Cancer Epidemiology 37 (2013) 474–478

Contents lists available at SciVerse ScienceDirect

Cancer Epidemiology

The International Journal of Cancer Epidemiology, Detection, and Prevention

journal homepage: www.cancerepidemiology.net

Molecular subtypes of ductal carcinoma in situ in African American and Caucasian American Women: Distribution and correlation with pathological features and outcome

Bashar Sharaf Aldeen [a,*], Jining Feng [b], Yun Wu [c], Hind Nassar Warzecha [d]

[a] Department of Pathology, Wayne State University, Detroit, MI, USA
[b] Department of Pathology, William-Beaumont Hospital, Royal Oak, MI, USA
[c] Department of Pathology, UT MD Anderson Cancer Center, Houston, TX, USA
[d] Department of Pathology, Universitätsklinikum Tübingen, Tübingen, BW, Germany

ARTICLE INFO

Article history:
Received 1 January 2013
Received in revised form 11 March 2013
Accepted 19 March 2013
Available online 30 April 2013

Keywords:
DCIS
Molecular subtypes
Ethnicity
Basal-like

ABSTRACT

Background: Molecular subtypes of breast cancer have been extensively studied in invasive carcinoma. They were shown to have a different distribution within the various ethnic populations. Few studies have applied the same classification to Ductal Carcinoma in Situ (DCIS). We report the distribution of the molecular breast cancer subtypes in DCIS between African American (AA) and Caucasian American (CA) women, their association with pathological features and outcome. *Materials and methods*: Tissue microarrays were constructed from paraffin blocks of 94 DCIS cases (67 AA and 27 CA) selected from a cohort of AA and CA patients diagnosed with DCIS between 1996 and 2000; mean age at diagnosis was 61 ± 12 for the AA and 58 ± 11 years for the CA group. TMA blocks were labeled with antibodies for ER, PR, HER2, Ki-67, and CK5/6. The cases were subtyped as Luminal A (ER+ and/or PR+; HER2−), Luminal B (ER+ and/or PR+; HER2+), HER2+ (ER−, PR−; HER2+), basal-like (BL) (ER−, PR−, HER2−; CK5/6+) or unclassified triple negative (UTN) (ER−, PR−, HER2−, CK5/6−). Information on grade, size and follow-up were obtained. *Results*: (1) Most DCIS cases were Luminal A, comprising 80% of the DCIS cases in AA and 92.6% in CA patients. (2) HER2+, BL and UTN DCIS subtypes were not seen in the CA population, and formed 9% of the DCIS cases in the AA population; these cases were all high grade. (3) In the cases with recurrence (8 AA and 1 CA patients), DCIS was Luminal A in 6 AA and 1 CA and Luminal B in 2 AA patients. *Conclusion*: The distribution of the molecular subtypes of DCIS did not show a significant difference between the two ethnic groups in our study. In addition, the risk of recurrence might not be higher in the non-luminal subtypes than in Luminal A and B.

1. Introduction

Molecular profiling studies have identified at least 4 types of breast cancer strongly associated with different clinical outcomes, Luminal A, Luminal B, HER2+ and Basal-like (BL) [1–4]. Later studies have shown that immunohistochemical (IHC) labeling for ER, PR, HER2, CK5/6 or EGFR permits reliably to classify breast cancer into the same molecular categories [5]. Currently, it is widely accepted to group breast carcinomas that are positive for hormone receptors by IHC under Luminal A or B subtypes, tumors that are hormone receptor negative and HER2 positive under the HER2+ subtype, tumors that are negative for ER, PR and HER2 but positive for CK5/6 or EGFR under the BL subtype and carcinomas that are negative for all these markers under the term Unclassified Triple Negative (UTN). The distribution of these molecular phenotypes was shown to be different among various ethnic groups [6,7]; for example, the BL subtype appears to be more common in African American (AA) patients than in Caucasian Americans (CA). This is especially true for young premenopausal AA women, a finding that could explain why this group of patients with breast cancer has a poor outcome [7–11].

Recently, few studies have shown that, by using IHC, it is possible to classify Ductal Carcinoma in Situ (DCIS) into the same major molecular subtypes identified in invasive breast cancer [12,13]. However, the correlation between molecular phenotypes and clinical outcome is not as clear in DCIS as it is in invasive breast carcinoma. That may be due to the relatively smaller numbers and lower rate of recurrence of DCIS in comparison with invasive breast carcinoma. In addition, the distribution of the molecular phenotypes in DCIS is not consistent among the various studies and has not been documented within the different ethnic populations.

* Corresponding author at: Wayne State University, Pathology Department, 540 East Canfield Avenue, Detroit, MI 48201, USA. Tel.: +13137458555; fax: +1 3139932900.
E-mail addresses: bsharafa@med.wayne.edu, aldeen76@yahoo.com (B. Sharaf Aldeen).

1877-7821/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canep.2013.03.018

Knowing that DCIS is a (non-obligate) precursor for invasive breast cancer, we showed in a previous report that, unlike invasive breast carcinoma, DCIS has similar pathological characteristics and outcome in AA and CA patients [14]. In the current study, we analyze the IHC profile and molecular subtypes of DCIS in both ethnic groups and correlate these findings with various pathological parameters and follow-up.

2. Materials and methods

The study was approved by the institutional review boards at Wayne State University and The Johns Hopkins Medical Institutions.

