original article

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Androgen receptor expression is significantly associated with better outcomes in estrogen receptor-positive breast cancers

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Background: The objective of the study was to evaluate the implications of androgen receptor (AR) in breast cancers.

Patients and methods: We investigated immunohistochemical AR expression from the tissue microarrays of 931 patients between 1999 and 2005, and analyzed demographics and outcomes using uni-/multivariate analyses. Tumors with ≥10% nuclear-stained cells were considered positive for AR.

Results: AR was expressed in 58.1% of patients. AR was significantly related to older age at diagnosis, smaller size, well-differentiated tumors, higher positivity of hormone receptors, non-triple-negative breast cancers (non-TNBCs), and lower proliferative index. In estrogen receptor (ER)-negative tumors, AR was distinctively associated with human epidermal growth factor receptor type 2 (HER2) overexpression. With a mean follow-up of 72.7 months, AR was positively related to survival in ER-positive but not in ER-negative tumors. In Cox's models, AR was an independent prognostic factor for disease-free survival in ER-positive cancers. Interestingly, molecular apocrine tumors (ER negative and AR positive) with HER2 positive status showed trends of poorer outcome, but AR had no impact on survival in patients with TNBC.

Conclusions: AR is significantly associated with favorable features in breast cancers and related to better outcomes in ER-positive not in ER-negative tumors. These results suggest that AR could be an additional marker for endocrine responsiveness in ER-positive cancers and a candidate for therapeutic targeting of ER-negative tumors.

Key words: androgen receptor, breast cancer, estrogen receptor, molecular apocrine, prognosis

introduction

Recently, it has been suggested that androgen receptor (AR) has an emerging role in patients with breast cancer [1, 2]. The molecular mechanisms of AR signaling pathway in breast cancer biology are not clearly understood, but a significant number of patients with breast cancer express AR in a primary or metastatic setting [3, 4]. A few retrospective case–control studies have demonstrated the important prognostic or predictive role of AR both in estrogen receptor (ER)-positive and in ER-negative tumors [5, 6]. Furthermore, AR expression is observed in patients with triple-negative breast cancer (TNBC) who have limited therapeutic options, and it has been implied that AR could be used as a therapeutic target for these patients [3, 7].

Serum androgen is a precursor for estrogen biosynthesis and also acts directly via AR. This complexity may make it difficult to define the role of AR in breast cancers. *In vitro* models demonstrate both stimulatory and inhibitory effects of androgen on growth of various breast cancer cell lines, and epidemiological evidence has shown controversial associations between serum androgen levels and risk for breast cancer [7–9]. Therefore, dual impacts of androgen and AR have been proposed that androgen might act as an estrogen antagonist in premenopausal patients with a highly estrogenic milieu and, by contrast, dehydroepiandrosterone (DHEA) would be more likely to serve as an estrogen agonist in postmenopausal women with much lower serum estradiol levels [9, 10]. In addition, because breast cancer is considered to be a heterogeneous disease demonstrated by gene expression profiling [11], all patients with breast cancer are not expected to have a consistently positive or negative reaction to AR as shown in those with prostate cancer [12]. To improve outcomes, it is important not only to define the role of AR in breast cancer

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patients but also to identify the subgroups that (i) react positively to AR, enhancing AR signaling pathways, and (ii) react negatively to AR, blocking these pathways.

A molecular apocrine breast tumor is one of subgroups having a possible advantage from blocking AR signaling. This subtype is characterized by apocrine features upon histological examination, ER-negative and AR-positive tumors, and a high association with human epidermal growth factor receptor type 2 (HER2) amplification [13]. Subsequent studies demonstrate that the molecular apocrine subtype is regulated in an AR-dependent and ER-independent manner and a functionally significant cross talk appears to be present between AR and HER2 pathways [14, 15]. A combined blockade of AR and HER2 signaling may provide therapeutic synergy to those with the molecular apocrine subtype [15].

