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Invasive breast carcinomas in Ghana: high frequency of high grade, basal-like histology and high EZH2 expression

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Abstract

Breast cancer in African-American women has a worse outcome than in Caucasian women. The ancestors of most African-American women come from West Africa, including Ghana. The Polycomb group protein EZH2 is a marker of poor outcome in breast cancers from Caucasian women. The histopathological features and biomarker expression of African breast cancers remain obscure. Here, we investigated a cohort of Ghanaian breast cancers to better define the prevalent tumor types and to test if EZH2 protein may identify aggressive tumors. A group of 169 breast tissues (100 invasive carcinomas and 69 benign) from women treated at Komfo Anoyke Teaching Hospital between 2006 and 2011 were histologically classified and investigated for EZH2 expression. EZH2 nuclear expression we defined as high or low following previously published criteria. Of the 100 invasive carcinomas, 89 % were ductal, 2 % were lobular, and 9 % were metaplastic. Basal-like pathological features were present in 30 % of the tumors. Of the invasive carcinomas, 7 % were grade 1, 41 % grade 2, and 52 % grade 3. EZH2 protein was overexpressed

in invasive carcinomas compared to benign breast ($p < 0.0001$). In invasive carcinomas nuclear EZH2 overexpression was significantly associated with basal-like subtype ($p = 0.03$) and high histologic grade ($p < 0.05$). Cytoplasmic EZH2, which has not been previously reported, was present in 16 % of invasive carcinomas and it was associated with triple negative status ($p = 0.02$). Our results provide the first comprehensive histopathological study of this patient population and uncover the association of EZH2 with high grade and basal-like tumors. We provide the basis for further detailed investigations on this cohort to advance diagnosis and treatment of African and African-American women.

Keywords

African-American; Breast cancer; EZH2; Enhancer of zeste 2; Triple negative; Basal like; Health disparity

Introduction

Compared to White American women, African-Americans have a lower lifetime incidence of breast cancer, yet they are likely to be diagnosed at a younger age and have higher mortality rates [1]. Invasive carcinomas in African-American women are more frequently high grade tumors that are negative for estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu overexpression (triple negative), and exhibit histological features of basal-like breast carcinomas [2, 3].

Basal-like breast cancers are one of at least five subtypes of invasive breast carcinomas originally defined by gene expression analysis. Basal-like tumors typically affect young patients, demonstrate rapid progression and are associated with poor outcome [4–7]. In the United States, basal-like breast cancers comprise approximately 15–20 % of all breast carcinomas and the majority of triple negative tumors [4–7]. Basal-like breast cancer occurs more frequently in African-American women compared to White Americans [5, 8]. The most distinctive pathological features of basal-like breast carcinomas are a pushing border, geographic necrosis, basaloid cells with high proliferation, areas of poorly differentiated squamous cell carcinoma and/or metaplastic, non-glandular elements [9, 10]. Over 75 % of basal-like carcinomas exhibit high histologic grade and triple negative status [4–7].

We have identified EZH2, a Polycomb group protein (PcG), as a critical oncogene specifically upregulated in hormone receptor negative carcinomas and their metastasis when compared to normal breast tissues [11, 12]. EZH2 expression is significantly associated with poor clinical outcome [12–16]. Our laboratory has reported that EZH2 downregulation in basal-like breast cancer cells MDA-MB-231 decreased their growth in vivo [17]. Recently, Polycomb group proteins, and especially EZH2, were suggested as candidates for targeted treatment for breast and other malignancies [18].

The genetic determinants for the high incidence of basal-like breast cancer phenotype in African-American women are currently unknown. Studies have shown that the ancestors of African-American women reside largely in West Africa [19, 20]. However, little is known about breast cancers in African women, and the histopathological features of invasive carcinomas from Ghanaian women are ill defined. We hypothesized that women from Ghana may develop breast carcinomas with basal-like histologic features, providing clues to the pathobiology of these tumors in African-American women. We further hypothesized that EZH2 levels increase in Ghanaian breast cancers compared to benign tissues, and that EZH2 expression may be associated with features of aggressive disease.

