RESEARCH ARTICLE

Clinicopathological Significance of CD133 and ALDH1 Cancer Stem Cell Marker Expression in Invasive Ductal Breast Carcinoma

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Abstract

Background: Biomarkers in breast neoplasms provide invaluable information regarding prognosis and help determining the optimal treatment. We investigated the possible correlation between cancer stem cell (CSC) markers (CD133, and ALDH1) in invasive ductal breast carcinomas with some clinicopathological parameters. Aim: To assess the correlation between expression of cancer stem cell (CSC) markers (CD133, and ALDH1) and clinicopathological parameters of invasive ductal breast carcinomas. Materials and Methods: Immunohistochemical analysis of CD133 and ALDH1 was performed on a series of 120 modified radical mastectomy (MRM) specimens diagnosed as invasive ductal breast carcinoma. Results: Expression of both CD133 and ALDH1 was significantly changed and related to tumor size, tumor stage (TNM), and lymph node metastasis. A negative correlation between CD133 and ALDH1 was found. Conclusions: Detecting the expression of CD133 and ALDH1 in invasive ductal breast carcinomas may be of help in more accurately predicting the aggressive properties and determining the optimal treatment.

Keywords: Breast carcinoma - cancer stem cell markers - CD133 - ALDH1

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Introduction

Breast cancer is the most common primary site in women (30% on average), followed by colorectal (13%), lung (8%), and cervical carcinoma (5%) and the most likely cause that a woman will die from cancer worldwide (Ferlay et al., 2013).

According to official statistics of the National Cancer Institute (Cairo University), breast cancer accounts for 35.1% of the cases of cancer in Egypt and is the most prevalent cancer among Egyptian women (Ibrahim et al., 2014).

In spite of advances in diagnosis and treatment of breast cancer, almost one-fourth of women with this neoplasm will die. The major causes of treatment failure and/or death for patients with breast neoplasms are tumor invasion and metastasis. Decisions regarding the use of adjuvant and palliative therapies in patients with breast neoplasms rely primarily on prognostic factors, such as tumor grade and size, axillary nodal status, distant metastasis (Lamy et al., 2013) and candidate biomarkers, such as hormone receptor (estrogen receptor (ER) and progesterone receptor (PR) expression, and c-erbB2/Her-2/neu) amplification/overexpression. Therefore, such biomarkers in breast neoplasms provide information regarding the outcome of patients (Tessari et al., 2013; Turner et al., 2014). A study in search of additional biomarkers is necessary for patients with breast neoplasms.

The cancer stem cell model suggests that in many cancers, tumor initiation and propagation is driven by a population of self-renewing tumor cells known as cancer stem cells (CSCs) (Reya, et al., 2001). CSCs also promote tumor cell heterogeneity, metastasis, and therapeutic resistance, and are potentially driven by known oncogenic signaling pathways. The study of CSCs would be greatly enhanced by the availability of specific markers to identify and isolate these cells (Meacham and Morrison, 2013). Through examinations using putative stem cell markers or side population, unique subsets of cancer cells from different types of tumors have been detected. These markers include CD133, CD44, CD24, and CD166. Among them, both CD133 and CD44 are widely used for isolating CSCs from solid tumors (Clark and Fuller, 2006; Visvader and Lindeman, 2008; Navin et al., 2011).

CD133 is a glycoprotein also known in humans and rodents as Prominin 1 (PROM1). It is a member of pentaspan transmembrane glycoproteins (Bertolini et al., 2009). CD133 is expressed in hematopoietic stem cells (Timothy et al., 2013), endothelial progenitor cells (Liao et al., 2010), gastric cancer (Saricanbaz et al., 2014), glioblastoma (Yan et al., 2011), neuronal and glial stem cells (Bexell et al., 2009), squamous cell carcinoma (Satpute et al., 2013) various pediatric brain tumors (Rasha et al., 2012) as well as adult kidney, mammary glands, trachea, salivary glands, placenta, digestive tract, testes, and some other cell types (Tirino et al., 2009; Shi et al.,

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2010; Bozzi et al., 2011).