The aims of this study were to investigate the association between AR expression and clinicopathological parameters and to evaluate the implications of AR expression in patients with breast cancer, including stratified analyses by ER status.

patients and methods

patient selection and analysis of clinicopathological parameters

We prospectively collected tumor tissues from specimens of surgically resected breast carcinoma at the Department of Surgery, Yonsei University College of Medicine, between November 1999 and August 2005. All tumor tissues were fixed in 10% buffered formalin and embedded in paraffin. Archival hematoxylin and eosin (H&E)-stained slides for each case were reviewed by two pathologists who specialize in breast pathology and have extensive experience. The interpretation of immunohistochemistry (IHC) results was carried out blindly, without any information regarding clinical parameters or outcome. Patients with pure *in situ* carcinoma of the breast, recurrent or metastatic disease, bilateral breast cancers, or non-epithelial-origin breast cancer such as phyllodes tumor, sarcoma, or lymphoma, as well as those receiving neoadjuvant chemotherapy, were excluded. Patients with invasive breast carcinoma who did not present an invasive focus by review of archival H&E-stained slides were also excluded. Among a total study population of 1153, after additional exclusion of 13 cases with unreadable AR expression, 931 patients with invasive breast carcinoma were finally enrolled. This study was reviewed and approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System, Seoul, Korea (4-2010-0136).

Data regarding patient demographics, histopathology of the primary tumor, treatment patterns, and survival were retrospectively obtained by reviewing medical records. The type of surgery and adjuvant therapies were determined not by AR expression but by international guidelines. Patients were treated with either mastectomy or breast-conserving surgery and sentinel lymph node biopsy or axillary lymph node dissection. After surgery, local radiotherapy or adjuvant systemic treatments were administered if the patient was able to tolerate it. Clinical follow-up included history taking, physical examination, laboratory tests, and radiological imaging tests every 6–12 months for detection of relapse. Tumor stage was based on the American Joint Committee on Cancer 6th edition criteria. Histological grade was assessed by the modified Bloom–Richardson classification.

Local recurrence was defined as the reappearance of carcinoma in the treated remnant breast, skin, or chest wall. Events determining regional relapse were defined as recurrences to the ipsilateral axillary, supraclavicular, or internal mammary lymph nodes. Any recurrence at a distant site including the contralateral axillary or supraclavicular lymph nodes was considered to be distant metastasis. Disease-free survival (DFS) time was measured from the date of the first curative surgery to the date of the first locoregional or systemic relapse, or death without any type of relapse. Overall survival (OS) time was calculated from the date of the first definite operation to the date of the last follow-up, or death from any cause.

tissue microarray blocks and immunohistochemical staining

Formalin-fixed, paraffin-embedded tissue blocks were arrayed using a tissue-arraying instrument (AccuMax™ Array; Petagen Inc., Seoul, Korea). Briefly, representative areas of each tumor were selected and marked on the H&E slide by breast pathologists. The designated zone of each donor block was punched with a tissue cylinder 3 mm in diameter and the sample was transferred to a recipient block in a grid pattern.

Immunohistochemical staining was carried out in the tissue microarray blocks. Thick sections of 5 μm were obtained with a microtome, transferred into adhesive slides, and dried at 62°C for 30 min. After deparaffinization and rehydration, the sections were treated with a 3% hydrogen peroxide solution for 10 min to block endogenous peroxidase and then pretreated for antigen retrieval in 10 mM citrate buffer (pH 6.0) in a microwave oven for 20 min. After incubation with primary antibodies against AR (AR 441; Thermo Scientific, Fremont, CA), ER (SP1; Thermo Scientific), progesterone receptor (PgR 636; DAKO, Glostrup, Denmark), HER2 (polyclonal; DAKO), and Ki-67 (MIB-1; DAKO), immunodetection was carried out with biotinylated anti-mouse/rabbit immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3'-diaminobenzidine chromogen as the substrate. Slides were counterstained with Harris hematoxylin. Normal breast tissue entrapped within the block and appropriate control tissues were used as positive controls.

Tumors with ≥10% positively nuclear-stained cells were considered positive for AR, ER, PgR, and Ki-67 expression. HER2 staining was scored by counting the number of positively stained cells on the membrane and expressed as a percentage of total tumor cells according to the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines [16] using the following categories: 0, no immunostaining; 1+, weak incomplete membranous staining in any proportion of tumor cells; 2+, complete membranous staining, either nonuniform or weak in at least 10% of tumor cells; and 3+, uniform intense membranous staining in >30% of tumor cells. HER2 results were considered positive in cases with 3+ membranous staining of IHC or gene amplification by FISH regardless of the IHC results using the diagnostic criteria described in the following. TNBC was defined as a tumor showing negative expression for ER, PgR, and HER2 by IHC or FISH.