Materials and methods

Case selection

This research was approved by the University of Michigan Institutional Review Board 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) in Kumasi, Ghana. Formalin-fixed, paraffin-embedded breast tissue from women receiving treatment for benign and malignant disease at KATH between 2006 and April 2011 were transported to the University of Michigan where they were recut and stained by hematoxylin and eosin for histologic classification by the pathologists in the study (JCP and CGK). Given the limited financial resources available to support computerized/electronic records and lack of comprehensive hard-copy medical records, the clinical information obtained on the study patient population is not comprehensive. In order to standardize the clinicopathologic assessment for this study population, we relied exclusively on cases for which medical records were available in the KATH Department of Pathology.

Histopathological study and immunohistochemical analyses

Histopathological evaluation was performed by two participating pathologists (JCP and CGK) with expertise in breast disease. Differences in opinion were rare and were resolved by consensus evaluation of the case among the pathologists. Morphological parameters evaluated in this study included type of the invasive carcinoma, Nottingham tumor grade, and the presence of basal-like histologic features including geographic necrosis, basaloid cells with mitotic activity, areas of squamous differentiation, and the presence of metaplastic elements. Benign breast tissues were also evaluated and diagnosed.

Immunostaining for EZH2 was performed on both malignant and benign breast tissues, following the manufacturer's protocol. We utilized standard biotin-avidin complex technique using a monoclonal antibody against EZH2 (1:300, BD Biosciences, San Diego, CA). EZH2 protein expression was scored based on a validated 1–4 scale: no nuclear staining = 1+, nuclear staining in <50 % of cells = 2+, intermediate staining in >50 % of cells = 3+, and strong staining in >50 % of cells = 4+. Scores of 1–2+ were considered low expression and scores of 3–4+ were considered high expression based on previous tissue based and functional biologic studies [11, 12, 17, 21, 22]. Cytoplasmic staining was noted as present or absent. Immunostaining for ER, PR, and HER-2/neu were performed previously on all invasive carcinomas by the University of Michigan immunohistochemistry core laboratory.

Statistical analysis

Statistical analyses were carried out to determine whether significant associations existed between EZH2 expression in benign tissues and invasive carcinomas. We also investigated whether EZH2 expression in invasive carcinomas was associated with Nottingham grade, estrogen and progesterone receptors and HER-2/neu overexpression. Statistical analyses were conducted using the SAS System, version 9.3 (Cary, NC, USA). Associations were measured by creating contingency tables and using either the Chi-square test statistic or the Fisher's exact test statistic, if table cell frequencies were below 5. *P* values at or below 5 % were considered significant. All statistical analyses were performed by the biostatistician in the study (KAG).

Results

Histopathological characteristics of Ghanaian breast carcinomas

The clinicopathological features of the Ghanaian benign biopsies and invasive carcinomas are summarized in Table 1. All patients were female. In 79 of the 100 patients, information was available on age at diagnosis. In these patients, the median age was 46 years (range 24–92 years). 75 % of invasive carcinomas were negative for ER, 76 % were negative for PR and 87 % were negative for HER-2/neu overexpression by immunohistochemistry.

