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Characterizing breast cancer in a population with increased prevalence of triple negative breast cancer: Androgen Receptor and ALDH1 expression in Ghanaian women

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Abstract

Introduction—Androgen Receptor (AR) is the most commonly-expressed nuclear hormone receptor in breast cancer and may be a marker of response to targeted anti-androgen therapy, a particularly attractive option in the setting of triple negative breast cancer (TNBC). Gene expression studies suggest that AR-positivity may distinguish a luminal/AR TNBC subtype from mesenchymal, stem cell-like, and basal-like subtypes. Furthermore, frequency of TNBC is 2–3-times higher in African American and African compared to White American and European breast cancer pts, yet little is known regarding the distribution of TNBC subtypes in the high-risk African-ancestry populations. We sought to characterize AR expression and TNBC patterns among a series of breast cancers from Ghana, Africa.

Methods—Formalin-fixed, paraffin-embedded invasive breast cancer specimens from 147 pts treated at a single teaching hospital in Ghana were studied at a comprehensive cancer center in the United States and analyzed for estrogen receptor (ER), progesterone receptor (PR), HER2/neu, ALDH1 and AR expression via immunohistochemistry.

Results—Median patient age was 45 (range, 28–76yrs). Only 31 cases (21%) were ER-positive, and 14 (10%) were HER2-positive; 89 tumors (61%) were TNBC. For the entire group, 44% were AR-positive and 45% were ALDH1-positive. ER/PR-positive tumors were more likely to be AR-positive compared to ER/PR-negative tumors (87% versus 26%; $p<0.0001$) but there was no association between ALDH1 and AR expression. Among the TNBC cases, 45% were ALDH1-positive and 24% were AR-positive. ALDH1-positivity versus negativity was associated with AR-positivity within the subset of TNBC tumors (36% versus 14%; $p=0.019$).

Conclusions—We confirmed the results of others showing that the majority of African breast cancers are triple-negative. We also found that AR expression is lower than that reported in other populations. Surprisingly, a marker of mammary stem cell expression was found to correlate with AR expression among triple negative tumors in this series, suggesting that novel TNBC subtypes may be identified by studying TNBC patterns among more diverse international populations.

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Keywords

triple negative breast cancer; androgen receptor; ALDH1

Introduction

Gene expression studies[1,2] have ushered in the era of intrinsic breast cancer subtypes, with several general categories identified, including four predominant subtypes: luminal A; luminal B; HER2/*neu*-overexpressing; and basal. The basal subtype tends to be the most prognostically-unfavorable. In clinical practice we identify patients with basal breast cancer as those whose cancers are negative for expression of the estrogen receptor (ER); the progesterone receptor (PR); and the HER2/*neu* marker, and this phenotype is commonly called triple negative breast cancer (TNBC). While the majority (approximately 80%) of TNBC tumors are indeed of the basal subtype, there is actually substantial diversity in the biologic behavior of these tumors[3,4]. Some of this variation in TNBC tumor biology can be appreciated in relation to histologic patterns, and recent gene expression studies of TNBC cases have identified at least six different TNBC subtypes with varying degrees of luminal versus mesenchymal/stem cell-like features[5,6]. Distinguishing the TNBC subtypes is likely to be clinically relevant, as the luminal subtype is less-likely to respond to neoadjuvant chemotherapy[7], but may be amenable to novel endocrine therapies, such as anti-androgen manipulation[8–13]. Generating a complete profile of TNBC subtypes is therefore important, however the studies reported to date have been based upon data from European, American (predominately Caucasian), and Asian populations. TNBC is more prevalent among African American and sub-Saharan African breast cancer patients yet little is known TNBC subtypes in these populations. We used immunohistochemistry (IHC) evaluations of Androgen Receptor (AR) and the mammary stem cell marker ALDH1 to generate novel information regarding luminal versus stem cell-like characteristics of TNBC cases from the sub-Saharan African country of Ghana.

Methods

The conduct of this research was approved by the University of Michigan (UM) 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.

The malignant nature of all specimens was first confirmed at UM by histopathologic evaluation of the hematoxylin and eosin-stained slides. Immunohistochemistry was then performed at UM for expression of estrogen receptor (ER); progesterone receptor (PR); HER2/*neu* (HER2); and ALDH1.

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 Cerb-2 (Her 2/*neu*). Pretreatment in Citrate Buffer pH 6.0 for 15 minutes was used for ER and PR. EDTA for 15 minutes was used for ALDH1. All slides were stained on the Dako Automated

Immunostainer. Cerb-2 (Dako North America) was used at a dilution of 1:100, ER (Dako North America, clone ID5) at 1:50, ALDH1 (BD Biosciences, clone 44) at 1:500 or 1:1000 and PR (Dako North America, clone PgR636) at 1:50. Antibodies were detected with either Envision^{+}Rabbit HRP (Cerb-2), 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 University of Michigan 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/*neu* negative and positive if it received IHC score of 3^{+}. Fluorescent in situ hybridization (FISH) typically used to assess amplification of the HER2/*neu* gene in cases with a score of 2+ was not needed as 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. Androgen receptor expression was initially scored as 1+ (negative); 2+; 3+; or 4+. These scores were dichotomized as positive if at least 10% nuclear staining was observed, corresponding to 1+ versus 2+; 3+; and 4+.

