·Original Article·

Expression and clinical significance of androgen receptor in triple negative breast cancer

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[**Abstract**] **Background and Objective:** Androgen receptor (AR) is involved in the pathogenesis of breast cancer, but its role is not clearly defined. This study was to explore the expression of AR and its relationship with clinicopathologic parameters in triple negative breast cancer (negative estrogen receptor, negative progesterone receptor, and negative Her-2). **Methods:** Immunohistochemical assays were performed to determine the expression of AR in 137 cases of triple negative breast cancer and 132 cases of non-triple negative breast cancer. The relationships between AR expression and clinicopathologic data and prognosis were analyzed. **Results:** The positive rate of AR was significantly lower in triple negative breast cancer than in non-triple negative breast (27.7% vs. 83.3%, $\chi^2 = 83.963$, $P < 0.001$). AR expression was correlated with menorrheal status ($\chi^2 = 6.803$, $P = 0.009$), tumor grade ($\chi^2 = 5.173$, $P = 0.023$), node status ($\chi^2 = 7.787$, $P = 0.005$), 5-year disease-free survival ($\chi^2 = 5.012$, $P = 0.025$) and 5-year overall survival ($\chi^2 = 5.552$, $P = 0.018$) in triple negative breast cancer, but was not correlated with clinicopathologic parameters and survival in non-triple negative breast cancer. The 5-year overall survival rate was 78.8% in triple negative breast cancer and 83.3% in non-triple negative breast cancer. **Conclusions:** The expression of AR is related to biological behaviors of triple negative breast cancer, and plays a role in endocrinotherapy and prognostic prediction.

Key words: Breast neoplasm, androgen receptor, clinicopathologic characteristic, immunohistochemistry

Breast cancer is the most common malignancy for women. Multiple oncogenes, tumor suppressor genes, sex steroid hormones and their receptors are involved in the genesis and development of breast cancer. Breast cancer is also a heterogeneous tumor including a variety of subtypes with different biological behaviors, clinicopathologic features and molecular characteristics. The responses to treatment and the prognosis of different subtypes of breast cancer are also markedly different. Triple negative breast cancer (TNBC) is a newly proposed subtype of breast cancer, with poor biological behaviors, high invasiveness and poor prognosis. It can not benefit from endocrine therapy and HER2-targeted therapy due to the negative estrogen receptor (ER), negative progesterone receptor (PR) and negative human epidermal growth factor receptor-2 (HER2) [1]. Therefore, TNBC is mainly treated by chemotherapy. Currently, the clinical study for TNBC mainly focuses on prognostic indicators and the selection of therapeutic drugs. In recent years, it has been showed that androgen and androgen receptor (AR) also play an important role in the genesis and development of breast cancer, but their effects in different subtypes of breast cancer are still unclear. In the present study, we investigated the expression of AR in TNBC, and explored its correlation with the clinicopathologic features and prognosis of TNBC.

Materials and Methods

General data

A total of 137 TNBC patients with complete follow-up data who underwent surgical resection and did not undergo preoperative radiochemotherapy in Sun Yat-sen University Cancer Center between January 2001 and November 2004 were enrolled in our study; 132 non-triple negative breast cancer (non-TNBC) patients who underwent surgical resection during the same period were randomly selected as the control. All patients were female aging from 25–80 years old, with a median age of 49 years. All patients were finally diagnosed as breast cancer by postoperative pathology. The criterion of TNBC was ER-, PR- and HER2-negative. HER2 with – and + immunohistochemical staining was judged as negative expression; for the cases with ++ expression, further fluorescence in situ hybridization was

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performed to determine the negative or positive expression.

Methods

The specimens of breast cancer were fixed with 10% formaldehyde and routinely embedded with paraffin to make 4-μm serial tissue sections, and then HE staining and immunohistochemical staining were performed. Immunohistochemical staining was carried out according to the instruction of ZYMED PV-6000-G Kit. Briefly, after deparaffinization and hydration, the tissue sections were heated for antigen repair and treated with 3% hydrogen peroxide methanol solution to suppress endogenous peroxidase activity. After antigens were blocked with goat serum, the sections were incubated with AR antibody (1:100 working concentration) overnight, second antibody and ABC compound were biotinylated, DAB coloration was performed followed by hematoxylin counterstaining and sealing. The sections were observed under light microscope. PBS which replaced the primary antibody and served as the negative control, and the known positive prostate cancer samples served as the positive control.

