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Original Article

Clinicopathologic significance of androgen receptor expression and discordant receptor status during progression in breast cancer

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Abstract: The role of androgen receptor (AR) as a prognostic marker has been proposed in breast cancer. This study investigated AR status and its clinical significance in breast cancer, especially in triple negative breast cancer (TNBC). We also evaluated discordant AR status during the process of lymph node metastasis, locoregional recurrences (LRR) and distant metastasis. From January 2005 to December 2010, we retrospectively reviewed 120 patients including 55 TNBC patients diagnosed as invasive carcinoma with no special type (NST), who were treated at the Kangbuk Samsung Hospital. Tissue microarray was constructed and immunohistochemical expression of AR was performed for 120 invasive carcinomas, NST specimens and matching samples from 28 lymph node metastasis, 2 LRR and 8 distant metastases. AR expression was found in 35.0% (42/120) of the total patients and 14.5% (8/55) of those diagnosed as TNBC. Positive expression of AR was significantly correlated with smaller tumor size, early T stage, fewer lymph node metastases, early AJCC stage, lower histologic grade, estrogen receptor/progesterone receptor positivity, more luminal A type, less TNBC, longer disease-free survival and overall survival, fewer distant metastasis and no deaths from breast cancer (all $P < 0.05$). AR was a favorable prognostic marker for disease free survival in univariate analysis ($P = 0.041$). The discordance rate of AR status between primary and recurrent/metastatic disease was 21.6%. AR expression was associated with favorable clinicopathological outcomes in the whole study population. AR status can be altered during tumor progression.

Keywords: Androgen receptor, breast cancer, discordance, triple negative breast cancer

Introduction

Breast cancer (BC) is the most common malignancy in women, and invasive carcinoma of no special type (NST) is the most common form of BC [1, 2]. Important parameters with therapeutic significance and those that aid in the prognosis of BC have been identified. American Joint Committee on Cancer (AJCC) stage, histological grade, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) gene amplification are important parameters determining the therapeutic options for BC. Androgen receptor (AR) is a steroid receptor expressed in 70-80% of BC cells and is more frequently expressed in ER-positive than in ER-negative tumors [3]. AR signaling pathways play different roles according to BC subtypes. In ER-positive BC, AR often inhibits the growth effect of ER signaling. In HER-2 positive BC without ER expression, it plays a proliferative role and in triple-negative breast cancer (TNBC) it induces tumor progression [4]. Identifying the underlying mechanisms of AR in each subtype of BC will allow the design of appropriate target therapies for BC, especially TNBC. No targeted therapies are available yet for TNBC. AR is expressed in 10-43% of TNBCs, and one subset of gene expression profiles in TNBCs is androgen responsive [5, 6]. Therefore, it is important to investigate the prevalence of AR expression in each BC subtype.

Discordance of ER, PR and HER-2 receptor status between primary tumor and metastatic tissue has been observed in several studies [7, 8].

Table 1. Clinicopathological characteristics of patients

Parameters	AR status n (%)			*p*
	Total (n = 120)	Positive (n = 42)	Negative (n = 78)	
Age at Diagnosis (years)*	50.8±0.9	51.6±1.8	50.4±1.2	0.566
Tumor size (cm)*	2.6±1.6	2.2±1.4	2.9±1.7	0.025
T stage†				0.023
1	49 (40.8)	23 (54.7)	26 (33.3)	
2	58 (48.4)	17 (40.5)	41 (52.7)	
3	12 (10.0)	1 (2.4)	11 (14.1)	
4	1 (0.8)	1 (2.4)	0 (0)	
N stage†				0.366
0	61 (50.8)	26 (61.9)	35 (44.8)	
1	26 (21.7)	7 (16.7)	19 (24.4)	
2	18 (15.0)	5 (11.9)	13 (16.7)	
3	15 (12.5)	4 (9.5)	11 (14.1)	
Tumor size (cm)†				0.019
≤2.0	54 (45.0)	25 (59.5)	29 (37.2)	
>2.0	66 (55.0)	17 (40.5)	49 (62.8)	
LN metastasis†				0.126
Yes	59 (49.2)	16 (38.1)	43 (55.2)	
No	61 (50.8)	26 (61.9)	35 (44.8)	
Number of LN metastases*	3.7±0.6	2.1±0.6	4.5±0.8	0.028
AJCC stage†				0.010
I	30 (25.0)	18 (42.9)	12 (15.4)	
II	51 (42.5)	14 (33.3)	37 (47.4)	
III	33 (27.5)	9 (21.4)	24 (30.8)	
IV	6 (5.0)	1 (2.4)	5 (6.4)	
Histologic grade†				0.000
1	19 (16.1)	12 (28.6)	7 (8.9)	
2	45 (38.1)	22 (52.4)	24 (30.7)	
3	54 (45.8)	8 (19.0)	47 (60.4)	
EIC†				0.037
Yes	11 (9.2)	7 (16.7)	4 (5.1)	
No	109 (90.8)	35 (83.3)	74 (94.9)	
Skin/chest wall invasion‡				1.000
Yes	2 (1.7)	1 (2.4)	1 (1.3)	
No	118 (98.3)	41 (97.6)	77 (98.7)	
Paget disease‡				0.551
Yes	3 (2.5)	0 (0)	3 (3.9)	
No	117 (97.5)	42 (100)	75 (96.1)	
LVI†				0.221
Yes	37 (30.8)	10 (23.8)	27 (34.6)	
No	83 (69.2)	32 (76.2)	51 (65.4)	
ER status†				0.000
Positive	54 (45.0)	32 (76.2)	22 (28.2)	
Negative	66 (55.0)	10 (23.8)	56 (71.8)	
PR status†				0.000
Positive	45 (37.5)	29 (69.1)	16 (20.5)	