fluorescence *in situ* hybridization

FISH analysis using the PathVysion HER2 DNA Probe Kit (Abbott, Abbott Park, IL) was carried out manually in all patients. In brief, consecutive sections from formalin-fixed, paraffin-embedded tissue microarray blocks were mounted on ProbeOn Plus microscope slides (Fisher Scientific, Pittsburgh, PA), deparaffinized in xylene, and subsequently rehydrated in ethanol. They were then boiled for 10 min in pretreatment solution, incubated with pepsin solution for 10 min, dehydrated in ethanol for 6 min, and finally air-dried. For hybridization, the buffered probe (HER2/*neu* and centromere 17) was introduced onto the slide and protected by a coverslip sealed with rubber cement. For denaturation, slides were heated to 82°C and incubated overnight at 45°C in a dark humidified chamber. The rubber cement and coverslip were then removed, and the slides were transferred to stringent wash buffer for 10 min at 65°C. Afterward, they were dehydrated in ethanol for 6 min and air-dried. Finally, they were counterstained with

4′,6-diamidino-2-phenylindole (DAPI). Evaluation of signals was carried out using an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with a fluorescein, Cy3, and a DAPI filter set and a 100-W mercury lamp. Counting was carried out according to the manufacturer's manual (the HER2/*neu* gene appeared as orange and centromere 17 as green). As recommended by the ASCO/CAP guideline [16], an absolute HER2 gene copy number >6 or HER2 gene/chromosome 17 copy number ratio higher than 2.2 was considered HER2 positive. Lymphocytes, fibroblasts, and normal ductal epithelial cells were used as internal controls.

statistical analysis

The differences between the groups were evaluated by a chi-square test. Fisher's exact test was used when appropriate. Survival curves were determined and plotted using the Kaplan–Meier method and group differences in survival time were investigated by a log-rank test. A Cox proportional hazards model was used to identify variables that were independently associated with survival. All statistical tests were two-sided, and P values of <0.05 were considered statistically significant. SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

results

characteristics and outcomes in all patients

The mean age at diagnosis was 49.3 years [standard deviation (SD) 10.5 years] for all patients. AR expression was demonstrated in 58.1% (541 of 931) of patients. Positive expression of ER, PgR, and HER2 was observed in 72.2% (n = 672), 61.4% (n = 572), and 24.7% (n = 230) of patients, respectively. The clinicopathological characteristics according to AR expression in all patients are summarized in Table 1. Patients with AR-positive tumor showed a higher frequency of age over 35 years at diagnosis. A significant number of AR-positive tumors were associated with lower pathological tumor stage, grade I or II tumors, and lower proliferative Ki-67 index. AR was significantly expressed in ER-positive, PgR-positive, and non-TNBC tumors. Among our study population, 68.7% (474 of 690) of patients with endocrine-responsive tumor (ER-positive and/or PgR-positive tumor) expressed AR. However, 27.8% (67 of 241) of patients with endocrine-nonresponsive tumor (ER-negative and PgR-negative tumor) and 13.5% (21 of 156) of patients with TNBC expressed AR. No statistical difference was demonstrated in AR expression according to pathological nodal stage, HER2 overexpression, and locoregional treatment modalities. Patients with AR-negative tumor more frequently received adjuvant systemic chemotherapy, whereas those with AR-positive cancer were more often administered endocrine therapy.

With a mean follow-up duration of 72.7 months (SD 22.6 months), AR expression was a significant prognostic factor for DFS and OS in all patients (Figure 1). The 5-year DFS and OS of patients with AR-positive tumor were 88.2% and 93.8%, respectively. The 5-year DFS and OS of those with AR-negative tumor were 83.7% and 90.1%, respectively. AR expression was positively associated with survival outcomes in all patients. However, in the Cox regression models for DFS and OS, statistical significance disappeared when adjusting for age at diagnosis, pathological tumor and nodal stage, ER status, HER2 overexpression, and use of chemoendocrine therapy (data not shown).