Criterion for the result assessment

The cells in five high power fields were counted. The expression of AR in breast cancer tissues were assessed according to the percentage of positive cells and staining intensity: a percentage of positive cells of ≤ 1% scored 0, > 1% – ≤ 25% scored 1, >25% – ≤ 50% scored 2, >50% – ≤ 75% scored 3, >75% scored 4; weak expression (+) with slight yellow staining or only individual cells were stained in yellow to brown-yellow scored 1, strong expression (+++) with staining from brown-yellow to brown scored 3, moderate expression (++) with staining intensity between weakly positive and strongly positive scored 2. Scores of staining intensity × score of percentage of positive cells was the integrated score: a score of ≥2 represented positive result, and ≤1 represented negative result.

Statistical analysis

All data were analyzed with SPSS16.0 software. The χ^2 test was adopted to analyze the correlation between the positive rate of AR and the clinicopathologic parameters. The survival time was calculated from the date of final diagnosis. The Kaplan-Meier method was adopted for survival analysis, and the Cox risk model was adopted for multivariate analysis.

Results

Expression of AR protein

AR protein was expressed, mainly in cell nuclei with moderate intensity (Figure 1A), only in 38 of 137 TNBC patients. However, AR protein was expressed in 110 of 132 non-TNBC patients, mainly with strong intensity (Figure 1B). There was significant difference between these groups (χ^2 = 83.963, P < 0.001).

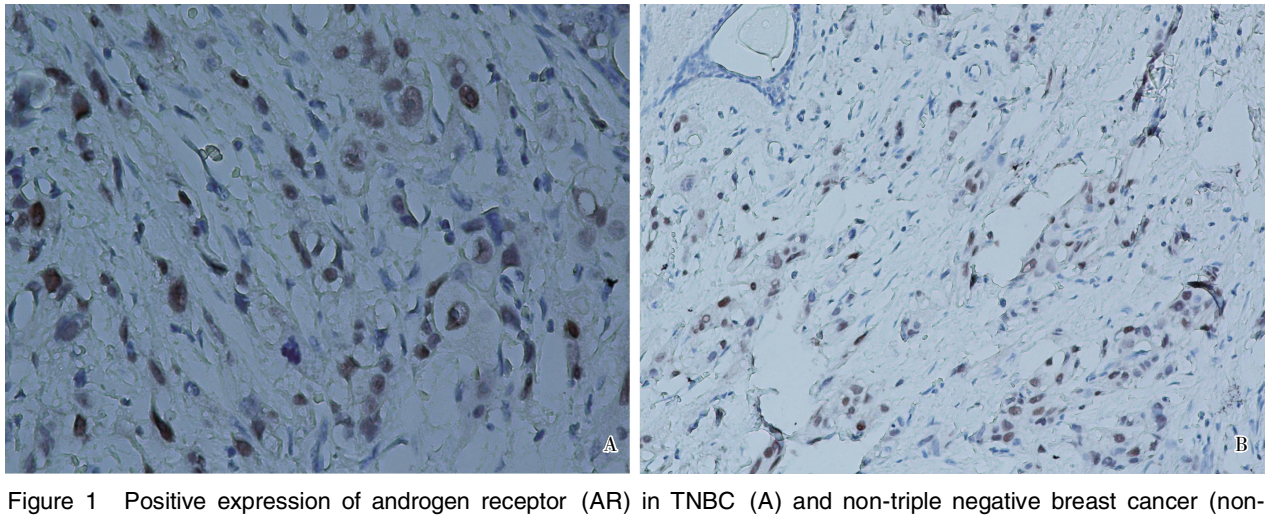

Figure 1 Positive expression of androgen receptor (AR) in TNBC (A) and non-triple negative breast cancer (non-TNBC, B) (IHC ×400)

Correlation between AR expression and clinicopathologic parameters

The correlation between AR expression and the clinicopathologic parameters of TNBC and non-TNBC is shown in Table 1. AR expression was correlated with the menorrheal status, histological grade and lymph node metastasis of the TNBC patients, but not correlated with the clinicopathologic parameters of non-TNBC patients.

