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Expression of Aldehyde Dehydrogenase 1 as a Marker of Mammary Stem Cells in Benign and Malignant Breast Lesions of Ghanaian Women

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Abstract

Background—Breast cancers that are negative for the estrogen receptor (ER), the progesterone receptor (PR), and the HER2 (human epidermal growth factor receptor 2) marker are more prevalent among African women, and the biologically aggressive nature of these triple-negative breast cancers (TNBCs) may be attributed to their mammary stem cell features. Little is known about expression of the mammary stem cell marker aldehyde dehydrogenase 1 (ALDH1) in African women. Novel data are reported regarding ALDH1 expression in benign and cancerous breast tissue of Ghanaian women.

Methods—Formalin-fixed, paraffin-embedded specimens were transported from the Komfo Anoyke Teaching Hospital in Kumasi, Ghana to the University of Michigan for centralized histopathology study. Expression of ER, PR, HER2, and ALDH1 was assessed by

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immunohistochemistry. ALDH1 staining was further characterized by its presence in stromal versus epithelial and/or tumor components of tissue.

Results—A total of 173 women contributed to this study: 69 with benign breast conditions, mean age 24 years, and 104 with breast cancer, mean age 49 years. The proportion of benign breast conditions expressing stromal ALDH1 (n = 40, 58%) was significantly higher than those with cancer (n = 44, 42.3%) ($P = .043$). Among the cancers, TNBC had the highest prevalence of ALDH1 expression, either in stroma or in epithelial cells. More than 2-fold higher likelihood of ALDH1 expression was observed in TNBC cases compared with other breast cancer subtypes (odds ratio = 2.38, 95% confidence interval 1.03-5.52, $P = .042$).

Conclusions—ALDH1 expression was higher in stromal components of benign compared with cancerous lesions. Of the ER-, PR-, and HER2-defined subtypes of breast cancer, expression of ALDH1 was highest in TNBC.

Keywords

breast cancer; stem cell marker; aldehyde dehydrogenase 1; triple-negative breast cancer; African ancestry

Introduction

Premenopausal breast cancer and tumors that are negative for the estrogen receptor (ER), the progesterone receptor (PR), and HER2/*neu* (HER2), a condition commonly known as triple-negative breast cancer (TNBC), are substantially more common among African and African American women compared with women of other racial/ethnic backgrounds,[1-3] as well as among women with BRCA1 mutation-associated breast cancer.[4,5] Approximately 80% of TNBCs belong to the basal breast cancer subtype, which has been identified as being particularly virulent. Shared ancestry between contemporary African and African American women raises the question of whether African heritage is associated with a heritable marker for this high-risk pattern of disease. Features of cancer progenitor cells, also known as cancer stem cells, may ultimately account for the biological nature of various breast cancer subtypes, and the presence of mammary stem cells in benign breast tissue has even been linked to future breast cancer risk.[6,7] Ongoing research seeks to clarify relationships between hereditary breast cancer, the basal subtype, and the mammary stem cells. It is therefore appropriate and necessary to study stem cells in association with breast cancer risk in women with African ancestry.

Mammary stem cells, as identified by cells expressing the marker aldehyde dehydrogenase 1 (ALDH1), appear to be correlated with malignant transformation of breast tissue and progression into the virulent triple-negative phenotype.[8] ALDH1 expression is found in a minority of the breast cancer specimens of white American and European women (19%-30%).[9] Little is known about the frequency of this marker in women of African descent, who are known to have an increased risk for triple-negative breast cancer, but recent studies suggest that breast cancers expressing this marker are more common among African women.[10] Our study presents novel data regarding ALDH1 expression in benign as well as malignant breast tissue of African women from Ghana.

Materials and Methods

The conduct of this research was approved by the Institutional Review Board of the University of Michigan (UM), Ann Arbor, Michigan, and the Committee on Human Research Publication and Ethics, Kwame Nkrumah University of Science and Technology College of Health Sciences-School of Medical Sciences, Komfo Anoyke Teaching Hospital (KATH), Kumasi, Ghana.