Table 1. Patient demographics

Factor	AR negative, n = 390 (%)	AR positive, n = 541 (%)	P value
Age (years)			
≤35	39 (10.0)	29 (5.4)	0.007
>35	351 (90.0)	512 (94.6)	
Pathological T stage			
T1	150 (38.5)	303 (56.0)	<0.001
T2	229 (58.7)	232 (42.9)	
T3–4	11 (2.8)	6 (1.1)	
Pathological N stage			
N0	214 (54.9)	315 (58.2)	0.260
N1	108 (27.7)	140 (25.9)	
N2	35 (9.0)	56 (10.4)	
N3	33 (8.5)	30 (5.5)	
Histological grade			
I	49 (12.6)	131 (24.2)	<0.001
II	177 (45.4)	312 (57.7)	
III	164 (42.1)	98 (18.1)	
Estrogen receptor			
Negative	188 (48.2)	71 (13.1)	<0.001
Positive	202 (51.8)	470 (86.9)	
Progesterone receptor			
Negative	223 (57.2)	136 (25.1)	<0.001
Positive	167 (42.8)	405 (74.9)	
HER2			
Negative	303 (77.7)	398 (73.6)	0.150
Positive	87 (22.3)	143 (26.4)	
Triple-negative breast cancer[a]			
No	255 (65.4)	520 (96.1)	<0.001
Yes	135 (34.6)	21 (3.9)	
Ki-67 (%, n = 929)			
<10	153 (39.2)	376 (69.8)	<0.001
≥10	237 (60.8)	163 (30.2)	
Type of surgery			
BCS	108 (27.7)	162 (29.9)	0.455
TM	282 (72.3)	379 (70.1)	
Radiotherapy			
Not done	225 (57.7)	305 (56.4)	0.689
Done	165 (42.3)	236 (43.6)	
Chemotherapy			
Not done	51 (13.1)	111 (20.5)	0.003
Done	339 (86.9)	430 (79.5)	
Endocrine therapy			
Not done	195 (50.0)	106 (19.6)	<0.001
Done	195 (50.0)	435 (80.4)	

[a]Triple-negative breast cancer represents tumors showing negative expression for estrogen receptor, progesterone receptor, and HER2 by immunohistochemistry or FISH.
AR, androgen receptor; T, tumor; N, node; HER2, human epidermal growth factor receptor type 2; BCS, breast-conserving surgery; TM, total mastectomy.

characteristics and outcomes stratified by ER status

The patient and tumor characteristics stratified by ER expression are shown in Table 2. AR expression was determined in 69.9% (470 of 672) of patients with ER-positive breast cancer

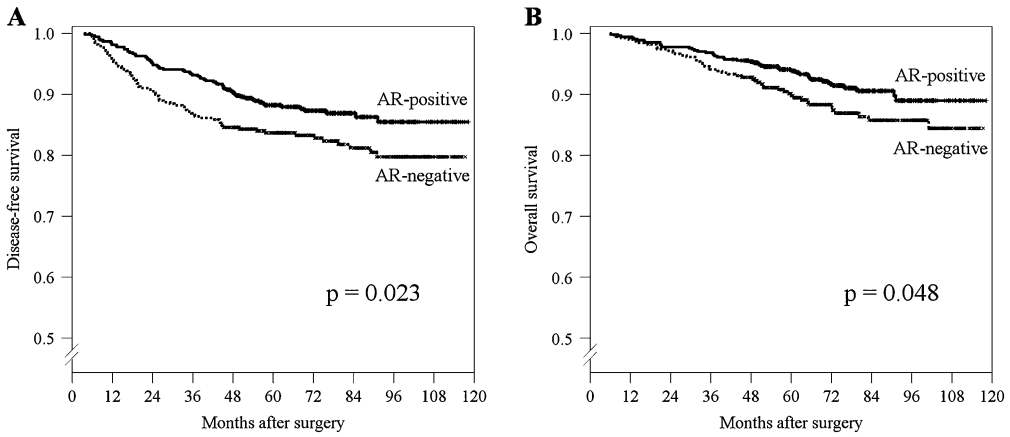

Figure 1. Disease-free (A) and overall (B) survival according to androgen receptor (AR) expression in all patients.

and in 27.4% (71 of 259) of those with ER-negative tumor. In patients with ER-positive tumor, AR expression was significantly associated with smaller tumor size, nodal uninvolvement, grade I or II tumors, higher PgR expression, and lower proliferation index. In patients with ER-negative cancer, however, AR expression was statistically related to well differentiation, lower Ki-67 index, and distinctively HER2 overexpression. Locoregional and adjuvant treatment modalities according to AR expression in ER-positive and ER-negative tumors, respectively, are summarized in Table 2.