Results

Invasive breast cancer samples from 147 Ghanaian women were available for analyses, and 134 had adequate tissue available for immunohistochemical evaluation of all five markers (ER, PR, HER2/*neu*, AR, and ALDH1). Thirteen cases (8.8%) had inadequate tissue for evaluation of AR expression. Clinicopathologic characteristics for the study patient population are shown in Table 1. Due to the sparse medical record-keeping and tumor registry resources at KATH, very limited clinical information was available for the cases analyzed. Median patient age was 45 years (range, 28–76). Three-quarters of all cases were ER-negative (116/147, 78.9%) and 60.5% (89/147) were triple negative. For the entire group, 59 (44%) were AR-positive and 66 (45%) were ALDH1-positive. Hormone receptor-positive tumors were more likely to be AR-positive compared to ER/PR-negative tumors (87% versus 26%; $p<0.0001$) but there was no association between ALDH1 and AR expression (Tables 2 and 3). Among the TNBC cases, 45% were ALDH1-positive and 24% were AR-positive. As shown by Table 4, ALDH1-positivity was associated with AR-positivity within the subset of TNBC tumors (36% versus 14%; $p=0.019$).

Discussion

The biologic heterogeneity of breast cancer is clearly documented by data from genetic profiling studies[1,2]. The four general categories (subtypes) of invasive tumors that have been most extensively studied are: luminal A; luminal B; HER2-positive; and basal. These subtypes vary prognostically as well as with regard to treatment sensitivity and targeted

therapies. The luminal subtypes tend to be the most biologically favorable tumors because they can be manipulated with endocrine therapies; the HER2/*neu*-overexpressing tumors can be managed with the expanding array of commercially-available anti-HER/*neu* agents. In contrast, the basal subtype tends to be a biologically more aggressive pattern of disease and in clinical practice we identify these as the triple negative breast cancers (TNBC).

TNBC is defined as an invasive breast tumor that is negative by immunohistochemistry (IHC) for both the estrogen receptor (ER) and the progesterone receptor (PR); and negative for evidence of HER2/*neu* overexpression by IHC and/or fluorescence in situ hybridization (FISH) analysis. It is important to emphasize that while there is approximately 80% overlap between TNBC and the basal breast cancer subtype, these two patterns of disease are not synonymous. Substantial diversity in histology and tumor biology exist within the group of cancers that are negative for all three tumor markers[3,4], and genetic profiling studies are now defining TNBC subtypes that have varying degrees of chemosensitivity, risk of relapse, and response rates to neoadjuvant chemotherapy[5,7,14].

Lehmann et al evaluated the gene expression profiles from 21 publicly-available datasets (including 587 cases of TNBC) and identified six different TNBC subtypes by cluster analyses. These TNBC subtypes featured a spectrum of inherent degrees of aggressiveness and prominence of particular pathways, such as the luminal androgen receptor subtype (LAR) and the mesenchymal stem cell-like (MSL), with expression of the mammary stem cell marker ALDH1[15] being prominent in the latter MSL subtype. Furthermore, distinguishing between TNBC subtypes may have significant clinical relevance, since the LAR subtype is associated with substantially lower rates of response to neoadjuvant chemotherapy, as shown by a study from the M.D. Anderson Cancer Center[7]. Expression of the androgen receptor (AR) by IHC may therefore play a role in the clinical applicability of TNBC subtyping, and AR expression among TNBC cases opens the door to possible targeted anti-AR therapy for these tumors[9–11,14,16,17]. The mammary stem cell marker ALDH1 also appears to be prominent in differentiating the genetics of TNBC subtypes.

The hypothesis-generating prospect of more refined insights regarding TNBC prognoses and treatments based upon expression of AR and is exciting, but data regarding the full spectrum of TNBC pathology and genetics are unfortunately limited. In particular, data regarding TNBC subtypes have been predominantly generated by populations with European and East Asian backgrounds. These populations typically have TNBC frequencies of 20% or lower. In contrast, populations known to have a higher frequency of TNBC, such as those with African ancestry (African Americans and sub-Saharan Africans) have been underrepresented in studies of both TNBC subtypes and AR expression. The landmark Lehmann et al study included no datasets from Africa. Although no details are available on the racial/ethnic distribution of the six American datasets among the total twenty-one analyzed, it is likely that African Americans contributed a minority of the cases evaluated.

As insights regarding TNBC subtypes advance, it will be essential to insure that the full spectrum of TNBC cases are studied, including those that represent the diverse international breast cancer patient population. We therefore embarked upon this study with the goal of assessing frequency of AR and ALDH1 expression among a population known to have an

increased prevalence of TNBC. We have previously shown that the majority of breast cancers from the Komfo Anoyke Teaching Hospital (KATH) in Kumasi, Ghana are TNBC, and we have also reported on the increased expression of ALDH1 among Ghanaian breast tumors (especially those of the TNBC phenotype)[18,19].