Possible mechanisms proposed for discordance include (a) a genuine switch in the biology of the cancer, (b) sampling error, (c) limited accuracy and reproducibility of receptor assays and (d) intra-tumoral heterogeneity [8]. Discordance rates are reported in the range of 10% to between 35% and 40% [9]. However, discordance of AR expression are not been reported widely in the literature. Therefore, it is important to investigate discordance of AR expression in primary tumor, lymph node metastasis, recurrence and distant metastasis. The aim of this study is to evaluate AR expression in BC population and assess how it correlates with patient outcomes. We also aimed to measure AR expression across different subtypes and correlate discordance during tumor progression.

Materials and methods

Patient selection

From January 2005 to December 2010, we retrospectively reviewed 120 patients including 55 TNBC, diagnosed as invasive carcinoma of NST, who were treated at the Kangbuk Samsung Hospital. Patients were characterized based on clinicopathological characteristics of age at diagnosis, TNM stage, axillary lymph node (LN) status, American Joint Committee on Cancer (AJCC) stage, histologic grade, extensive intraductal component (EIC), skin or chest wall invasion, Paget's disease,

Negative	75 (62.5)	13 (30.9)	62 (79.5)	
HER-2 status†				0.486
Positive	18 (15.0)	5 (11.9)	13 (16.7)	
Negative	102 (85.0)	37 (88.1)	65 (83.3)	
Tumor subtypes†				0.000
Luminal A	47 (39.2)	29 (69.0)	18 (23.1)	
Luminal B	7 (5.8)	3 (7.2)	4 (5.1)	
HER-2	11 (9.2)	2 (4.7)	9 (11.5)	
Triple negative	55 (45.8)	8 (19.1)	47 (60.3)	
Type of surgery†				0.406
Breast conserving	21 (17.5)	9 (21.4)	12 (15.4)	
Mastectomy	99 (82.5)	33 (78.6)	66 (84.6)	
Disease free survival, (month)*	68.9±39.0	81.6±34.9	61.9±39.6	0.008
Overall survival, (month)*	76.1±38.3	86.8±33.1	70.4±39.9	0.025
Locoregional recurrence†				0.444
Yes	12 (10.0)	3 (7.1)	9 (11.5)	
No	108 (90.0)	39 (92.9)	69 (88.5)	
Distant metastasis†				0.010
Yes	24 (20.0)	3 (7.1)	21 (26.9)	
No	96 (80.0)	39 (92.9)	57 (73.1)	
Death from breast cancer†				0.022
Yes	9 (7.5)	0 (0)	9 (11.5)	
No	111 (92.5)	42 (100)	69 (88.5)	

Values are number of individuals (n) or mean ± SE. LN, lymph node; AJCC, American Joint Committee on Cancer; EIC, extensive intraductal component; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2. *Student's T-test; †Pearson's chi-square test; ‡Fisher's exact test.

lymphovascular invasion (LVI), ER positivity, PR positivity, HER-2 overexpression and subtypes of tumor, type of surgery, locoregional recurrence (LRR), distant metastasis and death from BC. Overall survival (OS) was defined as the time interval between the date of surgical resection and the date of disease specific death or last follow-up. Disease-free survival (DFS) was defined as the time between the date of surgical resection and the date of documented relapse, including LRR and distant metastasis. All studies were conducted with prior approval from the Institutional Review Board of Kangbuk Samsung Hospital (Approval No. 2014-10-027).