The DFS and OS according to AR expression in patients with ER-positive and ER-negative tumors, respectively, are presented in Figure 2. In patients with ER-positive tumor, AR expression was positively associated with DFS and OS (Figure 2A and B), as shown in all patients with statistical significance. However, in ER-negative breast cancers, there was no significant difference in survival according to AR expression. AR-positive tumors presented a trend toward relatively poorer outcomes (Figure 2C and D). In the multivariate Cox's models, AR expression was an independent prognostic factor for DFS (hazard ratio, 0.654; $P = 0.049$), but it did not have statistical significance for OS (hazard ratio, 0.647; $P = 0.119$) in patients with ER-positive cancers when adjusting for age at diagnosis, pathological tumors and nodal stage, HER2 overexpression, and use of chemoendocrine therapy (Table 3). In ER-negative tumors, pathological tumor stage, nodal stage, and use of chemotherapy were independent prognostic factors for survival outcomes (Table 4). AR expression distinctively increased in hazard ratio without statistical significance in the multivariate analyses.

outcomes of subgroups with ER-negative tumor

The implications of AR expression on breast cancer outcome were investigated in the subgroup of patients with ER-negative tumor. As shown in Table 2, 53.8% (49 of 91) of patients with ER-negative and HER2-positive tumors expressed AR. The DFS and OS of 91 patients with ER-negative and HER2-positive tumors are presented in Figure 3 according to AR expression. Interestingly, tumors with molecular apocrine features (ER-negative, AR-positive, and HER2-positive tumors) showed a trend of poorer DFS ($P = 0.483$) and OS ($P = 0.074$). However, in our study population, only 13.5% of patients with TNBC expressed AR (Table 1), and there was no impact of AR expression on the survival of the 156 patients with TNBC (Figure 4).

discussion

Breast cancer is a highly hormone-dependent tumor. The role of estrogen and its receptor is well established in the carcinogenesis and tumor progression; therefore, indirect or direct inhibition of ER pathways is the mainstay for treatment of breast carcinoma [17]. Although androgen is a dominant steroid hormone throughout a woman's life and a necessary precursor for estrogen biosynthesis, AR has only recently been considered as an emerging biomarker in breast cancer [2, 9].

AR is expressed in >60%–70% of both *in situ* and invasive breast cancers and is frequently coexpressed with ER and PgR but rarely with HER2 [18, 19]. In the present study, we also demonstrated an association between AR expression and favorable clinicopathological parameters, which is consistent with our own previous study as well as others [3, 18–20]. AR expression was observed in significant levels in endocrine-responsive tumors including non-TNBC. Of note, however, is that approximately one-fourth of patients with ER-negative tumors also expressed AR. There was no significant interaction between AR and HER2 in all patients of this study.

The prognostic role of AR in breast cancer patients has recently been suggested [5, 6, 21]. Our results showed an important implication of AR in all study population. In univariate analyses, AR was a significant factor for survival outcome. After controlling for well-established prognostic factors including ER status, however, AR was found to have no independent statistical significance. Because previous studies have proposed a contradictory hypothesis according to estrogenic milieu [9], we analyzed the prognostic significance of AR according to ER expression status.

In patients with ER-positive tumor, AR expression was significantly associated with better prognostic markers as shown in all patients. In addition, the prognostic significance of AR was demonstrated in multivariate analyses for DFS but not for OS. These results were consistent with previous reports [5,

Table 2. Patient demographics stratified by estrogen receptor (ER) expression

Factor	ER positive ($n = 672$)			ER negative ($n = 259$)		
	AR negative, $n = 202$ (%)	AR positive, $n = 470$ (%)	*P* value	AR negative, $n = 188$ (%)	AR positive, $n = 71$ (%)	*P* value
Age (years)						
≤35	17 (8.4)	24 (5.1)	0.100	22 (11.7)	5 (7.0)	0.274
>35	185 (91.6)	446 (94.9)		166 (88.3)	66 (93.0)	
Pathological T stage						
T1	82 (40.6)	277 (58.9)	<0.001	68 (36.2)	26 (36.6)	0.946
T2–4	120 (59.4)	193 (41.1)		120 (63.8)	45 (63.4)	
Pathological N stage						
N0	92 (45.5)	275 (58.5)	0.002	122 (64.9)	40 (56.3)	0.204
N1–3	110 (54.5)	195 (41.5)		66 (35.1)	31 (43.7)	
Histological grade						
I/II	156 (77.2)	404 (86.0)	0.005	70 (37.2)	39 (54.9)	0.010
III	46 (22.8)	66 (14.0)		118 (62.8)	32 (45.1)	
Progesterone receptor						
Negative	49 (24.3)	69 (14.7)	0.003	174 (92.6)	67 (94.4)	0.786
Positive	153 (75.7)	401 (85.3)		14 (7.4)	4 (5.6)	
HER2						
Negative	157 (77.7)	376 (80.0)	0.504	146 (77.7)	22 (31.0)	<0.001
Positive	45 (22.3)	94 (20.0)		42 (22.3)	49 (69.0)	
Ki-67 (%)						
<10	128 (63.4)	350 (74.8)	0.003	25 (13.3)	26 (36.6)	<0.001
≥10	74 (36.6)	118 (25.2)		163 (86.7)	45 (63.4)	
Type of surgery						
BCS	41 (20.3)	146 (31.1)	0.004	67 (35.6)	16 (22.5)	0.044
TM	161 (79.7)	324 (68.9)		121 (64.4)	55 (77.5)	
Radiotherapy						
Not done	125 (61.9)	264 (56.2)	0.169	100 (53.2)	41 (57.7)	0.511
Done	77 (38.1)	206 (43.8)		88 (46.8)	30 (42.3)	
Chemotherapy						
Not done	27 (13.4)	95 (20.2)	0.035	24 (12.8)	16 (22.5)	0.052
Done	175 (86.6)	375 (79.8)		164 (87.2)	55 (77.5)	
Endocrine therapy						
Not done	27 (13.4)	39 (8.3)	0.043	168 (89.4)	67 (94.4)	0.215
Done	175 (86.6)	431 (91.7)		20 (10.6)	4 (5.6)	