Formalin-fixed, paraffin-embedded specimens of breast tissue from women receiving treatment for benign and malignant diseases at KATH between 2006 and 2010 (> 90% of specimens were retrieved in 2008 and 2009) were transported to UM for centralized histopathology review. These specimens were matched to limited clinico-pathology data retrieved from KATH pathology reports. The benign versus malignant nature of all specimens was confirmed at UM by histopathologic evaluation of slides stained with hematoxylin and eosin. Immunohistochemistry (IHC) was then performed at UM for expression of ER, PR, HER2, and ALDH1. Malignant specimens were further characterized by nuclear grade.

Briefly, paraffin-embedded tissue blocks were sectioned at 5 μm and placed on charged slides. Slides were deparaffinized in xylene and rehydrated through graded alcohols to buffer. Peroxidase blocking was performed. No slide pretreatments were used for HER2. Pretreatment in citrate buffer (pH 6.0) for 15 minutes was used for ER and PR. Ethylene diamine tetraacetic acid pretreatment for 15 minutes was used for ALDH1. All slides were stained on the Dako Automated Immunostainer. HER2 (Dako North America) was used at a dilution of 1:100, ER (clone ID5; Dako North America) at 1:50, ALDH1 (clone 44; BD Biosciences) at 1:500 or 1:1000, and PR (clone PgR636; Dako North America) at 1:50. Antibodies were detected with either EnVision+ Rabbit horseradish peroxidase (HRP) (HER2), EnVision+ Mouse HRP (ER, ALDH1), or LSAB+ HRP (PR) all from Dako North America. HRP staining was visualized with the DAB+ Kit (Dako North America), and slides were counterstained in hematoxylin. IHC was done by the UM Comprehensive Cancer Center Tissue Core Research Histology and IHC Laboratory.

Specimens were scored as being positive for ER and/or PR if at least 2% nuclear staining was observed. Benign breast ducts present in the sections of tumor served as internal positive controls for the hormone receptors. The expression of HER2 was scored as either 0 (no staining), 1^+ (weak staining in < 10% of tumor cells), 2^+ (weak complete membrane staining in > 10%), or 3^+ (strong complete membrane staining in > 10%). For the purpose of the present study, HER2 status was dichotomized as either positive or negative. A specimen scored as 0 or 1^+ was classified as HER2 negative and positive if it received an IHC score of 3^+. Fluorescent in situ hybridization, typically used to assess amplification of the HER2 gene in cases with a score of 2^+, was not needed, because none of the specimens in this study had a score of 2^+. ALDH1 was scored as positive if any staining was seen in the cytoplasm and negative if no staining was detected. ALDH1 staining was further characterized by its presence in stromal versus epithelial and/or tumor components of tissue.

Descriptive statistics were used to summarize clinical and histopathology information. We applied parametric and nonparametric statistics, as appropriate, to compare the distribution of variables between women diagnosed with benign breast conditions and breast cancer. Expression of ALDH1 in stroma and epithelial cells was correlated and statistically significant (r = .403, $P = .001$); consequently, we analyzed expression of ALDH1 stratified by the site of tissue, whether stroma or epithelial, and by their joint expression. The latter category, joint expression, therefore was defined as a sample positive for ALDH1, if the specimen had been scored positive for ALDH1 expression either in epithelial and/or in stroma tissue.

The association between clinicopathologic variables and expression of ALDH1 biomarkers among women diagnosed with invasive breast cancer was calculated using univariate logistic regression analysis. Because a small number of women (n = 6) were diagnosed with well-differentiated (grade 1) tumors, the grade categories of “well-differentiated” and “moderately differentiated” were collapsed into 1 group, yielding 2 categories of histological grades “well and moderately” and “poorly” differentiated for statistical analyses. The variable ALDH1 was dichotomized as positive or negative, with positive defined as weakly to strongly staining. Finally, we categorized breast cancers into 4 groups on the basis of IHC results for ER, PR, and HER2 biomarkers: [$HER2^+$ and ER^+ and PR^+], [$HER2^-$ and ER^+ and/or PR^+/PR^-], [$HER2^+$ and $ER^-$and/or PR^+/PR^-], [$HER2^-$ and ER^- and PR^-].For the statistical analysis, we used the category [$HER2^-$ and ER^+ and/or PR^+/PR^-] as the reference group because of its histological similarities to luminal A subtype, which has the most favorable outcome. All statistical tests were 2-sided, and analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC)