Tissue selection and tissue microarray (TMA) construction

We obtained primary invasive ductal carcinoma, NST tissue, metastatic cancer tissue from LNs, other organ and recurred tissue for TMA construction. Surgical specimens were fixed using 10% buffered formalin, processed and embedded in paraffin using a standard protocol. Hematoxylin and eosin (H&E) stained slides from all patients were reviewed by the same pathologist (SID). Histological data including T and N stage, lymphatic invasion and other characteristics were reconfirmed. All H&E stained slides were individually reviewed and the most representative tumor area was selected and marked on individual paraffin blocks. The most representative tissue core was obtained from each tumor specimen. TMA specimens were assembled using a tissue-array instrument (Tissue-Tek; Quick-Ray, Netherlands) consisting of thin-walled stainless steel punches and stylets for emptying and transferring the needle contents. The assembly was held in an X-Y position guide with a 1-mm increment between the individual samples, a 4-mm punch depth stop device and semi-automatic micrometers. The instrument was used to create holes in a recipient block with defined array cores. The fit needle was used to deliver the tissue cores into the recipient block. Taking into account the limitations of the representative areas of the tumor, we used duplicate 2-mm-diameter tissue cores from each donor block. The percentage of tissue cores taken from within the tumor exceeded 70%.

Immunohistochemistry

Immunohistochemical staining was performed on 3 μm-thick TMA block sections. Sections were dehydrated and deparaffinized in xylene and rehydrated in a graded series of alcohol solutions. We used primary antibodies against ER (1:200, clone SP1; Lab Vision Corporation, Fremont, CA, USA), PR (1:200, clone PgR 636; DakoCytomation, Glostrup, Denmark), HER-2 (1:200, clone SP3, Lab Vision Corporation), and AR receptor (1:200, clone AR 441; Abcam, Cambridge, UK). Immunostaining was per-