AR, androgen receptor; T, tumor; N, node; HER2, human epidermal growth factor receptor type 2; BCS, breast-conserving surgery; TM, total mastectomy.

20, 21]. Although we did not consider types or regimens of systemic therapies in this study, AR might provide an additional predictive role for systemic treatments including endocrine therapy because 90.2% (606 of 672) of our ER-positive subgroup received endocrine therapy or chemoendocrine therapy. The *in vitro* study suggests a significant inhibitory interaction between ER and AR [21], and that potential therapeutic effects of aromatase inhibitors may be due not only to a reduction in estrogen levels but also to an increase in inhibitory AR signaling pathways [22]. Another large retrospective study demonstrated a prognostic and predictive role of AR in the subset of ER-positive tumors [5]. Taken together, AR expression could be an additional significant biomarker for endocrine responsiveness in ER-positive cancers, and further prospective study is necessary to determine the role of AR in patients with ER-positive tumors.

There have been limited data regarding AR in ER-negative breast cancers. Most studies have determined higher expression rates of AR in ER-positive tumors; however, as many as half of the ER-negative tumors expressed AR [3, 5, 6]. The present study showed positive AR expression in ∼30% of ER-negative cancers, which is lower than that of our previous report on AR positivity in the ER-negative subgroup [3]. There was statistically no prognostic impact of AR in our ER-negative and TNBC subset; there was however a trend showing an inverse relationship between AR expression and clinical outcome for ER-negative tumors.

Approximately one-third of breast cancer cases are constituted by ER-negative tumors, and these patients do not benefit from endocrine therapy and show a poorer prognosis than patients with ER-positive tumor [17]. Among ER-negative breast cancers, however, active AR signaling was demonstrated in the molecular apocrine tumors as described by Farmer et al. [13] in 2005. Subsequent study showed that this subgroup was characterized by AR-dependent, androgen-regulated transcriptional program and by significant interaction between