Results

A total of 173 women, of whom 69 were diagnosed with benign conditions of the breast and 104 with breast cancer, contributed to this study. At the initial clinical presentation, the mean age of women with benign conditions of the breast was 24 (± 8.4) years and for women with breast cancer was 49 (± 13.4) years ($P < .001$). The proportion of women diagnosed with benign breast conditions and having stromal ALDH1 expression (n = 40, 58%) was statistically significantly higher than the stromal ALDH1 expression among women with cancer (n = 44, 42.3%; $P = .043$). We did not detect statistically significant differences in the proportions of cases staining positive in the epithelial cells expressing ALDH1 or in the category of joint expression (Table 1). ALDH1 expression was similarly low in the epithelial cell–positive proportions of benign and malignant specimens (15.9% and 17.3% of cases, respectively).

Of the 104 women with breast cancer, 5 were diagnosed with ductal carcinoma in situ and 99 with invasive carcinoma (Table 2). Among women with invasive carcinoma, 52 (52.5%) were diagnosed with poorly differentiated, 41 (41.4%) with moderately differentiated, and 6 (6.1%) with well-differentiated histologic grades. A total of 78 (75.7%) women were diagnosed with ER-negative breast cancers and 83 (80.6%) with HER2-negative biomarker. A total of 58 (56.3%) women were diagnosed with the triple-negative subtype ($HER2^-$ and ER^- and PR^-). Among the 4 subtypes of breast cancer, we observed the highest prevalence

of ALDH1 expression, either in stroma or in epithelial cells, in the triple-negative subtype. However, the observed distributions did not reach the level of statistical significance, presumably due to the small numbers within each subtype (Table 3).

Results from our univariate logistic regression analysis yielded a 3-fold higher likelihood (odds ratio = 2.99, 95% confidence interval = 0.94-9.46, $P = .06$) for expression of ALDH1 in the triple-negative subtype. In our second analysis, we opted to keep the non–triple-negative subtypes as the referent and evaluate the probability of ALDH1 expression in the triple-negative subtype. The likelihood of ALDH1 expression in the triple-negative subtypes was more than 2-fold (odds ratio = 2.38, 95% confidence interval = 1.03-5.52, $P = .042$) relative to non–triple-negative subtypes (Table 4). Comparison results were unchanged when the cases of ductal carcinoma in situ were excluded from analysis.

Discussion

Differences in breast cancer incidence and outcome between African American and white American women are well-documented by population-based data from the Surveillance, Epidemiology, and End Results Program. African American women have a lower lifetime risk of being diagnosed with breast cancer, accounting for approximately 8% of all estimated new cases in the United States; however, they account for approximately 13% of all breast cancer–related deaths.[11,12] African American women are also well known to have higher frequencies of ER-negative, triple-negative, and early-onset breast cancer.[1,13-15] The increased prevalence of these patterns among women with known hereditary susceptibility for breast cancer through germline BRCA1 mutations then raises the question of whether African ancestry might also be associated with inherited predisposition for these high-risk breast cancer patterns.[15]

Substantial genetic admixture has occurred between various race/ethnicity-defined subsets of the American population, resulting in a significant degree of ancestral heterogeneity within many African American families. Nonetheless, a disproportionate risk for breast cancer mortality has been consistently demonstrated for women who identify themselves as African American. Poverty and inadequate health care access are also more prevalent among African Americans, and it is therefore challenging to disentangle the effects of socioeconomic disadvantage from those of inherited susceptibility on breast cancer risk. To learn more about breast cancer predisposition that might be related to inherited factors associated with African heritage, it is appropriate to study available data on the breast cancer burden of African populations that share ancestry with African Americans. Most of the colonial slave trade from the 1500s to the 1800s occurred between ports along the Atlantic coast and western, sub-Saharan Africa, including the country of Ghana.[16,17]

Existing studies of breast cancer in European/white American women, African American women, and women from sub-Saharan Africa reveal provocative patterns. Frequencies of early-onset, ER-negative, and triple-negative breast cancer are lowest for women with European heritage, highest for African women, and intermediate for African American women.[1,2,19] These patterns suggest that extent of African ancestry could be associated with

an inherited susceptibility for aggressive patterns of breast cancer diagnosed at younger ages.

