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Glucocorticoid receptor expression in breast cancer associates with older patient age

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Abstract

Breast cancer can be classified according to estrogen (ER), progesterone (PR), and HER2 receptor expression. Recent evidence suggests that activation of the glucocorticoid receptor (GR) contributes to breast cell survival, although the incidence of GR expression in primary human breast tumors is not well established. We therefore evaluated ER, PR, HER2, and GR by immunohistochemistry from 231 patients and found that while African American (AA) patient tumors were much more likely to be ER negative compared to tumors from non-AA patients, GR expression was significantly higher in tumors from patients 50 regardless of ancestry. Prospective examination of GR expression in tumors should be considered to determine whether GR contributes to long-term clinical outcome.

Keywords

Estrogen receptor; Progesterone receptor; HER2; Glucocorticoid receptor; Triple-negative disease; Health disparities

Introduction

Recently, several groups have made the unexpected observation that the prevalence of ER and PR expression in invasive breast cancer differs among pre-menopausal women from diverse ethnic and racial populations. This observation may explain some of the differences that have been noted in treatment outcome among subsets of breast cancer patients. Although additional nuclear hormone receptors have been observed to exist in breast cancer, these receptors have not been examined from ethnically diverse populations. However, we do know that additional nuclear receptors can exhibit distinct cell type-specific expression patterns in human breast tissue. For example, the human glucocorticoid receptor (GR) is expressed predominantly in normal human myoepithelial cells [1], and appears to be expressed with a highly variable frequency in breast cancers reported from Asian [1] versus European [2] populations. GR function is also cell-specific. Thus, GR activation in lymphocytes and lymphoproliferative tumors results in apoptosis [3], while glucocorticoids demonstrate an anti-apoptotic role in mammary epithelium following lactation [4], as well as in breast cancer cells subjected to chemotherapy-induced apoptosis [5]. Furthermore, activation of the GR has been demonstrated to favor cell survival in other epithelial cell types such as normal and malignant ovary [6].

In addition to endogenous glucocorticoids (cortisol) produced in response to acute and chronic stressors, synthetic glucocorticoids (e.g., dexamethasone) are routinely administered to breast cancer patients to decrease treatment-related allergic reactions and nausea. Of concern with this practice is that it has been shown in vitro that GR activation following glucocorticoid treatment decreases chemotherapy-induced apoptosis in breast cancer cells through up-regulation of pro-survival genes [7]. Furthermore, human breast cancer xenograft studies demonstrate that systemic glucocorticoid administration to immunocompromised host mice decreases chemotherapy-induced apoptosis [5]. In view of the routine use of glucocorticoids in breast cancer treatment, as well as the pro-cell survival potential for endogenous GR signaling in breast cancer, determining the expression of the GR in primary human breast cancers is of clear importance to breast cancer biologists, clinicians, and patients.

The GR is expressed in a cell type-specific fashion in almost all human tissues including immune, epithelial, and mesenchymal cells as well as in most non-small cell lung and human digestive system cancers [8–12]. We have previously examined GR expression using Western analysis in several commonly studied breast cancer cell lines and found varying steady-state expression levels of GR in cell lines with various combinations of ER, PR and HER2 expression; interestingly the lowest GR expression was seen in T47D cells that demonstrate overexpression of the PR [13]. In addition, two previous studies from Taiwan [1] and Spain [2], have examined GR expression in primary human breast tissues as well as in in situ and invasive carcinomas of the breast. These studies reported very different percentages of GR positivity in infiltrating ductal carcinomas (IDC) (2% vs. 40% GR); the reason for this discrepancy might include differences in the sensitivity and/or specificity of the anti-GR antibodies used, the uniform ancestry of the populations studied, or differing age groups represented in the two populations. In order to understand GR expression in primary human breast cancer more thoroughly, we retrospectively examined breast tissue

biopsies from an ethnically diverse patient population; these patients represent allcomers to an urban Chicago academic hospital. We used a well-characterized anti-human GR antibody as well as clinically validated anti-ER, -PR and -HER2 antibodies to examine receptor expression in primary tumors.