Aldehyde dehydrogenase 1 (ALDH1) is an enzyme having the role of oxidating intracellular aldehydes and that could have the responsibility to oxidize retinol to retonic acid in stem cells (Jiang et al., 2009; Alison et al., 2010). Sorting cells based on high ALDH activity has been shown to enrich for CSC in several cancers (Douville et al., 2009), including carcinoids (Gaur et al., 2011). The use of ALDH1 as a target molecule to select cancer stem cells has been facilitated by a combination of live cell ALDH1 activity detection and cell sorting techniques that proposed it as a diagnostic marker, and a therapeutic target as well as a prognostic marker in a number of cancers including cervical carcinoma (Rao et al., 2012) and urothelial carcinoma of the urinary bladder (Keymoosi et al., 2014).

In this study, we evaluated the expression and distribution of the representative CSC markers CD133 and ALDH1 in breast cancer by immunohistochemistry, and studied their relationship with clinicopathologic features. We studied the interrelationship between the expressions of these two proteins.

Materials and Methods

Tissue samples

One hundred twenty patients who underwent MRM for treatment of invasive ductal breast carcinomas between years 2006-2013 at the Suez Canal University Hospital were retrospectively included in this study. No patients had evidence of distant metastasis at the time of primary surgery based on the preoperative examination. Tumor tissue specimens were retrieved from the archives of the Department of Pathology. The slides were reviewed by the researchers to ensure that the cases were consistent with breast ductal carcinoma. Clinical information, tumor size, and axillary lymph node status were obtained from medical records and the pathology reports.

Immunohistochemical examination

All samples were fixed in 10% buffered formalin and embedded in paraffin. Four- micrometer thick tissue sections were used for analysis. All sections were deparaffinized and dehydrated with graded alcohol. Then, the sections were washed for ten minutes in PBS at pH 7.2. The endogenous peroxidase activity was quenched by incubation in methanol containing 3% H2O2 for ten minutes at room temperature, then heated for 30 minutes at 95°C to repair the antigens and finally rinsed in PBS. After several washes in PBS, sections were blocked with goat serum for 20 minutes at room temperature, and then incubated with mouse monoclonal CD133 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and anti-ALDH1 antibody (Santa Cruz Biotechnology, INC) 1:200 primary antibodies overnight at 4°C in a humidified chamber. The slides were treated with polymer enhancer (reagent A) for 20 minutes at room temperature. Washing in PBS, the slides were treated with goat anti-mouse antibody (reagent B) for 30 minutes at room temperature. After a complete wash in PBS, the slides were developed in freshly prepared diaminobenzidine (DAB) solution for eight minutes, and then counterstained with hematoxylin, dehydrated, air-dried, and mounted.

Serial sections of invasive duct carcinoma were run in parallel with the primary antibody replaced by PBS and rabbit IgG1 as blank and negative controls.

Evaluation of immunohistochemistry

Antigen expression was evaluated independently by the two authors using light microscopy. Both assessors were unaware of the clinical outcome. Equivocal cases were re-assessed on a double-headed microscope to establish a final score. For each sample, at least five fields (inside the tumor and in the area exhibiting tumor invasion; ×400) were analyzed. Scores were applied as follows: score 0, negative staining in all cells; score 1+, weakly positive or focally positive staining in <10% of the cells; score 2+, moderately positive staining covering 10% to 50% of the cells; and score 3+, strongly positive staining, including >50% of the cells. For statistical analysis, as well as to reduce intraobserver variability, the immunohistochemical scores were further grouped into two categories: negative or weakly positive (0 and 1+) and moderately-to-strongly positive (2+ and 3+).

The positive expression of CD133 was found mainly on the membrane and cytoplasm of tumor cells. The positive expression of ALDH1was found mainly on the membrane and cytoplasm of tumor cells. They were presented as a brown granular material.

Statistical analysis

Fisher's exact test and Spearman's correlate analysis for univariate or multivariate analysis were used to assess the associations among the positive staining of CD133 or ALDH1and clinicopathological indices. SPSS 16.0 software for windows (Chicago, IL, USA) was used for this purpose. A value of $P<0.05$ was considered statistically significant.