The concept of cancers arising from stem cells was introduced more than a century ago,[19] and has been applied to research in the study of hematologic malignancies, multiple myeloma, and melanoma as well as brain, prostate, colon, pancreatic, and head and neck cancers.[20-27] Hematopoietic stem cells are multipotent cells that are normally found in the bone marrow and are responsible for producing all of the adult hematopoietic lineages. These cells have the ability to self-renew and undergo differentiation into phenotypically diverse populations of tumor cells. It is theorized that cancer stem cells drive the growth and spread of malignant tumors. It stands to reason that cancer stem cells have a phenotype defined by the cell of origin and by an oncogenic transformation event. In an attempt to find shared cancer stem cell markers, recent studies have focused on conserved stem and progenitor cell functions. These functional markers may be inherited by the malignant stem cell compartment across multiple histological subtypes of cancer from the same tissue of origin.[6,9,10,28-30]

ALDH1 is an intracellular enzyme that is responsible for the oxidation of aldehydes to carboxylic acids.[31] Ginestier and colleagues showed that stem cell–like populations in breast tissue are characterized by the expression of ALDH1, and breast cancer stem cells have been isolated on the basis of increased ALDH1 expression.[5,8] This group further demonstrated that in the breast, ALDH1 expression is considered to be a marker of both normal and malignant stem and progenitor cells. ALDH1 positivity has been associated with features of aggressive tumors, such as high histological grade, high mitotic count, p53 expression and ER/PR negativity. In addition, ALDH1 expression has been associated with poor clinical outcome.[7-9,33-39] Finally, ALDH1 expression within benign breast biopsies has been associated with future breast cancer risk.[8] These studies are summarized in Table 5.

ALDH1 can be detected by standard IHC staining techniques as a cytoplasmic protein. Consistent with the theory that stem cells comprise a minority of tumor tissue, specimens that are positive for ALDH1 frequently have fewer than 10% of cells expressing this marker. At this time, there is no consensus regarding optimal scoring and threshold levels for ALDH1 positivity, but published studies thus far generally reveal fewer than one-third of breast cancers to be positive for this marker. Two notable exceptions, however, are breast cancer cases related to BRCA1 mutations[38] and breast cancers from African women,[10] where ALDH1 positivity has been reported in 78% and 48% of cases, respectively.

Substandard and/or poor, delayed tissue fixation can result in diminished antigenicity and lower molecular marker expression on subsequent IHC evaluation. Although all IHC studies for this project were performed at UM, specimens were initially processed and formalin-fixed in Ghana. Although the understaffed surgery and pathology programs in developing countries such as Ghana can potentially yield decreased antigenicity because of suboptimal tissue fixation, it would be expected that a variety of markers would be affected in a comparably suppressed fashion. The observation of decreased ER, PR, and HER2 expression in contrast to relatively increased expression of ALDH1 suggests that our findings are not explained by generalized decreased antigenicity.

In summary, the cancer stem cell hypothesis has fundamental implications for cancer biology in addition to clinical implications for cancer risk assessment, early detection, prognostication, and prevention. Our study lends support to the stem cell hypothesis by demonstrating increased expression of ALDH1 in breast specimens from the western sub-Saharan country of Ghana, a population known to be characterized by higher prevalence of the TNBC pattern. Specifically, there appears to be significantly increased expression of this mammary stem cell marker in the stromal components of benign Ghanaian breast specimens, and expression of ALDH1 within Ghanaian cancers was highest for triple-negative tumors. These data, together with well-documented evidence of the high prevalence of TNBC among women with African ancestry in the United States as well as in women in continental Africa, suggests that stem cell marker expression in benign tissue may well be associated with future risk of these biologically aggressive tumors. Furthermore, some preliminary case-control data already suggest an association between ALDH1 expression in benign breast tissue and breast cancer risk. Compared to existing data on ALDH1 expression in the breast cancers of white American and European women, we found that mammary stem cells (as detected by ALDH1 expression) represent an expanded population of the breast tissue of women from Ghana.