Of the 100 invasive carcinomas, 89 were ductal, 2 were lobular, and 9 were metaplastic. Of the invasive ductal carcinomas, the most prevalent histological patterns included papillary and micropapillary (14, 16 %), poorly differentiated squamous (5, 6 %), and medullary-like features (9, 10 %) (Fig. 1a, b). Of the 9 metaplastic carcinomas, 7 (78 %) had squamous and/or spindle areas, and 2 (22 %) exhibited heterologous elements including mesenchymal and chondroid differentiation (Fig. 1c, d). Most invasive carcinomas had Nottingham grades 2 or 3 (41 and 52 %, respectively), while 7 % exhibited grade 1. Given the reported higher frequency of basal-like features in African-American women, we specifically evaluated for these features in our cohort. High histological grade, areas of geographic necrosis, medullary-like, squamous and/or metaplastic elements were found in 30 tumors (Fig. 1a–d). Twenty nine of the 30 (96.7 %) invasive carcinomas with basal-like features were Nottingham grade 3, 1 (3.3 %) was grade 2, and none were grade 1. The benign biopsies consisted of fibroadenomas, papillomas, fibrocystic changes and duct ectasia.

EZH2 protein is elevated in invasive carcinomas from Ghana and is associated with basal-like histologic features

To begin to elucidate the associations between EZH2 protein and the histopathological features of Ghanaian breast cancer, we first compared EZH2 nuclear expression in benign breast tissues and invasive carcinomas. EZH2 protein was overexpressed in invasive carcinomas compared to benign breast ($p < 0.0001$). High EZH2 nuclear expression was present in 42 % of invasive carcinomas but in none of the benign biopsies (Fig. 2a, b).

In invasive carcinomas, nuclear EZH2 overexpression was associated with higher tumor grade. High EZH2 expression was present in 0, 10 (24 %), and 32 (62 %) of Nottingham grade 1, 2, and 3 invasive carcinomas, respectively ($p = 0.0005$), Table 2. High nuclear EZH2 expression was associated with basal-like histologic features ($p = 0.03$) with the greatest intensity seen in those with medullary features (Fig. 2b). High nuclear EZH2 was associated with HER-2/neu overexpression ($p = 0.03$).

EZH2 was expressed in the cytoplasm of 16 % of invasive carcinomas, a pattern previously undescribed. Cytoplasmic EZH2 occurred in the absence of nuclear EZH2 expression in the majority of these cases (14 of 16, 87.5 %), Fig. 2c. Cytoplasmic EZH2 expression was significantly associated with negative ER ($p = 0.0099$) and triple negative status ($p = 0.02$), Table 2.

Discussion

Despite adjusting for accessibility to health care, socioeconomic status, stage of disease, and age, African-American women with breast cancer have a worse outcome than white Americans [23, 24]. It has been reported that breast cancer in African-Americans is more likely to be early-onset, high grade, and estrogen receptor negative compared to white Americans [2, 3]. The reasons for these differences remain ill defined, but genetic background may play an important role. Given the genetic link between African-American women in the US and native West African women from Ghana it is likely that tumors share

also phenotypical and clinical characteristics. In this study of 100 patients with invasive breast carcinomas diagnosed and treated in Ghana, we comprehensively characterized the pathological features and expression of the oncogenic protein EZH2 as an initial step to shed light into their pathobiology.

Several insights emerge from our study. Invasive carcinomas in Ghanaian women have a high frequency of high histological grade with 52 % of Nottingham grade 3 (of 3) tumors. We also noted that a large proportion of invasive carcinomas from Ghanaian women had histological features associated with the basal-like subtype of breast cancer. While in the United States, basal-like cancers represent an average of 16.7 %, expression profiling of invasive carcinomas from Nigeria and Senegal has shown that tumors with basal-like molecular features comprise 27 % of invasive carcinomas [20]. In our study, although immunohistochemical stains for basal markers (i.e., CK5/6, EGFR) were not performed; histological features of basal-like carcinoma including geographic necrosis, squamous metaplasia, medullary features (pushing border, syncytial arrangement of cells, stromal lymphocytic infiltrate) and metaplastic components were seen in 30 % of the invasive carcinomas. This is in agreement with recent studies which have found that a basal-like phenotype is frequent in West Africa (Nigeria and Senegal) and East Africa (Uganda), ranging from 22 to 27 % of all breast cancers [20, 25, 26]. However, the frequency of basal-like breast carcinomas is not uniformly high in Africa, as Awadelkarim et al. [27] reported that basal-like tumors constitute 10 % of invasive carcinomas in Sudanese women. Taken together, our data show that 30 % of breast cancers from Ghana have histological features of basal-like carcinomas and underscore the influence of genetic, ethnic, and racial factors in the determination of breast cancer subtypes.

