Breast Cancer: Basic and Clinical Research

ORIGINAL RESEARCH

Expression of the Androgen Receptor and its Correlation with Molecular Subtypes in 980 Chinese Breast Cancer Patients

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Abstract

Background: Recent studies have shown that androgen displays an inhibitory effect on breast cancer cell lines that express androgen receptor (AR) but not estrogen receptor (ER) and progesterone receptor (PR). We have previously reported that approximately 1/3 of ER negative high grade invasive ductal carcinomas express AR. Thus, AR can serve as a potential therapeutic target for this group of patients.

Aim: Here we investigated AR expression patterns in 980 consecutive breast carcinomas.

Results: We found that (1) AR was expressed more frequently (77%) than ER (61%) and PR (60%) in breast carcinomas; (2) AR expression was associated with ER and PR expression ($P < 0.0001$), small tumor size ($P = 0.0324$) and lower Ki-67 expression ($P = 0.0013$); (3) AR expression was found in 65% of ER negative tumors; (4) AR expression was associated with PR and Ki-67 in ER negative tumors, but not in ER positive tumors; (5) AR expression was higher in ER positive subtypes (Luminal A, Luminal B and Luminal HER2 subtypes, 80%–86%) and lower in ER negative subtypes [HER2, triple negative (TN), and TN EFGR positive subtypes; 52%–66%], with over 50% of TN tumors expressing AR.

Conclusion: More breast carcinomas express AR than ER and PR, including significant numbers of ER negative and TN tumors, for which AR could serve as a potential therapeutic target.

Keywords: androgen receptor, breast cancer, estrogen receptor, HER2, Ki-67, molecular classification, progesterone receptor

Breast Cancer: Basic and Clinical Research 2012:6 1–8

doi: 10.4137/BCBCR.S8323

This article is available from http://www.la-press.com.

Introduction

Breast cancer is one of the most common malignancies in women worldwide and the second most common cause of cancer-related death in women. Currently, breast cancer is treated by multi-modality therapies based on the pathologic characteristic of each tumor.[1,2] Targeting therapies to estrogen receptor (ER) and HER2 in breast carcinoma have shown remarkable clinical efficacy for the appropriate subsets of patients.[3,4] However, only about 15% and 70% of breast cancers are positive for HER2 and ER, respectively; and about 15% of the tumors are ER, progesterone receptor (PR) and HER2 negative [Triple Negative (TN) tumors],[5,6] which will not benefit from these targeted therapies.

The role of the androgen receptor (AR) in breast carcinomas has drawn great attention in recent years, especially due to its expression in ER and PR negative breast carcinomas.[7,8] We have previously reported that approximately 1/3 of ER negative high grade invasive ductal carcinomas express AR.[9] Breast cancer with BRCA1/2 mutants also shows a similar rate of AR expression.[10] AR expression also correlates with better prognosis for both primary and metastatic breast cancer.[11,12] A recent study has shown that androgen dehydroepiandrosterone sulfate (DHEAS) inhibits the growth of breast cancer cell lines that express AR but not ER and PR.[13] Thus, AR has been suggested to be able to serve as a potential therapeutic target for the subgroup of breast carcinomas that are AR positive/ER negative.[14]

Breast cancer is a very heterogeneous group of tumors that vary in clinical behavior and response to therapy. Gene expression profiling has provided the molecular basis for the heterogeneity of breast cancer; and also subdivided breast cancer into several clinically distinct molecular subtypes.[15–17] ER, PR, HER2, and more recently Ki-67, CK5/6 and EGFR have been used by many investigators as immunohistochemical (IHC) surrogates for molecular classification with comparable results.[18–22] Although no universally accepted definition for each subtype is present at this point, most studies have chosen to use definitions proposed by Nielsen,[18] Livasy[19] and Cheang.[20] This current study aims to investigate the expression patterns of AR in a large cohort of Chinese breast cancer patients, with a focus on ER negative and TN breast cancer, and breast cancer of different molecular subtypes.