In general, reviews of non-African breast cancer patient populations demonstrate that AR is expressed in 60–90% of cases[8,20], but usually fewer than half of TNBC cases[9]. We found a somewhat lower proportion of AR expression in the complete sample of Ghanaian breast cancers (44%) but a similarly low frequency of AR expression in TNBC cases (23%). Our finding that ALDH1-positivity among the TNBC cases was associated with an increased likelihood of AR-positivity was somewhat surprising, since the existing TNBC subtype studies suggest that androgenic pathways are distinct from the mesenchymal stem cell pathways in these subtypes. Our study is notably limited however in that we looked at very few molecular markers compared to the many pathways evaluated in the gene expression studies of TNBC subtypes. Furthermore, we do not know if IHC evaluation of tumor markers correlates closely with the various gene expression and cell cycle pathways. Nonetheless, our findings support the need for further investigation of TNBC subtypes in populations with African ancestry. These future studies may well elucidate other, as-yet uncharacterized TNBC subtypes and markers for targeted TNBC therapeutics.

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Synopsis

Recent research suggests that the androgen receptor and ALDH1 stem cell pathways are important in distinguishing between triple negative breast cancer subtypes. This project represents the first reported study of AR and ALDH1 in an African population known to have increased prevalence of TNBC compared to White Americans and Europeans.

Table 1

Distribution of Tumor Characteristics (n=147). Thirteen cases had inadequate tissue available for androgen receptor staining and analysis.

Feature		**N=147**
Median age, years (range)		43 (28–76)
Histology, n (%)	Ductal	134 (
	Mixed ductal and lobular	1
	Lobular	3
	Metaplastic	9
Estrogen receptor (ER)	Positive	31 (21.1%)
	Negative	116 (78.9%)
Progesterone receptor (PR)	Positive	41 (27.9%)
	Negative	106 (72.1%)
HER2/*neu*	Positive	14 (9.5%)
	Negative	133 (90.5%)
ER/PR	Positive	24 (16.3%)
	Negative	99 (67.3%)
	ER/PR Discordant	24 (16.3%)
Triple negative	Yes	89 (60.5%)
	No	58 (39.5%)
ALDH1	Positive	66 (44.9%)
	Negative	81 (55.1%)
Androgen receptor	1+ Negative	75 (56.0%)
	2+ Weak	23 (17.2%)
	3+ Moderate	28 (20.9%)
	4+ Strong	8 (6.0%)

Table 2

Distribution of Androgen Receptor expression among other molecular marker subsets*. Association of Characteristics with AR level for all samples with AR level (n=134)

	AR Level				
Characteristic	**1**	**2**	**3**	**4**	**p-value**
ER/PR					
Positive	3 (13.0)	10 (43.5)	8 (34.8)	2 (8.7)	<0.0001
Negative	65 (73.9)	9 (10.2)	12 (13.6)	2 (2.3)	
Triple Negative					
Yes	61 (76.3)	8 (10.0)	9 (11.3)	2 (2.5)	<0.0001
No	14 (25.9)	15 (27.8)	19 (35.2)	6 (11.1)	
ALDH1					
Positive	29 (48.3)	12 (20.0)	14 (23.3)	5 (8.3)	0.40
Negative	46 (62.2)	11 (14.9)	14 (18.9)	3 (4.1)	

AR level 1= AR negative

AR level 2= AR weakly positive

AR level 3= AR moderately positive

AR level 4= AR strongly positive

*Significant difference in AR level for ER/PR positive vs. ER/PR negative – most ER/PR negative have no AR level, whereas most ER/PR positive have weak-strong AR levels Significant difference in AR levels for Triple Negative vs. not TN-most TN tumors have no AR levels whereas most none-TN tumors have weak-strong AR levels

ALDH positivity distribution does not differ significantly between AR levels

Table 3

Association of Characteristics with Androgen Receptor (AR) level for all samples with AR level (n=134). AR level 1= AR negative; AR levels 2–4 =AR Positive.

	AR Level		
Characteristic	**1**	**2–4**	**p-value**
ER/PR			
Positive	3 (13.0)	20 (87.0)	<0.0001
Negative	65 (73.9)	23 (26.1)	
Triple Negative			
Yes	61 (76.3)	19 (23.8)	<0.0001
No	14 (25.9)	40 (74.1)	
ALDH1			
Positive	29 (48.3)	31 (51.7)	0.11
Negative	46 (62.2)	28 (37.8)	

Table 4

Association of ALDH1 expression with Androgen Receptor (AR) level among triple negative samples only (n=80).

	AR Level		
Characteristic	**1**	**2–4**	**p-value**
ALDH1			
Positive	23 (63.9)	13 (36.1)	0.019
Negative	38 (86.4)	6 (13.6)	

*Significant difference in ALDH1 positivity with AR level. Those which are ALDH1 positive are more likely to have AR 2+ to 4+