Previous studies have suggested that pre-menopausal African American (AA) women have a significantly higher incidence of ER-negative breast cancer when compared to pre-menopausal non-AA women [14, 15]. While ER-positive breast cancer risk has been positively linked to lifetime estrogen exposure [16], the risk factors for ER-negative breast cancers are much less well-defined, and the risk factors for GR-positive breast cancer are completely unknown. The importance of progesterone exposure to ER-negative breast cancer susceptibility is also not clear, with some evidence suggesting that progesterone is an anti-proliferative agent [17], while other studies implicate progesterone as a carcinogen [18]. Thus, while immunohistochemically detected combinations of receptor expression [14] [e.g., ER±/PR±/HER2-, ER±/PR±/HER2+, ER-/PR-/HER2+, and ER-/PR-/HER2- (triple negative)] correlate with distinctive gene expression signatures [i.e., Luminal A, Luminal B, HER2+, and basal-like, respectively], the genetic and environmental risk factors for these individual subtypes are not well understood. Therefore, the basis for the ethnic disparity observed in the incidence of triple negative disease in premenopausal women remains unclear [19].

Despite substantial evidence that GR activation plays an important role in epithelial tumor cell survival, the GR has not been extensively studied in primary invasive human breast cancers. Interestingly, an inverse relationship between ER and GR mRNA expression in breast cancer cell lines has been observed in vitro [20]. Furthermore, negative effects of estradiol treatment on GR mRNA expression [21] as well as ER-dependent proteasomal degradation of GR [22] have been reported in experimental systems. Taken together, these results suggest that the ligand-bound ER may negatively regulate GR expression in breast cancer cells. The observation that GR is often strongly expressed in myoepithelial cells of normal breast tissue, while ER and PR are infrequently expressed in these cells and more commonly expressed in luminal cells, further suggests a possible inverse relationship between ER and GR expression. In this study we examined this hypothesis, i.e., that ER negative breast cancers might correlate with GR positivity.

Using immunohistochemistry of tissue micro-arrayed breast cancers, we now show that in our mixed Northern American population of approximately 50% AA and 50% non-AA, 18% of IDC express GR (versus the 2% Taiwanese and 40% Spanish invasive breast cancers reported to be GR positive in previous publications). Interestingly, we find that GR expression is associated with tumors from older patients (> 50), perhaps reflecting the negative regulatory effect of estradiol on GR expression. Because, based on preclinical work, GR activation is believed to decrease chemotherapy sensitivity, the significantly higher expression of GR in older patient tumors may be a factor in the reported decreased sensitivity of older patients to polychemotherapy [23]. The unexpected finding of an association of GR expression with older patient age provides further evidence that human breast cancers from patients > 50 may demonstrate a different biological profile, independently of ER expression, from tumors in patients < 50 [24].

Material and methods

Tissue microarray (TMA) and Immunohistochemical (IHC) staining

Tissue microarrays were constructed by the Human Tissue Research Center (HTRC) of the University of Chicago with Institutional Review Board approval. Tissues from 231 breast cancer patients were obtained from patients who underwent surgical resections and agreed to tissue banking at the University of Chicago Medical Center between 1986 and 2003 under an Institutional Review Board Approved Protocol. H&E staining of formalin-fixed paraffin-embedded specimens was performed to define areas that represent either ductal carcinoma in situ (DCIS) and infiltrating ductal carcinomas (IDC). These areas were punched (2 mm cores) and arrayed with 60 cores per slide (non-duplicated). Tissue microarrays were cut into 4- μ m sections and mounted on slides. Fixation, blocking and staining followed standard clinical protocols. Omitting the primary antibody step served as a negative control. The following antibodies were used: anti-ERalpha (6F11, 1:80, Novocastra), anti-PR (RM-9102, 1:50, Thermo-Fisher), anti-GR (NCL-GCR, 1:20, Novocastra), and anti-HER2 (A0485 1:100, Dako).

Scoring

ER α , PR, and GR were scored by estimating the percentage of clearly positive (above background) nuclear staining using a 5-category scale (0: 0% expression; 1:<10%; 2: 10–50%; 3: 51–80%; and 4:>80%). Negative expression of ER, PR and GR was defined as 0% staining. HER2 was semi-quantitatively scored by estimating the percentage of membranous circumferential staining of the tumor cells using a 4-category scale (0: 0% expression; 1+: <10% of tumor cells show membranous stain; 2+: 10% of tumor cells show low/moderate stain; 3+: 10% of tumor cells show strong linear (not granular) circumferential membranous staining). Positive expression of HER2 was defined as 2+ and 3+ staining.