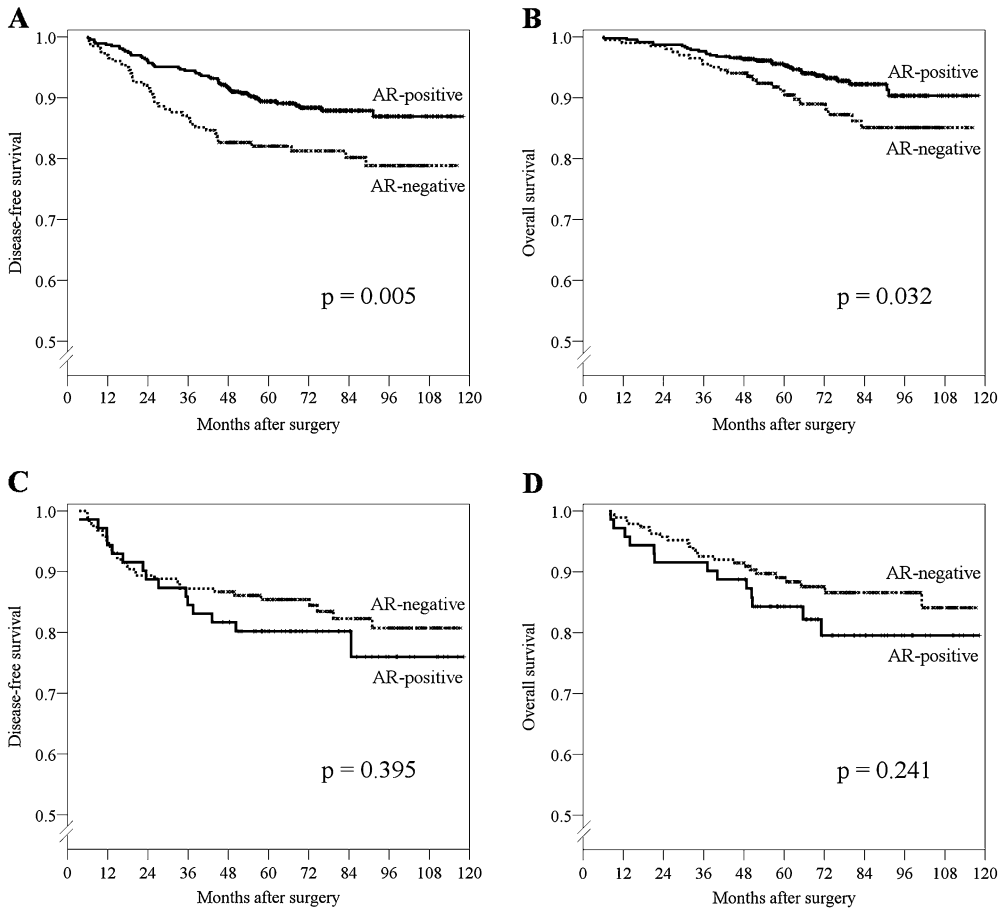

Figure 2. Survival curves stratified by estrogen receptor status. (A) Disease-free and (B) overall survival according to androgen receptor (AR) expression in patients with estrogen receptor-positive tumor. Panels C and D show the same in patients with estrogen receptor-negative tumor. ER, estrogen receptor.

Table 3. Multivariate analysis in patients with estrogen receptor-positive tumor

Factor	Disease-free survival			Overall survival		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	P value
Androgen receptor						
Negative versus positive	0.654	0.429–0.997	0.049	0.647	0.375–1.119	0.119
Age (years)						
≤35 versus >35	0.496	0.260–0.946	0.033	0.434	0.197–0.954	0.038
Pathological T stage						
T1 versus T2–4	1.622	1.011–2.602	0.045	2.108	1.109–4.008	0.023
Pathological N stage						
N0 versus N1–3	2.456	1.501–4.020	<0.001	2.167	1.154–4.070	0.016
HER2						
Negative versus positive	2.004	1.278–3.140	0.002	1.555	0.850–2.845	0.152
Chemotherapy						
Not done versus done	0.419	0.232–0.756	0.004	0.298	0.145–0.611	0.001
Endocrine therapy						
Not done versus done	1.147	0.583–2.256	0.691	1.001	0.436–2.297	0.998

CI, confidence interval; T, tumor; N, node; HER2, human epidermal growth factor receptor type 2.

AR and HER2 signaling [14, 15, 20, 23]. These associations have also been suggested by morphological studies of breast lesions. Morphologically, tumors with apocrine differentiation frequently showed ER-negative, AR-positive, and HER2-positive features [20, 24–26]. We previously described a possible correlation between AR and HER2 in these special subsets [3]. Using the independent dataset of ER-negative tumors, a statistically significant association between AR

Table 4. Multivariate analysis in patients with estrogen receptor-negative tumor

Factor	Disease-free survival			Overall survival		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	P value
Androgen receptor						
Negative versus positive	1.163	0.601–2.249	0.654	1.451	0.710–2.965	0.307
Age (years)						
≤35 versus >35	0.920	0.322–2.630	0.877	0.813	0.241–2.742	0.738
Pathological T stage						
T1 versus T2–4	2.904	1.240–6.801	0.014	4.324	1.522–12.286	0.006
Pathological N stage						
N0 versus N1–3	2.521	1.292–4.919	0.007	3.316	1.535–7.160	0.002
HER2						
Negative versus positive	1.026	0.546–1.930	0.936	0.814	0.401–1.655	0.570
Chemotherapy						
Not done versus done	0.332	0.135–0.814	0.016	0.192	0.076–0.487	0.001

CI, confidence interval; T, tumor; N, node; HER2, human epidermal growth factor receptor type 2.