These results have provocative implications regarding the possibility that breast cancer disparities between African American and white American women may have an inherited, genetic explanation. Although studies of mammary stem cell expression in African populations add compelling observations to the discussion of breast cancer disparities between race/ethnicity-identified populations, it is critical that future studies specifically seek to define expression of this marker in African American women. These data are presently unavailable but will be relevant to discussions of breast cancer disparities within the United States. Further studies are necessary to confirm our findings, and to fully understand their clinical significance regarding the biology of breast cancer in international populations. This work also demonstrates the value of global breast oncology collaborative efforts.

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Table 1

Aldehyde Dehydrogenase 1 (ALDH1) Expression in Benign and Cancerous Lesions of the Breast

Variable	Benign (n = 69) N (%)	Cancer (n = 104) N (%)	*P*
Mean age, y	24 (±8.4)	49 (±13.4)	<.001
Stromal ALDH1			.043
Negative	29 (42.0)	60 (58.0)	
Positive	40 (58.0)	44 (42.3)	
Epithelial ALDH1			.813
Negative	58 (84.1)	86 (82.7)	
Positive	11 (15.9)	18 (17.3)	
Joint expression			.099
Negative	29 (42.0)	57 (54.8)	
Positive	40 (58.0)	47 (45.2)	

Table 2

Distribution of Clinicopathologic Characteristics of Women Diagnosed With Breast Cancer

Variable	N (%)
Median age, y (range)	46 (24-92)
Progesterone receptor status[a]	
Negative	78 (75.7)
Positive	25 (24.3)
Estrogen receptor status[b]	
Negative	76 (73.8)
Positive	27 (26.2)
HER2 biomarker[c]	
Negative	83 (80.6)
Positive	20 (19.4)
Grade[d]	
1	6 (6.1)
2	41 (41.4)
3	52 (52.5)
Subtype	
$HER2^-$ and ER^- and PR^-	58 (56.3)
$HER2^+$ and ER^- and/or PR^+/PR^-	18 (17.5)
$HER2^-$ and ER^+ and/or PR^+/PR^-	19 (18.4)
$HER2^+$ and ER^+ and PR^+	8 (7.8)

Abbreviations: ER, estrogen receptor; HER2 human epidermal growth factor receptor 2/glioblastoma; PR, progesterone receptor.

[a] One patient missing estrogen receptor expression staining.

[b] One patient missing progesterone receptor expression staining.

[c] One patient missing HER2 staining.

[d] Five patients missing tumor grade assessment.

Table 3

Prevalence of Aldehyde Dehydrogenase 1 (ALDH1) Expression in Stroma, Epithelial Cells in Different Subtypes of Breast Cancers

Subtype	ALDH1-Negative N (%)	ALDH1-Positive N (%)
Stroma		
$HER2^-$ and ER^- and PR^-	30 (51.7)	28 (48.3)
$HER2^+$ and ER^- and/or PR^+/PR^-	13 (72.2)	5 (27.8)
$HER2^-$ and ER^+ and/or PR^+/PR^-	13 (68.4)	6 (31.6)
$HER2^+$ and ER^+ and PR^+	4 (50.0)	4 (50.0)
Epithelial		
$HER2^-$ and ER^- and PR^-	27 (46.6)	31 (53.4)
$HER2^+$ and ER^- and/or PR^+/PR^-	13 (72.2)	5 (27.8)
$HER2^-$ and ER^+ and/or PR^+/PR^-	13 (68.4)	6 (31.6)
$HER2^+$ and ER^+ and PR^+	4 (50.0)	4 (50.0)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2/glioblastoma; PR, progesterone receptor.

Table 4

Likelihood of Aldehyde Dehydrogenase 1 (ALDH1) Expression by Clinicopathologic Characteristics