Table 1. Clinicopathological Characteristics of the Patients and Tumors

Characteristic	n	(%)
Mean age±SD, yrs (range),	49.1±12.7, 28-70yrs	
Histologic grade (Nottingham Histologic score)		
Grade I	48	(40)
Grade II	56	(46.7)
Grade III	16	(13.3)
Tumor size (pT)		
pT1	48	(40)
pT2	56	(46.7)
pT3	12	(10)
p T4	4	(3.3)
Regional Lymph node metastasis (pN)		
Negative (pN0)	40	(33.3)
Positive	80	(66.7)
Stage (TNM)		
Stage I	40	(33.3)
Stage II	64	(53.3)
Stage III	16	(13.3)
Ductal carcinoma in situ component (DCIS)		
Minor (less than 25%)	80	(66.7)
Extensive (More than 25%)	40	(33.3)

Results

The age of the patients at time of diagnosis was ranged from 28 to 71 years, and the median age was 49 years. No patients had evidence of distant metastasis at the time of surgery. The majority of cases 64 (53.3%) had stage II disease and the tumor diameter between 2 and 5 cm was detected in 56 (46.7%) of the patients. The details of patient characteristics and descriptive statistics for the tumors are shown in Table 1.

Immunohistochemical staining

CD133 was expressed in the tumor cell membrane and the cytoplasm of cancer cells (Figure A). Expression of CD133 in tumor cells occurred in 53.3% (64/120) of the cases. As shown in Table 2, CD133 expression was significantly correlated with some clinicopathological parameters, including age (p=0.0435), tumor size (pT) (P= 0.0031), lymph node metastasis (P=0.0196) and highly statistically significant with tumor stage (TNM) (P=0.0001). CD133 was mainly expressed in larger tumors, lymph node metastasis and advanced tumor stage, indicating CD133 related to more aggressive characters of tumor. However, there was no significant correlation of CD133 expression with histopathological grade (Nottingham Histologic score) and Insitu Ductal Carcinoma component percentage.

Out of 120 cases of invasive ductal breast carcinoma, 50 cases (41.7%) were positive for ALDH1. ALDH1 was observed mainly in the cytoplasm and the membrane

Table 2. Correlations between CD133 or ALDH1 Expression and Clinicopathological Parameters

Variable	Number of Patients	CD133 expression			ALDH-1 expression		
		Positive, (n=64) n (%)	Negative, (n=56) n (%)	P value	Positive, (n=50) n (%)	Negative, (n=70) n (%)	P value
Age							
< 49	56	24 (20)	32 (27)	0.0435*	20 (17)	36 (30)	0.2664
> 49	64	40 (33)	24 (20)		30 (25)	34 (20)	
Tumor size (pT)							
pT1-pT2	104	50 (42)	54 (45)	0.0031*	35 (29)	69 (57)	0.0001*
pT3-pT4	16	14 (11)	2 (2)		15 (13)	1 (1)	
Histologic grade (Nottingham Histologic score)							
I	48	24 (20)	24 (20)	0.5796	15 (12)	33 (28)	0.0627
II-III	72	40 (33)	32 (27)		35 (29)	37 (31)	
Lymph node metastasis (pN)							
Negative	40	15 (12)	25 (21)	0.0196*	10 (9)	30 (25)	0.0107*
Positive	80	49 (41)	31 (26)		40 (33)	40 (33)	
Stage (TNM)							
I	40	10 (9)	30 (25)	0.0001*	16 (14)	24 (20)	0.846
II-III	80	52 (43)	28 (23)		34 (28)	46 (38)	
Ductal carcinoma in situ component (DCIS %)							
< 25%	80	42 (35)	38 (32)	0.8476	29 (24)	51 (43)	0.1163
	40	22 (18)	18 (15)		21 (17)	19 (16)	

