway²⁰.

This study aimed to investigate *ALDH1* expression, using real-time RT-PCR (qRT-PCR) in IBC tumors and to evaluate of its expression with clinical outcome and survival data in IBC Moroccan women.

Materials and methods

Patients and tissue specimens

A total of 83 patients with IBC from the medical oncology departments at the National Institute of Oncology in Rabat were recruited prospectively, according to the American Joint Committee on Cancer (AJCC). IBC is designated as T4d in the AJCC tumor, node, and metastasis staging system and as clinically defines it as "a clinicopathologic entity characterized by erythema occupying at least one-third of the breast, edema and/or orange peel of the breast, and/or a warm breast, a palpable mass is present in only a third of cases. The onset of these signs and symptoms should be rapid; the length of the time taken during its initial presentation should be 3 months"²¹. The diagnosis of IBC was confirmed clinically at least by two oncologists (H.E; N.B). Standardized

structured questionnaire was developed and used for the interviewed with patients. Demographic and pathologic profiles including age, histological grade, T stage, N stage, ER(estrogen receptor)/PR(progesterone receptor)/HER(human EGF receptor) 2 status, and treatments were recorded. FFPE tumors tissues were obtained from 81 cases. None of the patients had received any previous treatments including neo-adjuvant chemotherapy or radiotherapy.

Informed consent was obtained from all participating patients and the study was carried out with the approval of Ethical Review Committee of Faculty of Medicine and Pharmacy of Rabat.

Immunohistochemistry

ER status, PR status, and HER2 status were determined using immunohistochemical (IHC) analysis. Briefly, IHC analysis to determine ER and PR status was performed using standard procedures on 4- μ m sections of paraffin embedded tissue specimens stained with the monoclonal antibodies 6F11 and 1A6 for ER and PR, respectively. Nuclear staining 10% was considered a positive result. The Hercept test was realized in the institute since 2007 and was determined for all patient during the course of this study. Assays are scored with a 4-tiered system (0 - 3+). HER2 positivity was defined as strong complete membrane staining in at least 10% of tumor cells. Patients were considered HER2-positive if they had IHC 3+ by herceptest (DAKO, Agilent technologies, Sant Clara, CA, USA). Tumors exhibiting equivocal HER-2 expression, denoted as 2+ membranous staining of tumor cells, are confirmed by fluorescence in situ hybridization (FISH) at an outside laboratory. A signal ratios (HER2: CEP17) of ≥ 2.2 were classified as amplified. In the absence of positive FISH data, tumors scored 2+ by IHC were considered negative for HER-2.

Quantitative real time RT-PCR analysis

RNA extraction and cDNA synthesis

Total RNA was extracted from FFPE using Pure LinkTM FFPE Total RNA Isolation Kit (Invitrogen, Carlsbed, CA, USA) according to manufacturer's instruction. Total RNA was eluted and dissolved in RNase-free water. All samples were treated with DNase I. Amplification Grade (Invitrogen Carlsbed). RNA concentration measurements by fluorimetry were done on Qubit 2.0 fluorometer using Qubit[®] RNA Assay Kit (Thermo-Fischer Scientific, Waltham, MA, USA). For the first-strand cDNA synthesis, the SuperScriptTM First-Strand Synthesis System for RT-PCR Kit (Invitrogen), Random Hexamer and 2 μ g of total RNA were used. The synthesized cDNA was used for qRT-PCR analysis of relative expression levels of target genes.

Real-time RT-PCR

qRT-PCR were performed in technical triplicates

starting with the same cDNA using the Power SYBR Green PCR Master Mix (Thermo Fischer Scientific) and an ABI 7500® Fast Real-Time PCR (Thermo Fischer Scientific). cDNA was combined with primer sets and the Applied Biosystems™ Power SYBR™ Green PCR Master Mix. Each sample was normalized to glyceraldehyde phosphate dehydrogenase (*GAPDH*). The primers used were as follows: *ALDH1* forward 5'-TCCTGGTTATGGGCCTACAG-3', *ALDH1* reverse 5'-CTGGCCCTGGTGGTAGAATA-3'; *Notch1* forward 5'-GACCTCATCAACTCACACGC-3', *Notch1* reverse: 5'-CGGCATCCACATTGTTCA-3'; *GADPH* forward 5'-TGATGACATCAAGAAGGTGGTGAAG-3', *GADPH* reverse: 5'-TCCTTGGAGGCCATGTGGGCAT-3'. The following conditions were used: an initial denaturation at 95°C for 10 min followed by denaturation at 95°C for 15 s and extension at 60°C for 1 min for a total of 40 cycles. PCR products were analyzed on a 2% agarose gel. Amplification products were tested for specificity by melting curves. The relative expression levels of targets were calculated by the $2^{-\Delta\Delta CT}$ method (method to compute relative gene expression from quantitative real-time PCR Data).