Our data show that nuclear EZH2 protein is overexpressed in invasive carcinomas compared to benign breast tissues. High nuclear EZH2 levels were detected in 42 % of invasive carcinoma and in none of the 69 benign breast tissues. The 16 ductal carcinoma in situ lesions in our study cohort exhibited similar EZH2 expression levels to the adjacent invasive carcinoma. Collectively, these results suggest that EZH2 increases during breast cancer progression and is elevated before stromal invasion occurs.

In invasive carcinomas, EZH2 overexpression was associated with basal-like histologic features and high tumor grade, a measure of the degree of differentiation, mitotic activity, and an indicator of clinical outcome [28]. It has been reported that EZH2 overexpression in breast cancer tissues is associated with the proliferation rate as measured by Ki-67 staining [16, 29]. These data are consistent with studies which have shown that EZH2 plays a central role in cell proliferation [30, 11, 17, 18, 21]. Our laboratory has demonstrated that EZH2 knockdown in basal-like breast cancer cells decreases proliferation in vivo and in vitro, while EZH2 overexpression in benign breast cells increases their mitotic fraction [17, 21].

EZH2 overexpression has been consistently linked with negative hormonal receptors but its relationship with HER-2/neu status has not been established [12, 13, 15, 16]. Our previous data on 194 unselected invasive carcinomas of the breast found no association between EZH2 and HER-2/neu status [12]. EZH2 was found to be associated with HER-2/neu negative status [31], while a recent study from our laboratory including Stage I and IIA invasive carcinomas, found an association between high EZH2 and HER-2/neu overexpression [13]. A mechanistic link between EZH2 and HER-2/neu has not been reported. Our data suggest a complex relationship between these oncogenes which may vary with the stage or other clinical and pathological features of the invasive carcinomas, and warrants further investigation.

Mechanistic studies on EZH2 function in hematologic and solid malignancies demonstrate that EZH2 exerts transcriptional regulatory functions in the nucleus [18, 32–38]. Su et al. [39] have reported that EZH2 may regulate actin polymerization in various cell types and thus have proposed that EZH2 may be involved in nuclear and extra-nuclear signaling processes. Here, we discovered that EZH2 is expressed in the cytoplasm of 16 % of invasive carcinomas, with the majority of positive cases lacking nuclear EZH2 expression (14 of 16 cases, 87.5 %). Interestingly, we found that cytoplasmic EZH2 is associated with triple negative status. Taken together, our results suggest that EZH2 may have cytoplasmic functions in a subset of triple negative breast cancers, which open new testable hypotheses.

Therapeutic options against breast cancer in African and African-American women are not as efficacious as desired due to the high frequency of triple negative tumors. The Polycomb group proteins are rational targets for treatment [18]. Studies have shown that EZH2 overexpression induces histone deacetylase (HDAC) enzymatic activity [40] and that EZH2 mediated cell invasion is abrogated by the HDAC inhibitors trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) [12]. Several HDAC inhibitors, such as SAHA, have been shown to have clinical utility as anti-tumorigenic agents [41]. Another experimental approach to inhibit EZH2 activity is by utilizing the carbocyclic adenosine analog 3-deazaneplanocin (DZNep). This compound has a global inhibitory effect on histone methylation [42]. DZNep was reported to reduce proliferation in a subset of breast cancer cell lines [43] and was recently found to be effective against BRCA1-deficient breast cancer cells [44]. Efforts are also directed toward development of specific EZH2 inhibitors. It is possible that these inhibitors may be useful therapeutic compounds in EZH2 overexpressing tumors.