Statistical analysis

Associations between categorical variables (age group, ancestry, pathology and positive stain score) were analyzed using a Fisher's exact test. For univariate analyses of demographic characteristics and staining scores, the most positive single core score was used for patients with multiple cores of the same pathology present on the TMA. Multivariate analyses were done using generalized estimating equations (GEE) logistic regression models that appropriately account for the correlation between multiple cores per patient. Analyses were performed using the Stata v.10 statistical analysis program.

Results

Patient characteristics

As shown in Table 1, a total of 231 patients diagnosed with breast cancer were included for study. Among them, 90 patients (39%) were <50 years old and 141 (61%) were $\geq$ 50 years old, reflecting the usual age distribution of breast cancer within an ethnically mixed population. Patients were almost equally split in our study between African-Americans (AA) and non African-Americans (non-AA): 116 (51%) AA vs. 112 (48%) non-AA women; three patients had no ethnic designation. The non-AA group was mainly self-described as of

“European origin” with a few women of Hispanic ($n = 2$), Asian ($n = 3$), or Middle Eastern ($n = 2$) origin. The median age of AA patients was 61 and the median age of non-AA women was 53. The higher age of AA patients is inconsistent with published data suggesting that AA breast cancer patients are commonly younger than non-AA, but possibly reflects the referral pattern of predominantly older, Medicare-eligible AA patients to our Southside Chicago urban academic hospital.

IHC analysis of receptor expression

We analyzed the expression of ER, PR, HER2, and GR in individual patient specimens using tissue microarrays stained with appropriate antibodies at the concentrations listed in Table 2. Representative immunohistochemical staining of positive and negative receptor expression is shown in Fig. 1. The pathologic classification of samples as either DCIS or IDC is shown in Table 3. We observed increased expression of ER, PR, and HER2 in DCIS compared to IDC (79%, 59%, and 59% vs. 59%, 38%, and 29%, respectively). Likewise, GR expression was higher in DCIS compared to IDC (27% vs. 18%).

We next analyzed receptor expression in IDC only. Consistent with recent data [14], AA women had a higher prevalence of ER-negative IDC (58%), while non-AA women had only 30% ER-negative IDC ($P < 0.001$, Table 4). The difference in the prevalence of ER negative tumors was larger among women <50 (73% vs. 30%, AA vs. non-AA, $P < 0.001$). PR expression demonstrated a similar preponderance of negativity in AA women: 73% of AA women had PR-negative tumors while only 49% of tumors in non-AA women were PR-negative ($P = 0.003$). PR-negative tumors were more frequent in AA vs. non-AA women regardless of age group. Finally, the prevalence of HER2 positive tumors was higher in AA patients (29% vs. 16% in non-AA), although the difference was not statistically significant ($P = 0.06$).

Table 5 shows the distribution of immunohistochemical subtypes by patient age and ancestry. Consistent with recent observations [14, 25, 26], AA patients <50 had a very high percentage of ER-/PR-/HER2- i.e., “triple negative” tumors (55%); in contrast, non-AA patients <50 had a much smaller percentage (23%) of triple negative tumors ($P = 0.007$). Among AA patients, the prevalence of the ER/PR-positive subtypes increased significantly with age ≥ 50 (27% vs. 55%, $P = 0.015$). In contrast, non-AA patients did not demonstrate a significant difference in ER/PR-positive subtypes in the two age groups (70% vs. 72%).

The prevalence of tumor GR positivity is significantly increased in women ≥ 50

We next examined GR expression in order to determine whether similar relationships exist between GR, ancestry, and age. Surprisingly, we found that tumor GR expression significantly correlated with patient age, independently of ancestry ($P = 0.049$, Table 6). Specifically, GR was less frequently expressed in tumors from women <50 (12%) compared to patients ≥ 50 (25%). Subset analysis demonstrated that GR expression correlated with older patient age most significantly in non-triple-negative tumors [9% (<50) vs. 29% (≥ 50), $P = 0.01$]. In the triple-negative subtype, GR expression was higher in younger patients (19%) than older patients (13%), however, this correlation was not statistically significant ($P = 0.7$, Table 6). A logistic regression analysis confirmed that the relationship between age

and GR expression is indeed different depending on the triple negative status of the tumor ($P = 0.028$). Taken together, these data demonstrate for the first time that a significant interaction exists between triple negativity, age, and expression of GR; this interaction appears to be independent of ancestry.