Statistical analysis

All calculations were performed using SPSS V.20.0 statistical software. Quantitative variables were presented as means and standard deviations and qualitative variables by percentage and effective for description. The correlations between clinicopathologic parameters and *ALDH1* expression or *Notch1* were examined using a chi-square test for categorical variables and Student's t test for continuous variables. Variance (ANOVA) test was applied where appropriate. The Kaplan-Meier method was used to plot survival curves and the difference in survival time were compared by the log rank test. A $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

Clinical and pathological characterization of the IBC cases (n = 81) are summarize in Table 1. All patients were female and 50 (60.3%) of which were younger than 50 years old. Their median age at diagnosis was 48 (SD: 11.3) years with a range of 26–82 years old. Most of the women had a pre-menopausal status 51 (61%) and 17 (29.5%) were nulliparous. Obesity defined by BMI ≥ 30 was seen in 49 (59%) of cases. Tumor grade was II and III in 48 (57.8%) and 33 (39.8%) of cases, respectively. The histopathologic types of the IBC were invasive ductal carcinoma 78 (94%), while 2 (2.4%) were infiltrating lobular carcinoma and 3% as mixed types or other histology. Patients with positive axillary lymph nodes are

Table 1 Clinico-pathologic characteristics of cases with IBC

Variable	Number of cases	Percent (%)
Age of patients		
≤50 years	53	63.9
>50 years	30	36.1
Tumor Stages		
Stage III	59	71.1
Stage IV	24	28.9
Tumor Grades		
Grade II	50	60.2
Grade III	33	39.8
Tumor Types		
IDC	78	94
ILC	2	2.4
Other	3	3.6
Lymph node involvement or metastasis		
Negative	23	27.7
Positive	58	72.3
ER expression		
Negative	37	54.2
Positive	45	44.6
Not specified	01	1.2
PR expression		
Negative	42	50.6
Positive	40	48.2
HER2 expression		
Negative	51	63.5
Positive	27	35
No specified	01	1.5

IDC: Invasive Ductal Carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen Receptor, PR: Progesterone Receptor, HER2: Epidermal growth Factor Receptor 2.

Table 2 Clinico-pathologic characteristics of cases with IBC (continued)

Variable	Number	Percent (%)
molecular subtype		
Luminal	29	34.9
HER	32	38.3
TNBC	21	25.3
Not specified	01	1.5

TNBC: Triple negative breast cancer

similar to patients with negative axillary lymph nodes at the time of diagnosis. By standard IHC assay, 45% of the tumors showed positivity for ER, 40% for PR, and 31% for HER2. Luminal subtype and triple negative breast cancer were identified in 29 (34.9%) and 21 (25.3%) patients, respectively.

Expression of *ALDH1* in IBC tumor tissues

All specimens were categorized according to *ALDH1* and *Notch1* expression status using qRT-PCR as described in the materials and methods paragraph. There was a high prevalence of positive *ALDH1* (*ALDH1*+) and *Notch1* (*Notch1*+) expression among 69 (85.2%) and 69 (85.2%) cases, respectively (Table 2). Among the *ALDH1*+ tumors, 36 (44.4%) showed slight, 18 (22.2%) moderate, and 15 (18.5%) strong *ALDH1* expression. For *Notch1*+, 25 (30.8%) showed slight, 18 (22.2%) moderate, and 26 (32.1%) strong expression.