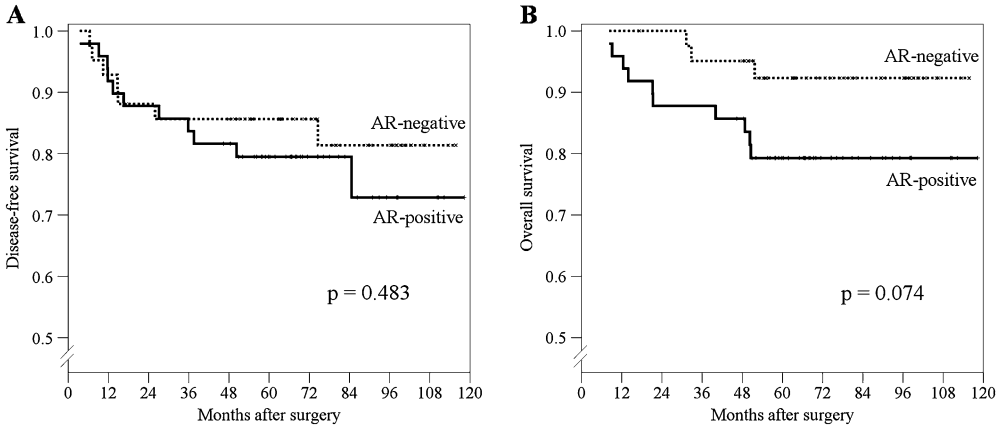

Figure 3. Disease-free (A) and overall (B) survival according to androgen receptor (AR) expression in 91 patients with estrogen receptor-negative and human epidermal growth factor receptor type 2-positive tumor.

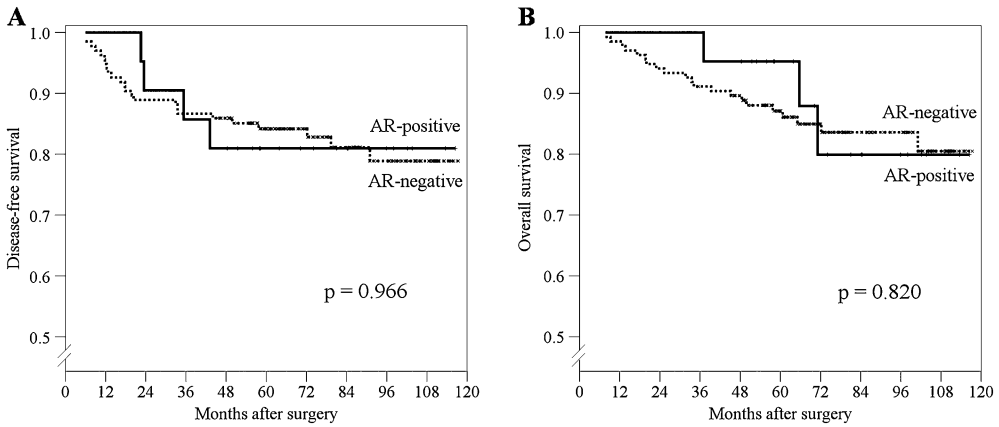

Figure 4. Disease-free (A) and overall (B) survival according to androgen receptor (AR) expression in 156 patients with triple-negative breast cancer.

expression and HER2 positivity was again demonstrated (Table 2) in addition to a borderline significantly poorer OS in the ER-negative, HER2-positive subset with AR expression (Figure 3). Although there were limitations that kept our study from including a retrospective analysis with a small sample size, it supports the idea that blockading both AR and HER2 pathways might be a rational and useful therapeutic option for this subgroup. Because many antiandrogenic agents, including

bicalutamide, are now clinically available and orally active, it is reasonable to validate the safety and efficacy of antiandrogens for breast cancer patients [7, 27]. A phase II feasibility study of bicalutamide in treating patients with metastatic breast cancer is being conducted (ClinicalTrials.gov identifier; NCT00468715). The preliminary results have been promising in demonstrating a high tolerability and possible antitumor effect of antiandrogen therapy in patients with AR-positive, ER-negative, and PgR-negative metastatic breast cancer [28].

In summary, the present study suggests that AR expression is significantly associated with favorable clinicopathological characteristics and better outcomes in ER-positive breast cancers. On the contrary, AR is related to growth factor signaling in ER-negative tumors, and molecular apocrine tumors show a trend of poorer survival. Taken together, this indicates that AR expression could be an additional marker for endocrine responsiveness in ER-positive cancers and a possible therapeutic target molecule for the ER-negative subgroup, including molecular apocrine tumors.

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disclosure

The authors declare no conflict of interest.

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