Materials and Methods

Patient selection

A total of 980 consecutive cases of invasive breast carcinomas between 2004 and 2009 were reviewed from the files of the Department of Pathology of the First Clinical Hospital of Harbin Medical University in China. 89 cases of DCIS were also identified from the same period and excluded from the study. All patients underwent surgical excision before receiving chemotherapy, radiotherapy or adjuvant hormonal therapy. Clinicopathological information including age of patients, tumor size and lymph node status were obtained from the files. The histological types and grades of the tumors were determined by following the WHO classification (2003).[23]

Immunohistochemistry analysis

The study protocol was approved by the Human Ethics Review Committee of Harbin Medical University. The information for primary antibodies for AR, ER, PR, HER2, Ki-67, and EGFR used in the study is listed in Table 1. Formalin-fixed, paraffin-embedded tissue blocks were cut into 3 micron thick serial sections that were mounted on pre-coated slides. The sections were de-paraffinized, rehydrated, and rinsed in distilled water, and then treated with 3% hydrogen peroxide

Table 1. Sources and dilutions of antibodies.

Antibody	Poly- vs. monoclonal	Clone no.	Manufacturer	Dilution
AR	monoclonal	AR441	Maixin_Bio, China	Working liquid
ER	monoclonal	EP1	ZSGB-BIO, China	Working liquid
PR	monoclonal	SP2	Maixin_Bio, China	Working liquid
Ki-67	monoclonal	EP5	ZSGB-BIO, China	Working liquid
HER2	monoclonal	EP3	ZSGB-BIO, China	Working liquid
EGFR	monoclonal	EGFR.113	Maixin_Bio, China	Working liquid

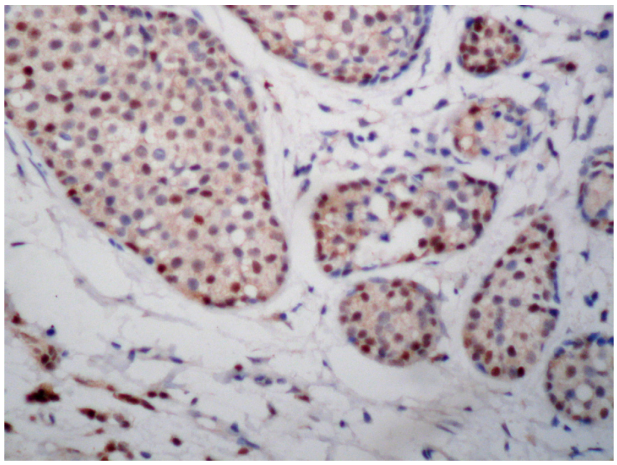

Figure 1. Immunohistochemical stain for AR, 40× of original magnification.

solution for 10 minutes to block endogenous peroxidases. Antigen retrieval was achieved by heating in a water bath with EDTA (pH 9.0; ZLI-9069; 901022, ZSGB-BIO, China) for AR, or in citrate buffer (pH 6.0) for ER, PR, HER2 and Ki67, or pretreated in pepsin (ZLI-9013; 572031A, ZSGB-BIO, China) for EGFR. The aforementioned primary antibodies were incubated for an hour, and then incubated with the secondary, biotinylated antibody for 30 minutes. After washing, sections were incubated with streptavidin-peroxidase for 40 minutes. 3,3′-diaminobenzidine (DAB) was used as chromogen. Samples were scored as positive for ER, PR and AR (Figure 1) when at least 10% of nuclei of tumor cells were immunoreactive. Ki67 expression was determined by mean positive nuclear staining percentage from 10 high power fields; over 14% is considered positive. HER2 was considered positive when >10% of tumor cells with 3+ membranous staining was observed; EGFR was considered positive when tumor cells with 1+ or greater staining were observed.

IHC-Based definitions for each subtype in the molecular classification

A 5 marker panel (ER, PR, HER2, Ki-67, and EGFR) was used to determine each subtype of breast cancer. The definition and number of breast cancer in each subtype for the current study are shown in Table 8.

Statistical analysis

Apart from the descriptive statistics, such as mean of continue variables and percent for categorical variables, the difference between AR positive and AR negative was tested by using two-sided Spearman two-sample test rank for continue variables and Fisher's exact test was used for categorical variables. The software SAS 9.2 (Cary, NC, USA) was used in the data analysis.