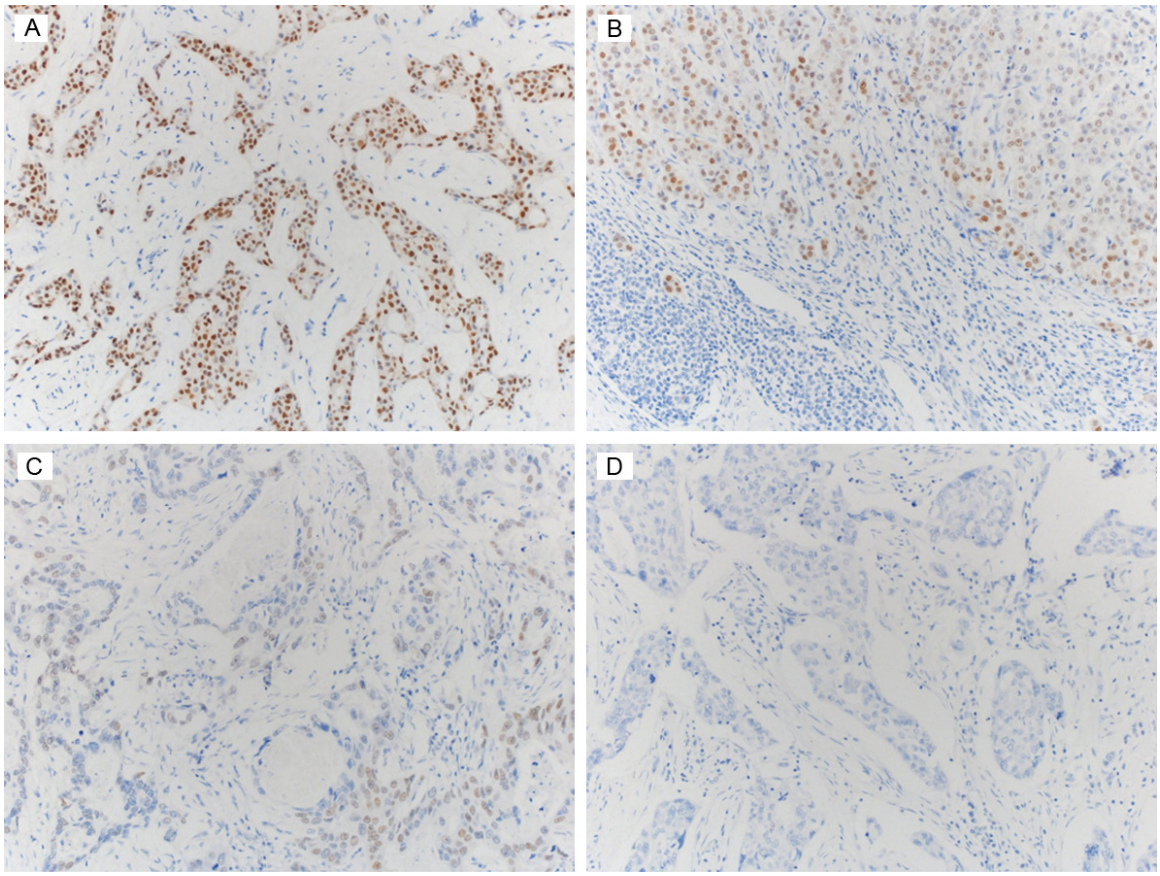

Figure 1. Androgen receptor expression in invasive carcinoma, NST. A: Strong expression; B: Moderate expression; C: Weak expression; D: Negative.

formed using a compact polymer method (Bond Intense Detection Kit; Leica Biosystems, Newcastle upon Tyne, UK). The primary antibodies were detected with an EnVision+ System utilizing horseradish peroxidase (DakoCytomation) according to the manufacturer's instructions. An EnVision+ Detection System incorporating peroxidase and 3,3'-diaminogenzidine (DakoCytomation) was used to perform chromogenic visualization. The slides were counterstained with hematoxylin and cover slipped. ER, PR and AR status was assessed using the Allred scoring method [10]. HER-2 overexpression was evaluated using American Society of Clinical Oncology/College of American Pathologists guideline recommendations. In cases with equivocal HER-2 staining (score 2), silver *in situ* hybridization (Ventana Medical Systems, Tucson, AZ, USA) was performed to determine *HER2* gene status. All slides were examined and scored by two board-certified pathologists blinded to the clinicopathological data and patient identity. Disagreements between the two pathologists were resolved by consensus.

Statistical analyses

Student's t-test for continuous variables and the Pearson's χ^2 test for categorical variables were used to evaluate the associations between AR expression and clinicopathologic parameters. Cox proportional hazards regression test was used to calculate the hazard ratio (HR) of data on DFS and OS. Multivariate Cox regression analysis was performed only for variables with significant univariate impact. Survival probability curves were calculated by the Kaplan-Meier method. A p-value < 0.05 (2-tailed) was considered statistically significant. All statistical analyses were performed with PASW Statistics for Windows, Version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient demographics

We retrospectively reviewed the clinicopathological data of 120 BC patients including 55

Table 2. Clinicopathological characteristics of 55 TNBC patients

Parameters	AR status n (%)			*p*
	Total (n = 55)	Positive (n = 8)	Negative (n = 47)	
Age at diagnosis (years)*	20.9±1.5	59.6±2.3	49.4±1.6	0.006
Tumor size (cm)*	2.9±0.2	1.8±0.3	3.1±0.3	0.032
T stage†				0.059
1	21 (38.2)	6 (75.0)	15 (31.9)	
2	26 (47.3)	2 (25.0)	24 (51.1)	
3	8 (14.5)	0 (0)	8 (17.0)	
4	0 (0)	0 (0)	0 (0)	
N stage†				0.100
0	25 (45.5)	6 (75.0)	19 (40.4)	
1	15 (27.3)	0 (0)	15 (31.9)	
2	7 (12.7)	0 (0)	7 (14.9)	
3	8 (14.5)	2 (25.0)	6 (12.8)	
Tumor size (cm)†				0.096
≤2.0	20 (36.4)	5 (62.5)	15 (31.9)	
>2.0	35 (63.6)	3 (37.5)	32 (68.1)	
LN metastasis†				0.089
Yes	26 (47.3)	6 (75.0)	20 (42.5)	
No	29 (52.7)	2 (25.0)	27 (57.5)	
Number of LN metastases*	3.6±0.8	3.2±2.2	3.7±0.9	0.576
AJCC stage‡				0.029
I	12 (21.8)	5 (62.5)	7 (14.9)	
II	24 (43.6)	1 (12.5)	23 (48.9)	
III	16 (29.1)	2 (25.0)	14 (29.8)	
IV	3 (5.5)	0 (0)	3 (6.4)	
Histologic grade‡				0.002
1	2 (3.7)	1 (12.5)	1 (2.2)	
2	17 (31.5)	6 (75.0)	11 (23.9)	
3	35 (64.8)	1 (12.5)	34 (73.9)	
EIC‡				0.272
Yes	53 (96.4)	7 (87.5)	46 (97.9)	
No	2 (3.6)	1 (12.5)	1 (2.1)	
Skin/chest wall invasion‡				1.000
Yes	1 (1.8)	0 (0)	1 (2.1)	
No	54 (98.2)	8 (100)	46 (97.9)	
Paget disease‡				1.000
Yes	1 (1.8)	0 (0)	1 (2.1)	
No	54 (98.2)	8 (100)	46 (97.9)	
LVI‡				0.696
Yes	21 (38.2)	2 (25.0)	19 (40.4)	
No	34 (61.8)	6 (75.0)	28 (59.6)	
Type of surgery†				0.859
Breast conserving	8 (14.5)	1 (12.5)	7 (14.9)	
Mastectomy	47 (85.5)	7 (87.5)	40 (85.1)	
Disease free survival, (month)*	68.9±39.0	81.6±34.9	61.9±39.6	0.008
Overall survival, (month)*	76.1±38.3	86.8±33.1	70.4±39.9	0.025