Table 3 Measurement of *ALDH1* and *Notch1* mRNA with qRT-PCR

Gene	expression			
	-	+	++	+++
<i>ALDH1</i>	12 (14.5%)	36 (43.4%)	18 (21.7%)	15 (18.1%)
<i>Notch1</i>	14 (16.9%)	23 (27.7%)	17 (20.5%)	27 (32.5%)

-: Negative expression, +: slight expression, ++: moderate expression, +++: strong expression

Table 4 Correlation analyses of *ALDH1* and *Notch1* expression and clinic-pathological parameters in IBC cases

Variable	<i>ALDH1</i> status		<i>p</i>	<i>Notch1</i> status		<i>p</i>
	Negative 15.7%	Positive 84.3%		Negative 18.1%	Positive 81.9%	
Age of patients						
≤50 years	5 (38.5%)	48 (68.6%)	0.041	7 (46.7%)	46 (67.6%)	0.12
>50 years	8 (61.5%)	22 (31.4%)		8 (53.3%)	22 (33.4%)	
Tumor Stages						
Stage III	11 (84.6%)	48 (68.6%)	0.2	8 (53.3%)	51 (75%)	0.08
Stage IV	2 (15.4%)	22 (31.4%)		7 (46.7%)	17 (25%)	
Tumor Grades						
Grade II	8 (61.5%)	42 (60%)	0.4	9 (69%)	41 (60.3%)	0.7
Grade III	5 (38.5%)	28 (40%)		6 (40%)	27 (39.7%)	
Tumor Types						
IDC	10 (76.9%)	68 (97.1%)	0.02	15 (100%)	63 (92.6%)	0.27
other	3 (23.1%)	2 (2.9%)		0 (%)	5 (7.4%)	
Lymph node involvement or metastatic						
Positive	10 (76.9%)	48 (70.6%)	0.46	8 (57.1%)	50 (74.6%)	0.18
Negative	3 (23.1%)	20 (29.4%)		6 (42.9%)	17 (25.4%)	
ER expression						
Negative	6 (46.2%)	31 (44.9%)	0.58	5 (33.3%)	32 (47.8%)	0.3
Positive	7 (53.8%)	38 (55.1%)		10 (66.7%)	35 (52.2%)	
PR expression						
Negative	9 (69.2%)	33 (47.8%)	0.15	5 (33.3%)	37 (55.2%)	0.12
Positive	4 (30.8%)	36 (52.2%)		10 (66.7%)	30 (44.8%)	
HER2 expression						
Negative	8 (61.5%)	46 (67.6%)	0.003	8 (53.3%)	46 (69.7%)	0.2
Positive	5 (38.5%)	22 (32.4%)		7 (46.7%)	20 (30.3%)	

IDC: Invasive Ductal Carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen Receptor, PR: Progesterone Receptor, HER2: Epidermal growth Factor Receptor 2.

Table 5 Correlation analyses of *ALDH1* and *Notch1* expression and clinicopathological parameters in IBC cases (continued)

Variable	<i>ALDH1</i> status		<i>p</i>	<i>Notch1</i> status		<i>p</i>
	Negative (%)	Positive (%)		Negative (%)	Positive (%)	
molecular subtype						
Luminal	4 (30.8%)	25 (36.8%)	0.81	3 (20%)	26 (39.4%)	0.149
HER	6 (46.2%)	25 (46.2%)		9 (60%)	22 (33.3%)	
TNBC	3 (23.1%)	18 (26.5%)		3 (20%)	18 (27.3%)	

TNBC: Triple negative breast cancer

We divided the 81 patients into four groups based on *ALDH1* and *Notch1* statut. Group 1 samples were negative for expression of both *ALDH1* and *Notch1* (*ALDH1*-/*Notch1*-), group 2 samples were negative for *ALDH1* and positive for *Notch1* (*ALDH1*-/*Notch1*+), group 3 samples were positive for *ALDH1* and negative for *Notch1* (*ALDH1*+/*Notch1*-), and group 4 samples were positive for both *ALDH1* and *Notch1* (*ALDH1*+/*Notch1*+). There were 1 (1.3%), 11 (13.5%), 11 (13.5%), and 58 (71.7%) patients in groups 1, 2, 3, and 4, respectively (Table 3).