Results

The age of patients ranged from 22 to 94 years old, with a mean age of 51 years. Among the 980 cases, 868 were infiltrating ductal carcinoma (IDC), 52 were infiltrating lobular carcinoma (ILC), 19 were medullary carcinoma, 21 were mucinous carcinoma, and 20 were rare-types of breast carcinomas including squamous cell carcinoma, neuroendocrine carcinoma, etc. The rate of AR expression was 77%, and the rates for ER, PR and HER2 expression were 61%, 60%, 17%, respectively. AR expression was highest in ILC (87%) and lowest in medullary carcinoma (37%) (Table 2); and highest in grade 1 tumors (91%) and lowest in grade 3 tumors (57%) (Table 3).

AR expression was significantly associated with the expression of ER and PR ($P < 0.0001$), and also inversely related to tumor size ($P = 0.0324$) and Ki-67 expression ($P = 0.0013$). There were no significant associations between the expression of AR

Table 2. Expression of AR, ER, PR and HER2 in different types of breast carcinomas.

Type	Percent of positive expression (%)		
	AR	ER	PR
IDC[a] (868 cases)	77 (668 cases)	62 (538 cases)	59 (512 cases)
ILC[b] (52 cases)	87 (45 cases)	67 (35 cases)	71 (37 cases)
Medullary (19 cases)	37 (7 cases)	11 (2 cases)	11 (2 cases)
Mucinous (21 cases)	88 (18 cases)	76 (16 cases)	71 (15 cases)
Others[c] (20 cases)	60 (12 cases)	60 (12 cases)	85 (17 cases)
Total cases (980)	77 (755 cases)	61 (598 cases)	60 (588 cases)

Notes: [a]IDC, Infiltrating Ductal Carcinoma; [b]ILC, Infiltrating Lobular Carcinoma; [c]Others, squamous cell carcinoma, neuroendocrine tumors, etc.

Table 3. Expression of AR, ER, PR and HER2 in different histologic grades of IDC.

Type	Percent of positive expression (%)		
	AR	ER	PR
IDC (868 cases)	77 (668 cases)	62 (538 cases)	59 (512 cases)
*G1 (55 cases)	91 (50 cases)	82 (45 cases)	84 (46 cases)
G1-2 (41 cases)	90 (37 cases)	76 (31 cases)	71 (29 cases)
G2 (669 cases)	76 (509 cases)	62 (415 cases)	59 (395 cases)
G2-3 (73 cases)	77 (56 cases)	53 (39 cases)	55 (40 cases)
G3 (30 cases)	57 (17 cases)	30 (9 cases)	23 (7 cases)

Note: *G, grade.

and the ages of patients, lymph node involvement, or expression of HER2 and EGFR (Table 4).

The rates of AR expression were 84% for ER positive tumors and 65% for ER negative tumors (Table 5). Interestingly, when comparing the expression of AR in ER positive and ER negative tumors, AR expression was significantly associated with PR expression ($P < 0.0001$) in ER negative tumors (Table 7), but not in ER positive tumors (Tables 6). Similar observations were seen for the mean Ki-67 levels. Other factors including the age of patients, tumor size, nodal metastasis, and HER2 and EGFR expression were not associated with AR expression in either ER positive or ER negative tumors.

Among the 980 cases, the distribution of Luminal A, Luminal B, Luminal HER2, HER2 over-expression, TN and TN EGFR+ subtypes were 27%, 24%, 21%, 12%, 10% and 6%, respectively (Table 8). AR expression was higher in three ER positive subtypes (Luminal A, Luminal B and luminal HER2 subtypes, range: 80%–90%) and lower in three ER negative subtypes (HER2 over-expression, TN, and TN EFGR+ subtypes, range: 52%–66% Table 9). AR expression was significantly different between luminal HER2 and HER2 over-expression subtypes ($P < 0.0001$), while there was no significant difference between luminal A and B subtypes, luminal B and luminal HER2 subtypes, HER2 and TN subtypes, HER2 and TN EGFR+ subtypes, and TN and TN EGFR+ subtypes (Table 10).