TNBC (**Table 1**). The mean age was 50.8±10.9 years (range, 27-82 years). Thirty patients (25.0%) were AJCC stage I, 51 (42.5%) were stage II, 33 (27.5%) were stage III and 6 (5.0%) were stage IV. LN metastasis was detected in 59 (49.2%) patients. Twelve patients (10.0%), 24 patients (20.0 %) and 9 patients (7.5%) had LRR, distant metastasis and death from BC, respectively, during the follow-up period. The mean duration of DFS and OS was 68 months (range, 1-140 months) and 76 months (range, 3-192 months), respectively.

AR expression and association with clinicopathological characteristics

AR expression was found in 42 (35.0%) of the 120 cases (**Figure 1**). Positive expression of AR showed significant correlation with smaller tumor size, early T stage, fewer number of LN metastases, early AJCC stage, lower histologic grade, ER/PR positivity, more luminal A type, lesser TNBC, longer DFS and OS, fewer distant metastasis and no death from BC (all $P < 0.05$) (**Table 1**). No statistically significant differences were observed between AR expression and age at diagnosis, histologic type, skin/chest wall invasion, Paget disease, LVI, HER-2 status, type of surgery and LRR. A summary of the relationship between AR expression and clinicopathological parameters is provided in **Table 1**.

Locoregional recurrence†				0.333
Yes	5 (9.1)	0 (0)	5 (10.6)	
No	50 (90.9)	8 (100)	42 (89.4)	
Distant metastasis†				0.089
Yes	13 (23.6)	0 (0)	13 (27.7)	
No	42 (76.4)	8 (100)	34 (72.3)	
Death from breast cancer†				0.243
Yes	7 (12.7)	0 (0)	7 (14.9)	
No	48 (87.3)	8 (100)	40 (85.1)	

Values are number of individuals (n) or mean ± SE. LN, lymph node; AJCC, American Joint Committee on Cancer; EIC, extensive intraductal component; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2. *Student's T-test; †Pearson's chi-square test; ‡Fisher's exact test.

AR expression and clinicopatholgic association in TNBC

AR expression in TNBC occurred in 8 (14.5%) of the 55 patients. AR expression in TNBC was associated with older age at diagnosis (59 vs. 49 years, respectively; P = 0.006), smaller tumor size (P = 0.032), early AJCC stage (P = 0.021) and lower histologic grade (P = 0.003) (**Table 2**). We could not evaluate association between AR expression in TNBC patients and prognosis, because there was no LRR, distant metastasis or death from BC in AR positive TNBCs.

AR expression and association with prognosis

AR expression was significantly independent favorable prognostic factor with distant metastasis (P = 0.010) and death from BC (P = 0.022) (**Table 1**). In univariate analyses, AR expression, tumor size, LN metastasis, skin/chest wall invasion, LVI, ER/PR positivity and HER-2 enriched subtype were all statistically significantly associated with DFS (all P < 0.05; **Table 3**). However, multivariate analyses showed that LN metastasis (HR 2.88, 95% confidence interval (CI), 1.19-6.97; P = 0.019) and skin/chest wall invasion (HR 10.562, 95% CI, 1.95-57.17; P = 0.006) were significantly associated with DFS (**Table 3**). Patients with AR expression had a more favorable DFS than those without expression in Kaplan-Meier curve analyses (χ^2 = 4.18; df = 2; P = 0.041) (**Figure 2**). We could not evaluate association between AR expression and OS, because there was no death from BC in AR positive BCs.