Correlation *ALDH1* clinicopathological features

Of the *ALDH1*+ tumours, 29 (34.9%) were of the luminal subtype, 21 (25.3%) were in the HER2 subtype

and one (1.5%) was in the unclassified category. In addition, 32 (38.2%) were triple-negative tumours. The majority 50 (60.2%) of *ALDH1*+ cases were high-grade ductal carcinomas. The correlation between expression of *ALDH1* and the clinicopathologic features of the tumors is summarized in Table 3. *ALDH1*+ correlated significantly with age ($p = 0.04$), Tumor Types ($p = 0.02$), and HER2 expression ($p = 0.003$), the combined *ALDH1*+ and *Notch1*+ group was significantly correlated with tumor stages ($p = 0.008$), interestingly, statistic analysis using the *Khi-2* test shows that the positively *ALDH1* and *Notch1* expressions have an interactive effect in IBC cases ($p < 0.05$) (Tables 4 and 5).

Discussion

Inflammatory breast cancer (IBC) is the most lethal form of locally advanced breast cancer. Although categorized as a rare disease, IBC accounts for more than 15% of breast cancer related deaths^{22, 23}. IBC is a designated health disparity due to its increased incidence and mortality amongst minority populations both in the US and globally²⁴. The comprehensive study of the epidemiology of IBC has been of great interest to a number of investigators, but epidemiological research has been hampered by (i) the use of inconsistent diagnostic criteria (ii) the lack of an agreed upon case definition and the relatively small number of patients available to any single investigator or institution²⁵, making it difficult to obtain a large sample size of IBC cases and to evaluate results across studies. On February 28th, 2018, which marked World Rare Disease Day, the Duke Consortium for IBC held its inaugural meeting and focused on three sessions: i) global partnership in the clinical management of IBC, ii) research challenges and opportunities in IBC, and iii) engaging advocates and community partners to improve IBC research and education. There is a clear need for collaboration to develop strategic plans and programs for addressing issues surrounding the disease. Measures to improve early detection and diagnosis may have the most benefit when considering prognosis, survival, and treatment costs to patients²⁶.

In order to perform a comprehensive genomic profiling of IBC tumors, a number of genetic variants have been examined globally. In some parts of the North Africa region, biological aspects of IBC show important similarities and differences^{27, 28}. Egyptian IBC tumors had the highest *RhoC* expression (87%) than Tunisians and Moroccan. *Rho* was over-expressed in 50–68% and 38.1% in Tunisian and in Moroccan tumors, respectively²⁸. Using mRNA expression, *RhoC* was expressed in 83% of Tunisian patients²⁷. *Wisp3* gene was studied only in Tunisian population and showed a positive immunohistochemistry staining in 17% and mRNA expression in 26.8% of IBC cases²⁹. In the present study, elevated expression level of *ALDH1* was identified in 85% (69 of 81) IBC tumor (including slight, moderate, and strong expression). Some recent studies corroborate our findings. Zhong, employed qRT-PCR and reported that a positive *ALDH1* expression was observed in 63% (92 of 146) of human invasive ductal carcinoma (IDC) tissues, and the incidence of moderate or strong staining were 21.4%³⁰. Additionally, the *ALDH1* phenotype is an independent predictor of early tumor relapse characteristic (specifically, incidence of early local recurrence and distant metastasis) of IDC³⁰. Charafe-Jauffret³¹ have found that *ALDH1* was expressed in 34% of IBC with approximately 3% – 5% positively stained cells in these tumors.

However, Park³² have found that *ALDH1* was expressed in few cases (less than 10 %) and no significant difference was observed in four histologic groups. A cohort of IBC tumors was tested for *ALDH1* expression using the IHC analysis and the results were similar to those of Charafe-Jauffret³¹; *ALDH1* expression was found in 32% of IBC patient tumor samples³³.

These apparent discrepancies of the percentage IBC specimen positive for *ALDH1* expression among the studies could be multifactorial including the variety of detection tools and lack of consistency in staining and scoring methods. Early methods for determining *ALDH1* levels relied on measuring enzyme kinetics or immunoblotting of enzymes released after cell lysis. However, these methods are flawed by antibody cross-reactivity between enzyme isoforms and enzyme structural instability³⁴. Furthermore, mixed results have been reported regarding the specific *ALDH1* isoenzyme involved in tumorigenesis and the AldefluorTM assay in breast cancer, with some groups reporting *ALDH1A1* as the main enzyme involved and others showing that *ALDH1A3* is the accountable isoform³⁴⁻³⁶. It is important to clarify that, by current nomenclature, the term *ALDH1* does not accurately describe any of the isoforms of the *ALDH* super family and could refer to *ALDH1A1*, *ALDH1A2*, *ALDH1A3*, *ALDH1B*, *ALDH1L1*, or *ALDH1L2*. This is a major problem in the *ALDH*/tumor cells literature, where *ALDH1A1* is often used interchangeably with *ALDH1* or *ALDH*. Further confusion arises when AldefluorTM positivity is referred to as *ALDH1* activity or positivity³⁴.