Table 4. Correlation between AR expression and age, tumor size, LN stage, and expression of ER, PR, HER2, EGFR and Ki67.*

	r_s	*P* value
Age	0.13350	0.6022
Tumor size	–0.31765	0.0324
Nodal metastasis	–0.04456	0.1454
ER expression	0.54546	<0.0001
PR expression	0.32267	<0.0001
HER2 over-expression	–0.03476	0.7348
EGFR expression	–0.03090	0.4782
Ki-67 expression	–0.31765	0.0013

Discussion

Like ER and PR, AR is also a steroid hormone nuclear protein, but the clinical significance and functional role of AR has not been well defined in breast cancer.[24,25] Studies have shown that AR expression is found in about 70% of breast cancers,[26,27] similar to the 77% we found with our Chinese patients. A study from Poland with 488 cases showed the AR expression rate was 43%, but their criterion for its positivity was not mentioned.[28] We confirmed that AR is more frequently expressed in our Chinese breast cancer patients than ER and PR and its expression is closely associated with ER and PR. We observed that 65% of ER negative tumors express AR, higher than the 30% rate from other studies.[7,9,11,26,27] One possibility is the higher cut off (10%) used in the current study as a positive value for ER, PR, and AR, which defined more negative cases compared to the current CAP/ASCO Guideline.[29] The higher rates for ER negative tumors (39%) compared to other studies[30,31] may also be explained by the higher cut off point used in

Table 5. Expression of AR in ER positive and ER negative tumors.

	AR positive cases	Totals cases
ER positive	503 (84%)	597
ER negative	248 (65%)	383
Total	751 (77%)	980

Table 6. Clinical-pathological features for AR expression in ER+ tumors (597 cases).

	AR+ (503)	AR– (94)	*P* value
Age (mean years)	51.08	51.15	0.8394
Tumor size (mean cm)	2.06	2.17	0.6322
Positive nodal metastasis (253 cases)	217 (86%)	36 (14%)	0.4267
Positive PR expression (485 cases)	415 (86%)	70 (14%)	0.0832
HER2 over-expression (182 cases)	154 (85%)	28 (15%)	0.9035
Positive EGFR expression (303 cases)	249 (82%)	54 (18%)	0.1777
High Ki-67 expression (>14%) (283 cases)	233 (82%)	50 (18%)	0.2605
Mean Ki-67 expression level	20.04	22.73	0.3825

current study. Another possibility for this difference may due to the specificity of the antibodies used in the current study.[32] Nevertheless, we confirmed that a significant portion of ER negative tumors expressed AR in our patient population, which provides this subgroup with a potential therapeutic target.

Comparing the relationship between AR expression and molecular classification, we found that AR expression is higher in ER positive subtypes than in ER negative subtypes, which is comparable with previous reports,[31,33] though the definitions for each subtype were not identical. It is very interesting to see the significant difference in AR expression between Luminal HER2 and HER2 over-expression subtype. Both subtypes over-express HER2, but they are different in their expression of ER and/or PR, suggesting the close relation between AR and ER/PR. A recent study from Micello et al[34] reported a 77% AR expression rate in HER2 positive breast carcinomas, comparable with what we observed in this study (66%–86%). Our prior studies with 17 breast cancer cell lines also showed that all 4 ER negative HER2 positive cell lines expressed AR.[35] This is especially interesting since an earlier study[36] has suggested that in breast cancer, the growth inhibitory effect resulting from AR activation can counterbalance the growth stimulating effect by HER2 over-expression.

We did not observe an age difference between AR positive and AR negative tumors, even when we subdivided the tumors into ER positive and ER negative groups. Hu et al[37] recently reported that in postmenopausal patients, AR expression is associated with a more favorable prognosis among women with ER-positive tumors, but not among patients with ER-negative tumors. In fact, AR expression is associated with increased breast cancer-related mortality in their patients who have triple negative breast cancer. AR expression is associated with more favorable pathologic features including small tumor size ($P = 0.0324$) and lower Ki-67 expression ($P = 0.0013$) in current study, similar to previously reported studies.[31]

With the high expression rate of AR, especially in ER negative or TN breast cancer, investigations of AR as a therapeutic target have been the subject of extensive research.[14,38] Preclinical studies with breast cancer cell lines have shown the inhibitory role of androgen (such as dehydroepiandrosterone) on ER–/PR–/AR+ cells.[13] It is thought that if androgen were used to treat ER–/AR+ tumors, its therapeutic effect would be maximized with aromatase inhibitors, since

Table 7. Clinical-pathological features for AR expression in ER– tumors (383 cases).