Clinicopathologic Feature	ALDH1-Positive	OR (95% CI)	*P*
Age group			
Age < 50 y vs age ≥ 50 y	23/46 vs 17/38	1.23 (.52-2.92)	.624
Estrogen receptor			
Negative vs positive	36/76 vs 10/27	1.54 (.62-3.85)	.355
Progesterone receptor			
Negative vs positive	36/78 vs 10/25	1.28 (.51-3.22)	.591
Grade			
3 vs 1 and 2	27/52 vs 18/47	1.84 (.82-4.14)	.139
HER2 biomarker			
Negative vs positive	38/83 vs 8/20	1.05 (.36-3.12)	.920
Subtype			
[$HER2^+$ and ER^+ and PR^+] vs [$HER2^-$ and ER^+ and/or PR^+/PR^-]	4/8 vs 6/19	2.60(.39-17.4)	.324
[$HER2^+$ and ER^- and/or PR^+/PR^-] vs [$HER2^-$ and ER^+ and/or PR^+/PR^-]	5/18 vs 6/19	1.18 (.27-5.18)	.825
[$HER2^-$ and ER^- and PR^-] vs [$HER2^-$ and ER^+ and/or PR^+/PR^-]	31/58 vs 6/19	2.99 (.94-9.46)	.063
TNBC vs none-TNBC[a]	31/58 vs 15/45	2.38 (1.03-5.52)	.042

Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2/glioblastoma; OR, odds ratio; PR, progesterone receptor.

[a]TNBC, triple-negative breast cancer ($HER2^-$ and ER^- and PR^-); None-TNBC, all other subtypes combined.

Table 5
Selected Studies of Aldehyde Dehydrogenase 1 (ALDH1) Expression in Breast Tissue

Study (Year)	Assessment of ALDH1 Positivity	Frequency of ALDH1 Positivity	Study Findings
Kunju et al[8] (2011)	≥5% epithelial cells with cytoplasmic staining.	Case-control study of benign breast biopsies in women who subsequently developed breast cancer versus those who did not develop breast cancer.	Epithelial and stromal ALDH1 expression associated with increased risk of future breast cancer.
	Expanded stromal staining defined as extension beyond intralobular stroma into interlobular stroma.	43% of cases.	
	Scoring based on foci with maximal staining.	13% of controls.	
Ginestier et al[9] (2007)	Any cytoplasmic staining (ALDH1-positive cases noted to have only 5% cells expressing stain).	19% University of Michigan cases.	ALDH1-positivity associated with high-grade, estrogen receptor–negative tumors and poor prognosis.
		30% Institut Paoli-Calmettes cases.	
Nalwoga et al[10] (2010)	Combination of ALDH1 staining intensity and proportion of cells staining positive.	48% of Ugandan breast cancer cases.	ALDH1 expression associated with high-grade tumors and estrogen receptor–negativity
Zhou et al[32] (2010)	N/A	N/A	Meta-analysis of cancer stem cell studies in breast cancer; studies using ALDH1 as a cancer stem cell marker demonstrated strongest association between cancer stem cells and poor prognosis.
Neumeister et al[33] (2010)	N/A	N/A	Multiplexed staining for ALDH1 coexpressed with CD44 as a marker of cancer stem cells was associated with poor prognosis.
Park et al[34] (2010)	≥10% ALDH1 staining	9% pure IDC	ALDH1 positivity correlated with basal-like and HER2/neu-overexpressing tumors.
		6% IDC plus DCIS	
		3% DCIS plus microinvasion	
		3% pure DCIS	
Charafe-Jauffret et al[35] (2010)	Combination of ALDH1 staining intensity and proportion of cells staining positive	31% of inflammatory breast cancer cases	ALDH1-positivity associated with poor prognosis among cases of inflammatory breast cancer.
Yoshioka et al[36] (2011)	Any cytoplasmic staining in cancer cells	26% IDC	ALDH1 expression associated with poor prognosis in node-positive breast cancer.
		14% DCIS	
Resetkova et al[37] (2010)	Combination of ALDH1 staining intensity and proportion of cells staining positive.	18% all cancers	Tumor ALDH1 expression correlated with basal-like tumors.
		39% basal tumors	Stromal ALDH1 expression correlated with improved outcome.
Heerma van Voss et al[38] (2011)	Combination of ALDH1 staining intensity and proportion of cells staining positive.	78% BRCA1 mutation-associated cancers and 41.5% sporadic cancers with tumoral expression.	BRCA1 mutation-associated cancers more likely to be ALDH1-positive.
		58.5% BRCA1 mutation-associated cancers and 43.9% sporadic cancers with stromal expression.	

Abbreviations: DCIS, ductal carcinoma in situ; IDC, invasive ductal carcinoma; N/A, not applicable.