ALDH1 is a detoxifying enzyme belonging to a family of 19 NADP-dependent enzymes that catalyze the oxidation of aldehydes to acids. Accordingly, this *ALDH* family of isoenzymes plays a self-protective, detoxifying role and therefore seems to influence the chemotherapy resistance observed in cancer stem cell (CSC) subpopulations³⁷. Notas *et al.* identified an increased of *ALDH1* expression and a modification of its intracellular distribution in relapsing tumors of human breast cancer after surgery and tamoxifen therapy³⁸. Recently, tamoxifen was reported as a specific activator of *ALDH1*, increasing its activity by 60–80%³⁹, which might help to explain the resistance of tumor cells to oxidative stress during tamoxifen administration. *ALDH1* expression in breast cancer has also been associated with advanced disease stages, triple negative cells, and poor prognosis⁴⁰. *ALDH1* is currently used as marker of pluripotency in epithelial cancers which is absent in normal breast tissue. The expression of *ALDH* isoforms have been analyzed in transcriptome and proteome data^{41, 42} of several cancer types and unraveled that some other *ALDH* isoforms represent positive and negative correlation with poor prognosis⁴³. *ALDH1A1* activity is predominantly expressed in prostate and thyroid cancer^{44, 45}. In the case of some *ALDH1* isoenzymes, *ALDH1A1* may be a major

contributor of *ALDH* activity and a biomarker for predicting the poor survival of patients with breast cancer⁴⁶ and for cervical carcinoma⁴⁷. High levels of *ALDH1A1* and *ALDH1A3* expression are associated with malignant transformation to lung adenocarcinoma⁴⁸. These apparent discrepancies between *ALDH1* expressions in different tumors could reflect the notion that *ALDH1*, a ubiquitous metabolic enzyme, may serve different functions in each organ system, and thus may play a specific role in each organ's tumor biology. Its positive correlation with prognosis suggests that *ALDH1* expression is unlikely to serve as a CSC marker for melanoma in the way it may in breast cancer, where *ALDH1* expression is believed to correlate with CSC status. Moreover, it may be a marker of differentiation rather than undifferentiating⁴⁹.

In spite of increased awareness and research, the etiology and pathogenesis of IBC remains poorly understood. Genetic data suggested that mutations in TP53 gene are more frequent in IBC (50%) than in non-IBC (20–30%)⁵⁰. Higher CXCR4 and CCR7 expression has been found to be associated with poor prognostic outcome in IBC phenotype⁵¹. Expression of *Notch-1*, E-cadherin, and lympho angiogenic factors (*VEGF-C*, *VEGF-D*, *VEGFR-3*, *Prox-1*, lymphatic vessel endothelial receptor1) have been shown to be increased in IBC tumors^{11, 52-54}. Recombinant human C protein (*RhoC*) guanosine triphosphatase (*GTPase*), a metastatic switch responsible for tumor phenotype and loss of WNT1-inducible-signaling pathway protein (*Wisp3*), a breast tumor suppressor gene located at 6q22–q23, have been shown to be over-expressed in 90% of IBC tumors when compared with stage-matched non-IBC tumors¹⁰. An observational analysis of 35 IBC patients (80% had metastatic disease) that failed therapies had plasma analyzed for ctDNA alterations, which were detected in the majority of patients (94%) with stage III or IV tumors. The most common mutations were *TP53* (49%), *PIK3CA* (20%), *ERBB2* (17%), *Notch1* (17%), and *ALK* (11%)⁵⁵. Using next-generation sequencing and comprehensive genomic profiling to identify potential therapeutic targets from metastatic lesions from the skin, chest wall, bone, liver, spine, and brain of 53 patients with relapsed IBC, Ross *et al.* identified *TP53* and *MYC* as well as components of the *RAS* pathway and *PI3K* pathways⁵⁶. Mutations of the *RAS* pathway included those encoded by the *ERBB2*, *KRAS*, *BRAF*, and *EGFR* genes, while in the *PI3K* pathway, *PIK3CA*, *PTEN*, *AKT1*, and *AKT3* were found to be mutated. This study highlights the diversity of genomic alterations occurring within IBC patients, indicating an opportunity for more personalized therapies that specifically target onco-proteins encoded by mutated or amplified genes⁵⁷.