2.1. Patient population

The population of this study derives from a cohort of 358 patients, 217 African American and 141 Caucasian American women, diagnosed with DCIS between 01/01/1996 and 12/31/2000 at the Detroit Medical Center/Wayne State University. The selection criteria and characteristics of this cohort were previously published in 2009 [14]. In summary, cases with invasive carcinoma before the diagnosis of the DCIS or 6 months after were excluded as well as patients with ethnicities other than AA or CA. From this cohort, we selected the cases that had 2 or more foci of DCIS or one focus larger than 5.0 mm and available paraffin blocks. The total number of patients included is 94 (67 AA and 27 CA).

2.2. Tissue microarrays

Tissue microarrays (TMA) were manually constructed. Each case was represented by two to five 1.0 mm diameter spot (average = 2.5 ± 1.1) depending on the amount of DCIS. These spots were arranged as nine rows and eleven columns. Column number 6 contained the control tissue, taken from benign breast tissue from the specimens and from non-breast tissue (brain, prostate, lymph node, kidney, duodenum, lung, pancreas, endometrium, liver, and small intestine).

2.3. Immunohistochemical staining

Three TMA blocks were obtained and labeled for ER, PR, HER2, Ki-67, and CK5/6 in one laboratory (the Johns Hopkins Hospital). Immunohistochemistry labeling for ER and PR were performed on the Benchmark XT autotimers (Ventana Medical Systems Inc., Tucson, AZ, USA) using I-View detection kit. The antibodies, dilutions, and sources were as follows: ER, monoclonal antibody; 1:100 dilution, Ventana Medical Systems Inc.; PR, monoclonal antibody, 1:60 dilution, DAKO; Ki-67, prediluted monoclonal antibody, Ventana Medical Systems Inc. HER-2 IHC was performed using the DAKO Herceptest kit according to the manufacturer's standard protocol. Cytokeratin 5/6 (CK 5/6) IHC using the Benchmark XT autostainer (prediluted monoclonal antibody, DAKO).

The immunostains were reviewed and scored by two authors (BSA, and HN). For the evaluation of ER and PR we used the Allred scoring [15,16]. After combining the proportion of positive DCIS cells and the intensity of staining, a score of 0–2 was considered negative, and 3–8 positive. HER2 was scored according to the CAP/ASCO criteria used for invasive carcinoma [17]. Cases with a score of (0–1) were considered negative; cases with (2+) and (3+) were counted as positive. Ki-67 proliferation index were scored as a percentage of nuclear staining in the neoplastic cells by semi-quantitative analysis; we reported the cases as negative when no staining was seen, weakly proliferative when 1–10% of the cells had positive labeling, and highly proliferative when >10% were positive. For CK5/6, any labeling in the neoplastic cells was considered positive and labeling of myoepithelial cells was not counted [12,18]. For each case each core was counted and an average final score was given. For the cases where a discrepancy between the two authors was present, the cases were re-reviewed and discussed until an agreement was reached.

2.4. Molecular subtypes

Using IHC, cases were classified into 4 categories (Luminal A, Luminal B, HER2+ or Basal-like) correlating with groups defined by gene expression profiling, as follows:

Luminal A: ER+ and/or PR+; HER2−.
Luminal B: ER+ and/or PR+; HER2+.
HER2+: ER−, PR− and HER2+.
Basal-like (BL): ER−, PR−, HER2−; CK5/6+.

If the tumors were negative for ER, PR, HER2 and CK5/6, they were grouped under the category of unclassified triple negative (UTN).

2.5. Statistical analysis

Continuous data were analyzed using Student independent sample t test (i.e., age at diagnosis), and Fisher exact and chi-square tests for categorical measures. Kaplan–Meier survival analyses were used to compare disease-free and overall survival times between groups. Significance values of $p < 0.05$ were considered statistically significant. All data were analyzed using SPSS v.15.0 for Windows (SPSS Inc., Chicago, Illinois).

3. Results

3.1. Clinical features and tumor characteristics

The population consists of 67 AA (71%) patients and 27 CA (29%) with a mean age at diagnosis of 61 ± 12 and 58 ± 11 years respectively ($p = 0.2$). The clinical features are summarized in Table 1. Radiology results and information on presentation were available for 31 AA and 11 CA patients. The lesions were diagnosed by screening mammography in 27 AA (87%) and 9 CA (82%) patients and were clinically symptomatic in 4 AA (13%) and 2 CA (18%) patients. Information on treatment was available for all patients. Surgical treatment consisted of breast conserving surgery in 45 cases (69%) and mastectomy in 20 cases (31%) in the AA group and 17 (63%) breast conserving surgery and 10 (37%) mastectomies in the CA group. Hormonal therapy was given to 11 AA (17%) and 6 CA (22%) patients and radiation therapy to 39 AA (60%) and 15 CA (56%).

The general tumor characteristics are summarized in Table 2. The mean size of the DCIS was 20.7 mm for AA and 12.9 mm for CA patients. In the group of AA patients, tumor grade was low (G1) in

Table 1
Clinical characteristics of the AA and CA patients with DCIS.

	AA	CA
Mean age (years)	61 ± 12	58 ± 11
Diagnosis (available cases 31 AA and 11 CA)		
Screening mammography	87%	82%
Clinical symptoms	13%	18%
Surgical treatment		
Mastectomy	31%	37%
Conservative surgery	69%	63%
Adjuvant therapy		
Radiation	60%	56%
Hormonal therapy	17%	22%