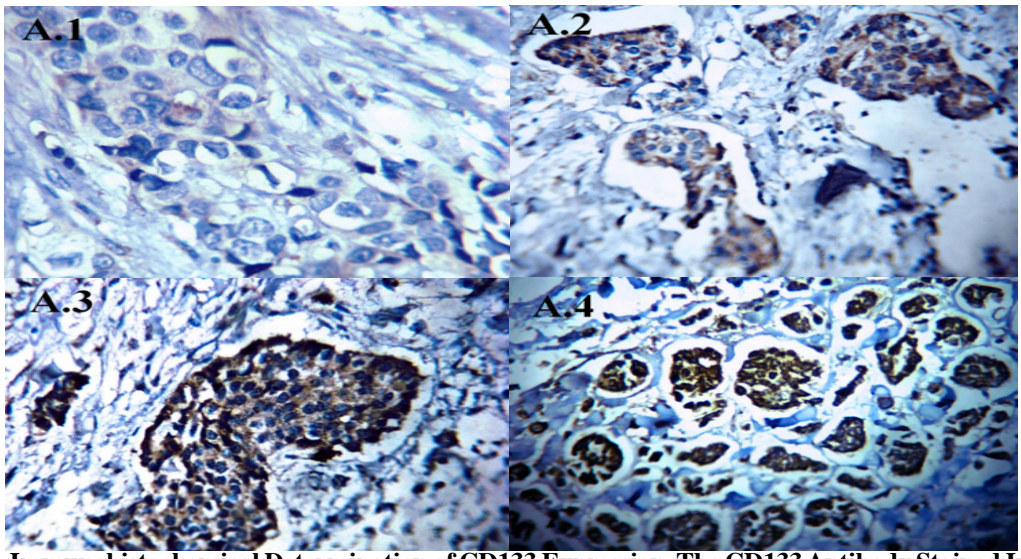

Figure 1A. Immunohistochemical Determination of CD133 Expression. The CD133 Antibody Stained Intensely at the Membrane and in the Cytoplasm of Cancer Cells. Scores were applied as follows: Score 0: negative staining in all cells; Score 1+: weakly positive or focally positive staining in <10% of cells (Figure A.1); Score 2+, moderately positive staining in 10%-50% of cells (Figure A.2); Score 3+, strongly positive-staining, involving 50% or more of the cells (Figure A.3 and Figure A.4)

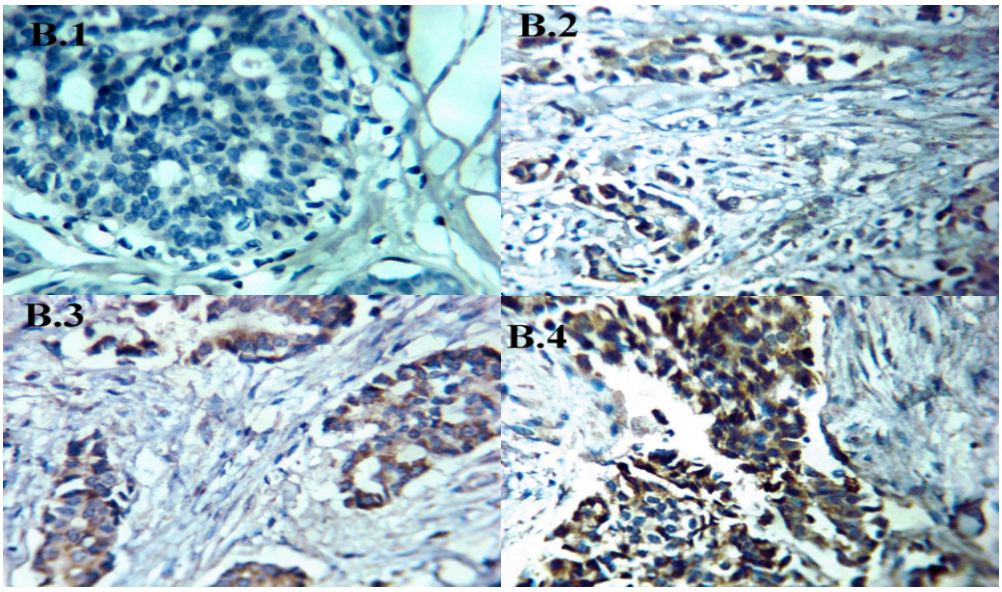

Figure 2B. Immunohistochemical Determination of ALDH1 Expression. The ALDH1 antibody stained intensely at the membrane and in the cytoplasm of cancer cells. Scores were applied as follows: Score 0: negative staining in all cells; Score 1+: weakly positive or focally positive staining in <10% of cells (Figure B.1); Score 2+, moderately positive staining in 10%-50% of cells (Figure B.2); Score 3+, strongly positive-staining, involving 50% or more of the cells (Figure B.3 and Figure B.4)

of the tumor cells (Figure B). ALDH1 expression was significantly correlated with some clinicopathological parameters including tumor size (pT) (p=0.0001), lymph nodes metastasis (p=0.0107). Although not significantly correlated ALDH1-positive cases are seen more with high histological grade (p=0.0627), advanced TNM stage (P=0.8460), minor DCIS % (P=0.1163) than ALDH1-negative cases (table 2).