Correlation between the clinicopathologic parameters and the survival of TNBC

Univariate analysis showed that the tumor size, lymph node metastasis, tumor stage, vascular invasion, and AR expression were correlated with 5-year overall survival (OS) of TNBC patients (Table 2). Multivariate analysis showed that tumor size, lymph node metastasis, and AR expression were correlated with 5-year OS of TNBC patients (Table 3).

Table 1 The correlation between androgen receptor (AR) and clinicopathologic characteristics of 137 patients with triple negative breast cancer (TNBC) and 132 patients with non-triple negative breast cancer (non-TNBC)

Clinicopathologic characteristic	TNBC					Non-TNBC				
	Cases	AR		χ^2	*P*	Cases	AR		χ^2	*P*
		+	−				+	−		
Age (years)				0.736	0.390				0.492	0.483
< 50	73	18	55			63	51	12		
> 50	64	20	44			69	59	10		
Menorrheal status				6.803	0.009				1.030	0.310
Premenopause	75	14	61			64	53	8		
Postmenopause	62	24	38			71	57	14		
Tumor size				0.146	0.702				0.000	1.000
T1 + T2	83	24	59			102	85	17		
T3 + T4	54	14	40			30	25	5		
Lymph node metastasis				7.787	0.005				0.492	0.483
No	71	27	44			69	59	10		
Yes	66	11	55			63	51	12		
Stage				1.732	0.423				1.517	0.468
I	28	10	19			29	25	4		
II	54	16	38			58	50	8		
III	55	12	43			45	35	10		
Tumor grade				5.173	0.023				2.723	0.099
I + II	48	19	29			57	44	13		
III	89	19	70			75	66	9		
Tumor necrosis				0.692	0.406				0.135	0.713
No	113	33	80			117	100	17		
Yes	24	5	19			15	10	5		
Vascular invasion				0.699	0.403				2.788	0.095
No	117	34	83			116	99	17		
Yes	20	4	16			16	11	5		
VEGF				0.546	0.460				2.481	0.115
Negative	58	18	40			56	50	6		
Positive	79	20	59			76	60	16		
P53				0.719	0.397				0.903	0.342
Negative	57	18	39			54	47	7		
Positive	80	20	60			78	63	15		
Ki67				1.027	0.311				1.438	0.230
Negative	52	17	35			51	45	6		
Positive	85	21	64			81	65	16		

Correlation between AR expression and the survival of breast patients

AR expression was correlated with the 5-year disease-free survival (DFS) and OS of TNBC patients (Table 4, Figure 2), but not correlated with the 5-year DFS and OS of non-TNBC patients (Table 4, Figure 3).

Discussion

ER and PR promote the genesis and development of breast cancer, and are associated with its prognosis. Clinically, they have been widely recognized to guide endocrine therapy of breast cancer[2]. However, the correlation between androgen as well as AR and breast cancer is still controversial. More and more basic and clinical studies have suggested that AR and androgen are closely associated with the genesis, development and prognosis of breast cancer. Moreover, clinical practice has proved that anti-androgen rescue therapy is effective for some advanced breast cancer patients who failed to routine endocrine therapy.

The effects of androgen and AR in breast cancer are still unclear. However, epidemiologic investigation and animal model showed that androgen is one of the etiologic factors for breast cancer. Kaaks *et al.*[3] found through a case-control study involving 1986 patients that the postmenopause women with high serum level of androgen had high risk for breast cancer. Tworoger *et al.*[4] found through a case-control study that the premenopause women with high serum level of androgen had high risk for breast cancer. If the serum levels of progesterone and/or estrogen were also increased simultaneously, the risk for breast cancer was even higher[4]. Wong *et al.*[5] found through an

Table 2 Univariate analysis of clinicopathologic characteristics and prognostic factors of 137 patients with TNBC

Clinicopathologic characteristic	Cases	5-year survival (%)	χ^2	*P*
Tumor size			5.682	0.017
T1 + T2	83	85.5		
T3 + T4	54	68.5		
Lymph node metastasis			4.431	0.035
No	71	85.9		
Yes	66	71.5		
Stage			6.304	0.043
I	28	85.7		
II	54	85.1		
III	55	69.1		
Vascular invasion			4.977	0.026
No	117	82.1		
Yes	20	60.0		
AR			5.552	0.018
Negative	99	73.7		
Positive	38	92.1		

animal model that testosterone combined with estrogen could induce the occurrence of breast cancer; if flutamide was used to rivalry the effect of estrogen, breast cancer would not be induced.