Discussion

It is becoming increasingly clear that breast cancer is a highly heterogeneous disease and is characterized by variations in gene expression and nuclear receptor profiles in addition to differences in tumor differentiation and tyrosine kinase receptor subtypes. Four subgroups of tumors termed Luminal A, Luminal B, HER2+, and basal-like were identified previously based on gene expression signatures [19]. These subtypes roughly correlate with four immunohistological subtypes: ER+/PR+/HER2-, ER+/PR+/HER2+, ER-/PR-/HER2+, and ER-/PR-/HER2- (triple negative). There have been a few studies that have shown the interaction of these tumor types with ancestry, age, and other clinical and socioeconomic factors [27]. Most commonly, the triple-negative type of tumors makes up a higher percentage of tumors afflicting young AA patients and may contribute to the overall poorer disease-free survival outcomes that are seen in AA women [14, 25, 26]. In this study, we have confirmed that AA women <50 years old have a significantly higher percentage of triple negative tumors when compared to non-AA women <50.

Demographic data have long suggested that breast cancer has a bimodal age structure [28] in which ER-negative cancers are overall more frequent in younger patients, while ER positive breast cancers affect more older patients. We found that AA >50 patients indeed had an increased prevalence of ER positive tumors compared to younger AA patients; however, the non-AA patients had similar proportions of ER-positive tumors in both age groups using age 50 as a cutoff. These results are similar to North Carolina [14] and London [25] studies.

Interestingly, we found that individual expression of ER, PR, GR, and HER2 is more commonly found in DCIS compared to IDC. This is consistent with previous observation [2] and implies that IDC acquires additional mutations that obviate the need for these receptors to be expressed during early tumorigenesis. Although we initially hypothesized that ER expression would inversely correlate with GR expression, no such inverse correlation was found. Instead we found that in IDC, GR expression did not correlate with any of the commonly examined receptors. However, independent of ancestry, GR expression in non-triple negative tumors clearly correlated with older age. However, in triple negative tumors, GR expression appeared to inversely correlate with age, although our numbers of GR positive, triple negative breast cancers are low and therefore larger studies are clearly needed to confirm this hypothesis-generating finding.

Because GR activates known pro-survival pathways in breast cancer cells [5, 13], our results suggest that tumor GR expression might contribute to the relative polychemotherapy resistance observed in older (>50) breast cancer patients [23]. A prospective study of adjuvant chemotherapy and its effectiveness with respect to percentage GR positivity would help answer this question.

While the role of GR expression in breast cancer biology and resistance to chemotherapy is just beginning to be elucidated, many in vitro and in vivo models suggest that GR contributes to tumor cell chemoresistance via sequential and networked transcriptional activation of pro-survival signaling pathways. Although two previous studies examining GR expression in primary human breast cancers demonstrated highly variable percentages of GR positivity [1, 2], these differences may be related to antibody specificity, tissue preservation, and perhaps patient age and ancestry. By examining age at diagnosis, ancestry, and histopathological subtypes, we were able to see a significant correlation of GR expression with older age in non-triple negative cancers. Additional studies, also with age and ancestry available, are needed to determine the correlation of GR expression with triple-negative cancers. The selective expression of GR in a subset of pre-invasive and invasive breast cancers suggests that selective GR antagonists could be considered for both prevention and treatment strategies [29].