Genetic alteration of the *Notch* pathway has the potential to cause solid tumors. Breast cancer was the first solid tumor in which *Notch* pathway was molecularly

implicated. In our study, increased expressions of *Notch1* have been associated with aggressive IBC tumors compared with non-IBC tumors. These results are consistent with previous data and other reports demonstrating that *Notch1* acts as an oncogenic in IBC tumors and promote breast cancer growth. Ibrahim *et al.*, using qRT-PCR, revealed that *Notch1* was significantly upregulated in IBC in comparison with non-IBC. The authors also explored the role of *Syndecan-1* in regulating expression of components of the *Notch* signaling pathway. They found a higher expression of *Notch1* mRNA and a significant positive correlation between *Notch1*, *Notch3* and *Syndecan-1* mRNA levels in carcinoma tissues of triple negative IBC versus non-IBC tumors⁵⁸. In the current investigation, a significant correlation was found between *Notch1* and *ALDH1* activity in IBC tumors compared with non-IBC tumors. Zhong *et al.* demonstrated that the *Notch1* signaling pathway is possibly involved in the negative association of the *ALDH1* phenotype with early malignant relapse in IDC 30 and this inconsistency seemed *Notch* signaling pathway may played a negative role on *ALDH1*-positive breast cancer cells, which was similarly with the finding that *Notch* signaling might serve as a tumor suppressor in some solid tumors^{59, 60}. Zhao *et al.*, suggest that *Notch* signaling can regulate *ALDH1* acetylation. Mechanistically, this appeared to be mediated by *Notch* signaling-induced *SIRT2* expression. Inhibition of *Notch* signaling decreased *SIRT2* levels and concomitantly increased *ALDH1* Lys-353 acetylation. Likewise, *Notch* activation induced expression of *SIRT2*, which could then deacetylate and activate *ALDH1*⁶¹. Crosstalk of the *Notch1* receptor and other genes was proven to contribute the formation of breast cancer⁶².

The results of the current study showed that the expression levels *ALDH1* were correlated to age and tumor types, as well as HER2 and *Notch1* expression. Nevertheless, there is no statistically significant association between the expression levels *ALDH1* and tumor stages, lymph node involvement, ER, and PR expression as well as triple negativity features in IBC specimens. The significant correlation between the expression levels of *ALDH1* with age may be explained by the age structure of the Moroccan population. There are simply far fewer women in their 60s relative to western populations. Thus the later-onset cases are largely missing, leaving possibly the same incidence of young cases, which thus account for a higher proportion. Furthermore, much attention has been focused on the *ALDH1* expression and the clinic-pathologic features as well as on the *ALDH1* expression and OS in IBC; however, the reported results are controversial. For example, Charafe-Jauffret. found that *ALDH1* positivity associated with metastasis-free survival and tumor specific survival among IBC cases. However, there was no significant correlation between *ALDH1* positivity and ER, PR, HER2, or nodal status¹⁵.

Only a trend toward an association between positive *ALDH1* expression and poor overall survival was observed by Gong *et al.* However, there was no significant association between *ALDH1* expression and clinical outcome or other clinicopathologic variables⁵². On the whole, studies are much too small and further studies involving a larger cohort will be needed to examine possible relationship.

In conclusion, the results of our study and those of de Ibrahim *et al.*⁵⁸ in North Africa have highlighted the importance of the *ALDH1* status in IBC tumors. This finding reflects that the assessment of *ALDH1* expression may have a potential role in incidence of IBC and be helpful tool in the future anticancer strategies. Further studies are needed to give us a better understanding of the etiology and biology of IBC.

Competing interests:

The authors have declared that no competing interest exists.

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