AR status in metastases and recurrence

Only 37 primary BCs had available AR status on LN metastasis, LRR, and distant metastasis. AR status was positive in 43.2% (16/37) of primary tumor, 64.2% (18/28) of LN metastasis, 50.0% (1/2) of LRR and 50.0% (4/8) of distant metastases. A discordant AR status between primary BC and matched metastatic samples was observed in 21.6% (8/37) of cases tested. Among 16 AR positive primary BCs, 1 case was negative in matching LN metastasis (**Figure 3**). The remainder of the 15 patients stayed AR positive during LN metastasis and distant metastasis. Among 21 AR negative primary tumors, 4, 1 and 2 tumors changed to AR positive on matching LN metastasis, both LN metastasis and LRR, and distant metastasis, respectively (**Figure 4**). Rest of 14 AR negative BCs stayed negative in LN metastasis, LRR and distant metastasis. The results are summarized in **Table 4**.

Discussion

AR is a member of the steroid hormone receptor family, which also includes ER and PR. Steroid hormone receptor plays significant roles in signaling pathways and as a transcription factor. ER and PR are well-known prognostic and predictive factors of endocrine therapies in BC. However, the role of AR in BC and its progression has been less profoundly studied and remains as an unanswered question.

In this study, we assessed how AR serves as a prognostic marker. Consistent with previous studies, our results showed that AR expression was related with favorable prognostic markers such as DFS [11]. AR negativity was associated with larger tumor size, LN metastasis, higher AJCC stage, higher histologic grade, ER/PR negativity, triple negativity and shorter DFS in univariate analysis.

Majority of the literatures described favorable prognostic impact of AR expression in BC [12]. However, others have reported mixed results such as patients with AR expressing ER nega-

Table 3. Univariate and multivariate analysis of disease-free survival in whole population

Parameter	Univariate		Multivariate	
	HR (95% CI)	*p*	HR (95% CI)	*p*
AR expression		0.041		0.346
Negative	1		1	
Positive	0.37 (0.14-0.99)		0.61 (0.22-1.71)	
Age at diagnosis		0.272		
≤40	1			
>40	0.98 (0.94-1.02)			
Tumor size (cm)		0.005		0.398
≤2.0	1		1	
>2.0	1.35 (1.09-1.66)		1.46 (0.61-3.50)	
LN metastasis		0.015		0.019
No	1		1	
Yes	2.98 (1.24-7.16)		2.88 (1.19-6.97)	
Histologic grade				
1	1			
2	1.21 (0.32-4.56)	0.320		
3	2.12 (0.61-7.41)	0.610		
EIC		0.242		
No	1			
Yes	0.30 (0.04-2.25)			
Skin/chest wall invasion		0.001		0.006
No	1		1	
Yes	12.41 (2.73-56.48)		10.562 (1.95-57.17)	
Paget disease		0.075		
No	1			
Yes	3.77 (0.88-16.19)			
LVI		0.035		0.334
No	1		1	
Yes	2.35 (1.06-5.21)		1.59 (0.62-4.08)	
ER positive		0.045		0.729
No	1		1	
Yes	0.42 (0.18-0.98)		1.25 (0.36-4.32)	
PR positive		0.019		0.123
No	1		1	
Yes	0.31 (0.11-0.83)		0.31 (0.07-1.37)	
HER-2 overexpression		0.338		
No	1			
Yes	1.57 (0.62-3.97)			
Subtype				
Luminal A	1			
Luminal B	0.71 (0.08-5.96)	0.750		
HER-2	3.79 (1.20-12.00)	0.023		
Triple negative	1.95 (0.76-4.97)	0.163		
Type of Surgery		0.091		
BCS	1			
Mastectomy	5.62 (0.76-41.55)			

AR, androgen receptor; LN, lymph node; EIC, extensive intraductal component; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2; BCS, breast conserving surgery.