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Fig. 1. ER, PR, HER2 and GR expression in representative IDC subtypes. (a) Positive staining for GR in (i) ER±/PR±/HER2-, (ii) ER±/PR±/HER2+, (iii) ER-/PR-/HER2+, and (iv) ER-/PR-/HER2- subtypes. (b) Negative staining for GR in an ER±/PR±/HER2- tumor. In this case, GR staining of some stromal cells serves as a positive control

Table 1

Patient characteristics

Characteristic	n (%)
All patients	231 (100)
Age, median (year)	56
Range (year)	22–89
<50	90 (39)
50	141 (61)
AA	116 (51)
Age, median (year)	61
Range (year)	22–89
<50	37 (32)
50	79 (68)
Non-AA	112 (48)
Age, median (year)	53
Range (year)	24–86
<50	52 (46)
50	60 (54)
European	105 (45)
Hispanic	2 (1)
Asian	3 (1)
Middle Eastern	2 (1)
Missing ancestry	3 (1)

Table 2

Antibodies used in this study

Antigen	Antibody clone (Source)	Dilution	Retrieval method
Estrogen receptor	NCL-ER 6F11 (Novocastra)	1:80	Microwave
Progesterone receptor	RM-9102 (Thermo-Fisher)	1:50	Steamer
Glucocorticoid receptor	NCL-GCR (Novocastra)	1:20	Microwave
HER2	A0485 (Dako)	1:100	Steamer

Table 3

Prevalence of receptor expression, all available cores

Receptor ^a	Pathology	Negative <i>n</i> (%)	Positive <i>n</i> (%)
ER (<i>n</i> = 277)	DCIS (<i>n</i> = 56)	12 (21)	44 (79)
	IDC (<i>n</i> = 221)	90 (41)	131 (59)
PR (<i>n</i> = 280)	DCIS (<i>n</i> = 51)	21 (41)	30 (59)
	IDC (<i>n</i> = 229)	141 (62)	88 (38)
HER2 (<i>n</i> = 269)	DCIS (<i>n</i> = 49)	20 (41)	29 (59)
	IDC (<i>n</i> = 220)	157 (71)	63 (29)
GR (<i>n</i> = 275)	DCIS (<i>n</i> = 48)	35 (73)	13 (27)
	IDC (<i>n</i> = 227)	187 (82)	40 (18)

^a Scoring: ER, PR, and GR were scored as negative with 0%, and as positive with >0% staining; HER2 was scored as negative with <10% membranous staining, and as positive with 10% membranous staining

Table 4
Correlation of estrogen, progesterone, and HER2 receptors with ancestry and age of patients, *n* (%)

Age	Ancestry	ER		PR		HER2		<i>P</i> -value
		-	+	-	+	-	+	
All	AA	49 (58)	35 (42)	61 (73)	23 (27)	60 (71)	24 (29)	0.06
	Non-AA	25 (30)	58 (70)	41 (49)	42 (51)	70 (84)	13 (16)	
<50	AA	24 (73)	9 (27)	25 (76)	8 (24)	24 (73)	9 (27)	0.25
	Non-AA	12 (30)	28 (70)	19 (48)	21 (52)	34 (85)	6 (15)	
50	AA	25 (49)	26 (51)	36 (71)	15 (29)	36 (71)	15 (29)	0.151
	Non-AA	13 (30)	30 (70)	22 (51)	21 (49)	36 (84)	7 (16)	

Table 5

Prevalence of breast cancer subtypes according to age and ancestry

Patient age	Ancestry	Immunohistochemical subtype, <i>n</i> (%)				Total (<i>n</i> = 167)
		ER±/PR±/HER2– (<i>n</i> = 79)	ER±/PR±/HER2+ (<i>n</i> = 17)	ER–/PR–/HER2+ (<i>n</i> = 20)	ER–/PR–/HER2– (<i>n</i> = 51)	
<50	AA	6 (18)	3 (9)	6 (18)	18 (55)	33
	Non-AA	25 (63)	3 (8)	3 (8)	9 (23)	40
50	AA	21 (41)	7 (14)	8 (16)	15 (29)	51
	Non-AA	27 (63)	4 (9)	3 (7)	9 (21)	43
Total cores		97 (48)	24 (12)	23 (11)	57 (28)	201

Table 6Glucocorticoid receptor correlation with age, *n* (%)

Age	GR		
	Negative	Positive	P-value
In all tumors			
<50	64 (88)	9 (12)	0.049
50	70 (75)	23 (25)	
In non-triple negative tumors			
<50	42 (91)	4 (9)	0.01
50	49 (71)	20 (29)	
In triple negative tumors			
<50	22 (82)	5 (19)	0.7
50	21 (88)	3 (13)	