Discussion

According to the CSC hypothesis, it is assumed that CSCs are responsible for cancer initiation and development. Expression of CSC markers has been reported to be present in normal adult stem/ progenitor cells as well as in CSC (Clevers (2011); Mills and Shivdasani, 2011). In the present study, we examined the expression and distribution of representative CSC markers (CD133 and ALDH1) in ductal carcinoma of the breast, the most common subtype of breast carcinomas. Originally considered a marker of hematopoietic stem cells, CD133/ prominin is a highly glycosylated trans-membrane protein expressed in various tissues, such as breast, in which it seems to regulate ductal branching but not regenerative capacity (Zobalova et al., 2011). The protein CD133 is one of the hot CSC markers in a variety of tumors (Wang et al., 2011; Zhao et al., 2011; Di Bonito et al., 2012; Schneider et al., 2012). In this study, we found that the positive expression of CD133 was 53.3% (64/120) in invasive duct carcinoma and it was significantly correlated with some clinicopathological parameters, including age (p=0.0435), tumor size (pT) (P= 0.0031), lymph node metastasis (P=0.0196) and highly significant with the tumor stage (TNM) (P=0.0001). CD133 was mainly expressed in larger tumors, lymph node metastasis and advanced tumor stage, indicating CD133 related to more aggressive characters of tumor. However, there was no significant correlation of CD133 expression with histopathological grade (Nottingham Histologic score) and Insitu Ductal Carcinoma component. As CD133 expression was more in more aggressive characters of tumor (higher stage tumors, larger tumors, and lymph node metastasis), suggesting the expression of CD133 might be a potential prognostic factor in invasive duct carcinoma of the breast. These results are consistent with previous reports that CSC marker expression is significantly upregulated in some solid carcinomas and are risk factors for worse clinical behavior (Shimada et al., 2009; Charafe-Jauffret et al., 2010). However, there was no significant correlation of CD133 expression with age, histopathological grade and Insitu Ductal Carcinoma component. Recent and numerous studies show that positivity for CD133 allows identifying CSCs in breast cancer (Wright et al., 2008). CD133 is expressed by several solid tumors, including invasive breast cancer triple negative, with very low levels of expression compared to other CSCs markers previously reported, like CD44 and ALDH1 (Wu and Wu, 2009). In early-onset breast cancers, associated with mutations on BRCA1, CD133+ cells show CSCs properties (Wright et al., 2008). The employment of this tumor stemness marker in breast cancers has become popular more recently and its expression is often described as associated with a worse prognosis (Ieni et al., 2011; Zhao et al., 2011).

The breast CSC marker ALDH1 has been described as a marker of both normal and malignant breast stem/ progenitor cells (Huang et al., 2009; Meyer et al., 2009; Deng et al., 2010). ALDH1hi tumor cells form visibly larger colonies and mammospheres, when compared with ALDH1low cells (Deng et al., 2010). Previous works also detected small percentages of ALDH1+ cases in invasive breast cancer, ranging from 4% to 19% (Morimoto et al., 2009; Park et al., 2010; Resetkova et al., 2010). In our study, we found 42% (50/120) of ALDH1 expression. ALDH1 expression was significantly correlated with some clinicopathological parameters including tumor size (pT) (p=0.0001), and lymph nodes metastasis

(p=0.0107). Although not significantly correlated ALDH1-positive cases were seen more with high histological grade (p=0.0627), advanced TNM stage (P=0.8460), or minor DCIS % (P=0.1163) than ALDH1-negative cases. These results are consistent with previous reports that ALDH1 expression in breast cancer accounts for 20-50%. High positivity in tumor cells is associated with high histological grade, ERBB2 over-expression, absence of hormone receptors ER and PgR and worse prognosis (Ohi et al., 2011; Sakakibara et al., 2012). However the study of (Madjd et al., 2012) couldn't find statistical correlation between ALDH1 expression and breast tumor characteristics except a noticeable trend relation between ALDH1 expression and high grade tumors.

CD133 and ALDH1 expression, which could be detected by immunohistochemistry, might be a useful molecular marker to predict the prognosis in invasive ductal carcinoma of breast patients. The current study concluded that the expression of CD133 and ALDH1 proteins could be correlated with lymph node metastasis, grade of tumor, and pTNM stage in invasive ductal carcinoma of breast. The combined detection of CD133 and ALDH1 can, to some extent, reflect the biological behavior of invasive ductal carcinoma of breast, thus giving the choice of molecular targeting therapy.

In conclusion, It is suggested that CD133 and ALDH1 may play an important role in the evolution of invasive ductal carcinoma of breast and CD133 along with ALDH1 should be considered as potential marker for the prognosis in patients with invasive ductal carcinoma.

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