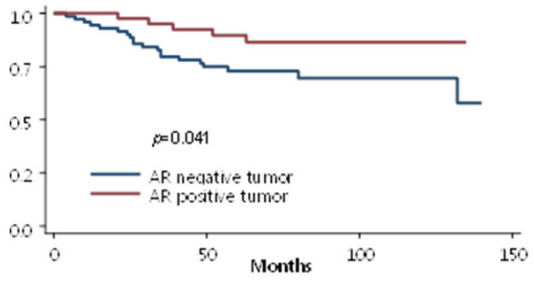

Figure 2. Disease-free survival curve of the whole study population.

tive BC had shorter survival [13]. Presently, AR expression in TNBC was associated with older age at diagnosis, smaller tumor size, early AJCC stage and lower histologic grade. To elucidate these mixed results among the studies, we should clarify unique role of the AR during tumorigenesis of TNBC.

There are currently no targeted therapies available for TNBC. AR is expressed in 10-43% of TNBCs, and one gene expression subset of TNBCs is androgen responsive [6]. The luminal group (LAR) was driven by AR signaling and sensitive to the effects of antiandrogens. The role and prognostic significance of AR expression in TNBC is unclear. AR expression in TNBC was correlated with postmenopausal status, lower histological grade, lack of LN metastasis and better OS [14]. Another study reported that AR expression in 287 TNBC patients was a favorable prognostic factor of DFS and OS [15]. However, contrary to these findings, several reports demonstrated that AR positivity in TNBC was associated with worse clinicopathological parameters and prognosis. AR positive TNBCs reportedly are associated with an 83% increase in overall mortality compared to AR negative tumors [16].

Although, there is disagreement as to the prognostic significance of AR expression in TNBC, it should be emphasized because AR positive TNBC patients may benefit from future targeted therapy. AR blockade could be a potential endocrine therapy for patients with ER negative BCs. Bicalutamide is an oral active nonsteroidal antiandrogen agent, in which ongoing clinical trials has designed the effect of bicalutamide in advanced AR positive and ER/PR negative BC [3].

We also found that AR status tended to be preserved in metastatic lymph node, recurrence and distant metastasis. A discordant AR status between primary BC and matched metastatic samples was observed in 21.6% (8/37) of cases tested. Preservation of AR in carcinoma cells between primary and metastatic/recurrent sites has previously been reported. In a recent study, 23 TNBC patients with matched recurrences (n = 16) and LN metastases (n = 46), AR discrepancies between primary tumors and metastasis did not occur [17]. In another study, AR status was performed on 356 primary BCs, 135 matching metastases and 12 recurrences [18]. A discrepant result was seen in 4.3% (5/117) of primary BC and matching LN metastases. No discrepancies were seen between primary BC and distant metastases or recurrence (n = 17). Compared to these two studies, we observed a higher discordant rate.

Several explanations can be offered for the high discordant rate of AR during tumor progression. First is the true molecular conversion during tumor progression. Conversion for ER-α and PR is mainly confined to the primary tumor and is absent in the metastasized tumors [7]. This finding may be explained by clonal selection of less differentiated receptor negative cells during the metastatic process. Likewise, discordant AR status could be a result from genetic drift during tumor progression. A second explanation is that the limited accuracy and reproducibility of receptor assays can lead to discordant AR status. Differences in tissue handling, tissue processing, interpretation of immunohistochemistry and different cut-off values that determine whether a tumor is positive or negative may have influenced discordance. The third explanation is that premature handling or insufficiently fixed specimens causes impairing of staining [19]. Finally, primary BCs could have exhibited marked intratumoral heterogeneity [20]. Remarkable heterogeneity in the mutational system and copy number alterations between primary tumors, circulating and disseminated tumor cells and metastases has been revealed [21]. Several studies reported discordance in receptor status between primary BC and synchronous nodal metastases as well as with metastatic sites [22, 23]. Many national and international guidelines for metastatic BC management recommend re-testing of at least one metastatic biopsy for hormonal and HER2 status [24, 25]. Therefore, it is important to re-biopsy metastatic sites during tumor progression for hormonal, HER2 as well as AR status, especially for TNBC