	AR+ (248)	AR– (135)	*P* value
Age (mean years)	50.12	51.23	0.3955
Tumor size (mean cm)	2.09	2.28	0.0956
Positive nodal metastasis (163 cases)	98 (60%)	65 (40%)	0.1062
Positive PR expression (106 cases)	86 (81%)	20 (19%)	<0.0001
HER2 over-expression (145 cases)	102 (70%)	43 (30%)	0.0786
Positive EGFR expression (221 cases)	142 (64%)	79 (56%)	0.8293
High Ki-67 expression (>14%) (241 cases)	150 (62%)	91 (38%)	0.1861
Mean Ki-67 expression	26.19	33.98	0.0307

Table 8. The definitions for each molecular subtype.

Subtype	ER	PR	Ki67	HER2	EGFR	# of cases
Luminal A (ER+ and/or PR+, Ki67 < 14%, HER2–) 262 cases (27%)						
Luminal A	+	+	–	–	+	90
Luminal A	+	+	–	–	–	106
Luminal A	+	–	–	–	+	17
Luminal A	+	–	–	–	–	12
Luminal A	–	+	–	–	+	17
Luminal A	–	+	–	–	–	20
Luminal B (ER+ and/or PR+, Ki67 > 14%, HER2–) 233 cases (24%)						
Luminal B	+	+	+	–	+	73
Luminal B	+	+	+	–	–	80
Luminal B	+	–	+	–	+	19
Luminal B	+	–	+	–	–	18
Luminal B	–	+	+	–	+	21
Luminal B	–	+	+	–	–	22
Luminal HER2 (ER+ and/or PR+, HER2+) 208 cases (21%)						
Luminal HER2	+	+	+	+	+	40
Luminal HER2	+	+	+	+	–	27
Luminal HER2	+	+	–	+	+	39
Luminal HER2	+	+	–	+	–	30
Luminal HER2	+	–	+	+	+	15
Luminal HER2	+	–	+	+	–	11
Luminal HER2	+	–	–	+	+	10
Lzuminal HER2	+	–	–	+	–	10
Luminal HER2	–	+	+	+	+	7
Luminal HER2	–	+	+	+	–	8
Luminal HER2	–	+	–	+	+	5
Luminal HER2	–	+	–	+	–	6
HER2 Over-expression (ER–, PR–, HER2+) 119 cases (12%)						
HER2	–	–	+	+	+	49
HER2	–	–	+	+	–	28
HER2	–	–	–	+	+	22
HER2	–	–	–	+	–	20
Triple Negative (TN), EGFR+ (ER–, PR–, HER2–) 100 cases (10%)						
TN, EGFR+	–	–	+	–	+	73
TN, EGFR+	–	–	–	–	+	27
Triple Negative (TN), EGFR– (ER–, PR–, HER2–) 58 cases (6%)						
Triple Negative	–	–	+	–	–	33
Triple Negative	–	–	–	–	–	25

Table 9. AR expression in different molecular subtypes.

Molecular subtypes	Total #	AR+	%
Luminal A (ER+ and/or PR+, Ki67 < 14%, HER2–)	262	235	90%
Luminal B (ER+ and/or PR+, Ki67 > 14%, HER2–)	233	186	80%
Luminal HER2 (ER+ and/or PR+, HER2+)	208	178	86%
HER2 over-expression (ER–, PR–, HER2+)	119	78	66%
Triple negative (TN), EGFR+ (ER–, PR–, HER2–)	100	52	52%
Triple negative (TN), EGFR– (ER–, PR–, HER2–)	58	32	55%

Table 10. *P*-value of AR expression between subtypes.

Molecular subtypes	*P*-values
Luminal A vs. Luminal B	0.0927
Luminal B vs. Luminal HER2	0.1317
Luminal HER2 vs. HER2 Over-expression	<0.0001
HER2 vs. TN	0.1909
HER2 vs. TN EGFR+	0.0531
TN vs. TN EGFR+	0.7426

they block the in vivo conversion of androgen to estrogen.[39,40] For ER+ tumors, androgen can stimulate cell growth;[41,42] thus, the therapy should inhibit both ER and AR. Further studies on the role of AR in both ER+ and ER− breast cancers are warranted in order to better understand how this receptor might be exploited as a potential target for therapy.

Acknowledgment

The authors wish to thank Ms. Mary Jackson for her excellent clerical support.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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