In conclusion, our study is the first detailed histological characterization of a large cohort of invasive breast carcinomas from Ghana. We found a high frequency of basal-like, high grade tumors with squamous differentiation. EZH2 overexpression occurs in 42 % of the invasive carcinomas and is associated with basal-like features. We made the novel observation that EZH2 is expressed in the cytoplasm of breast cancer cells in a subset of tumors. The main limitation of this study is that the patient population was restricted to those with medical records available, with the risk of not being entirely representative of the whole Ghanaian population. Our study provides the basis for further biological and clinical studies to better understand the pathobiology and therapeutic opportunities for breast cancer in African and African-American women.

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Fig. 1. Histopathological features of Ghanaian invasive carcinomas. **a** and **b** Invasive carcinoma with high Nottingham grade, and basal-like features including syncytial growth pattern and central geographic necrosis. **c** Focus of squamous differentiation in a Ghanaian invasive carcinoma. **d** Metaplastic carcinoma with chondroid differentiation

Fig. 2. EZH2 protein expression in benign tissue and in invasive carcinomas from Ghana. **a** Fibroadenoma showing occasional epithelial cells expressing low nuclear EZH2. **b** A basal-like carcinoma with high EZH2 nuclear expression. The inflammatory cells mixed in with the tumor cells are negative for EZH2. Also note the EZH2 localization to the mitotic figures, consistent with a reported role of EZH2 in mitosis [17, 21]. **c** Representative picture of an invasive carcinoma with cytoplasmic EZH2 expression. The nuclei of the cancer cells are negative for EZH2. This pattern of EZH2 expression has not been described

Table 1

Clinical and pathologic features on 169 breast biopsies from Ghana

Characteristic	
No. patients	169
Benign biopsies	69
Invasive carcinoma	100
Median age (range)	46 (24–92 years)
Histological grade, <i>n</i> (%)	
1	7 (7)
2	41 (41)
3	52 (52)
Histologic type, <i>n</i> (%)	
Ductal	89 (89)
NOS	53 (59)
Papillary/micropapillary features	14 (16)
Medullary-like	9 (10)
Poorly differentiated squamoid areas	5 (6)
Apocrine features	5 (6)
Mucinous features	3 (3)
Lobular	2 (2)
Metaplastic	9 (9)
Squamous/spindle	7 (78)
Mesenchymal	2 (22)

Table 2

EZH2 expression in relationship to the pathologic features of 100 invasive carcinomas from Ghana

Parameter	<u>Nuclear EZH2</u>		<u>Cytoplasmic EZH2</u>	
	Low	High	Negative	Positive
Diagnosis				
Benign lesion	69 (100)	0 (0)	67 (97)	2 (3)
Invasive carcinoma	58 (58)	42 (42)*	84 (84)	16 (16)
Invasive carcinomas				
Nottingham grade				
1	7 (100)	0 (0)	6 (86)	1 (14)
2	31 (76)	10 (24)	34 (83)	7 (17)
3	20 (38)	32 (62)*	44 (85)	8 (15)
Histologic type				
Basal-like	12 (40)	18 (60)*	25 (83)	5 (17)
Non basal-like	46 (66)	24 (34)	59 (84)	11 (16)
Hormonal receptors				
ER				
Negative	42 (56)	33 (44)	59 (79)	16 (21)*
Positive	16 (67)	9 (33)	25 (100)	0 (0)
PR				
Negative	42 (55)	34 (45)	61 (80)	15 (20)
Positive	16 (67)	8 (33)	23 (96)	1 (4)
HER-2/neu				
Not overexpressed	54 (62)	33 (38)	72 (8)	15 (92)
Overexpressed	4 (31)	9 (69)*	12 (92)	1 (8)
Triple negative	35 (58)	25 (42)	46 (77)	14 (23)*
Non-triple negative	23 (58)	17 (42)	38 (95)	2 (5)

*
 $p < 0.05$