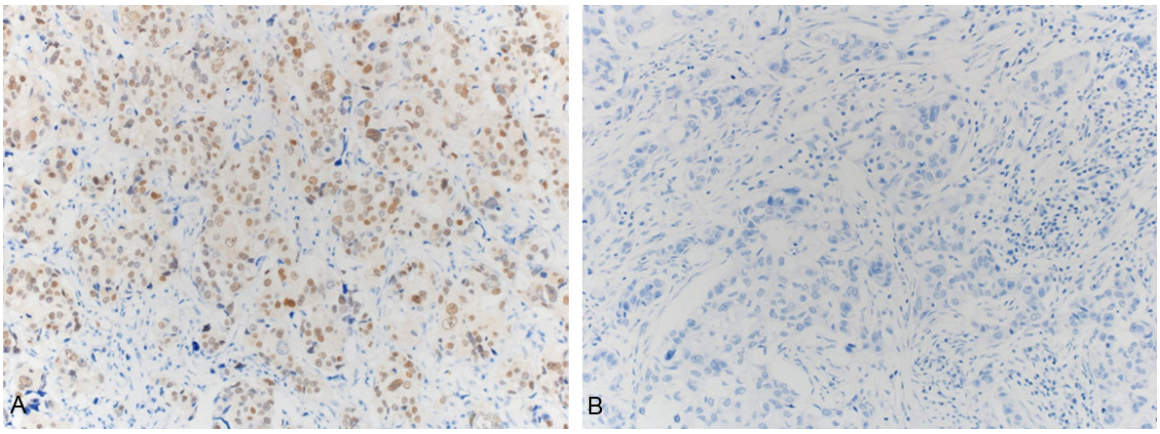

Figure 3. Discordant androgen receptor expression status. A. Primary breast cancer shows positive AR expression. B. Metastatic breast cancer of same patient shows negative AR status.

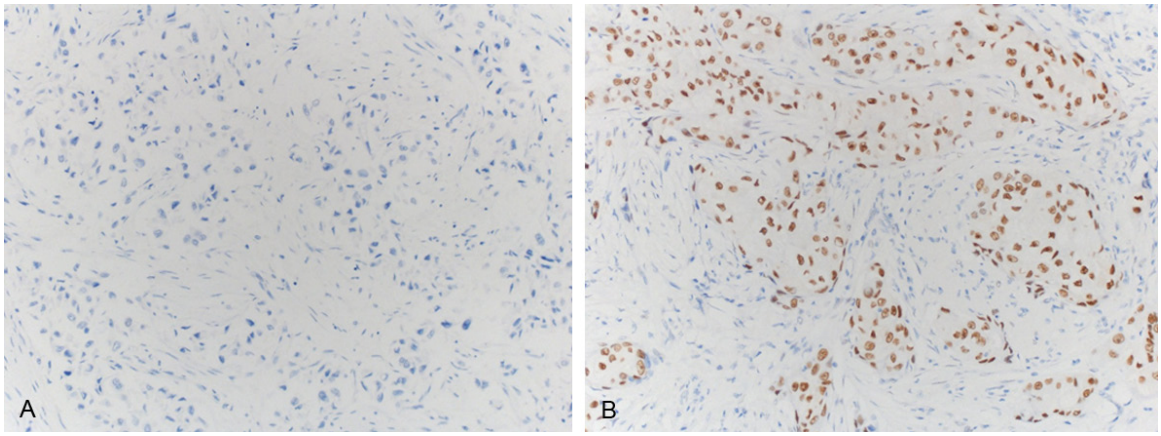

Figure 4. Discordant androgen receptor expression status. A. Primary breast cancer shows negative AR status. B. Metastatic breast cancer of same patient shows positive AR expression.

Table 4. Discordant AR status between primary BC and matched metastases

Number of patients	AR status			
	Primary BC	LN metastasis	Locoregional recurrence	Distant metastasis
13	Positive	Positive	-	-
2	Positive	-	-	Positive
1	Positive	Negative	-	-
9	Negative	Negative	-	-
1	Negative	-	Negative	-
4	Negative	-	-	Negative
4	Negative	Positive	-	-
1	Negative	Positive	Positive	-
2	Negative	-	-	Positive

AR, androgen receptor; BC, breast cancer; LN, lymph node.

patients, in which targeted therapies are not available yet. Further investigations are warranted to validate these findings.

Our study has several limitations. First, there were a small number of patients with too few LRR, distant metastasis and no death from BC. Larger sample size with better matched metastatic samples can effectively characterize survival differences. Second, due to the poor preservation status of tissue samples, immunohistochemical staining for LN metastasis, LRR and distant metastasis could not be performed for the entire population.

In conclusion, the presence of AR was significantly associated with favorable clinicopathologic and prognostic features. AR positive TNBC was associated with older age at diagnosis, smaller tumor size, early AJCC stage and lower histologic grade. In addition, AR status can be

changed during tumor progression such as LN metastasis, recurrent and distant metastatic tumors. Further evaluation will be needed to find out the mechanism of AR expression alteration.

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Disclosure of conflict of interest

None.

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