It has been reported that AR expression is correlated with the DFS and OS of patients with breast cancer. Bryan *et al.*[6] reported that AR expression was significantly correlated with the survival of breast cancer patients, even through the ER status was considered. Agoff *et al.*[7] analyzed AR expression and its significance in 69 ER-negative and 19 ER-positive breast cancer patients. Univariate analysis showed that AR expression was correlated with the DFS of ER-negative breast cancer patients (P = 0.049), but not correlated with the DFS of ER-positive breast cancer patients[7]. Gonzalez *et al.*[8] studied AR expression and it significance in 83 breast cancer patients, and found that AR expression was significantly correlated with the OS of breast cancer patients (P = 0.001). In our study, univariate analysis showed that AR expression, vascular invasion, primary tumor size, lymph node metastasis and tumor stage were significantly

Table 3 Multivariate Cox regression prognostic analysis of TNBC

Variate	Overall survival		Disease-free survival	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Age	1.012(9.958-1.070)	0.666	1.009(0.956-1.065)	0.740
Menorrheal status	1.590(0.454-5.567)	0.468	1.951(0.542-7.021)	0.306
Tumor size	3.118(1.782-5455)	0.000	3.563(1.957-6.485)	0.000
Lymph node metastasis	1.822(1.112-2.986)	0.017	2.177(1.308-3.624)	0.003
Stage	1.479(0.390-5.615)	0.565	1.064(0.271-4.167)	0.929
Tumor necrosis	1.702(0.651-4.452)	0.278	1.444(0.541-3.854)	0.463
Vascular invasion	1.908(0.706-5.518)	0.203	1.348(0.508-3.574)	0.549
VEGF	1.514(0.630-3.634)	0.354	1.104(0.471-2.587)	0.820
P53	1.601(0.662-3.870)	0.296	1.171(0.490-2.799)	0.722
Ki67	1.601(0.662-3.870)	0.496	1.501(0.575-3.919)	0.407
AR	1.721(0.891-2.132)	0.047	0.394(0.110-1.404)	0.151

Table 4 The correlation between AR and different subtypes of breast cancer and survival

Item	Cases	AR		χ^2	*P*
		+	-		
TNBC					
DFS (years)				5.012	0.025
> 5	96	32	64		
< 5	41	6	36		
OS (years)				5.552	0.018
> 5	108	35	73		
< 5	29	3	26		
Non-TNBC					
DFS (years)				0.941	0.332
> 5	97	79	18		
< 5	35	31	4		
OS (years)				2.138	0.144
> 5	110	94	16		
< 5	22	16	6		

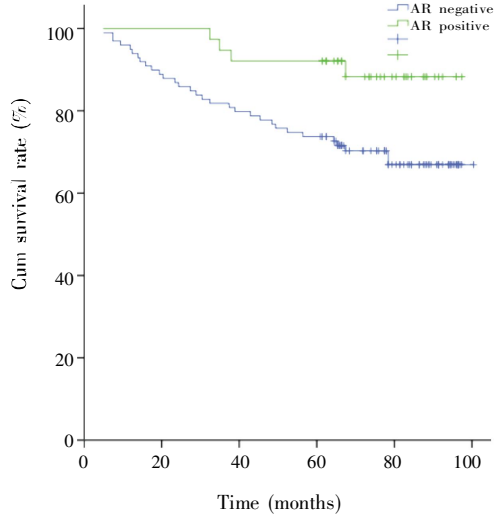

Figure 2 Correlation between AR expression and overall survival (OS) of 137 TNBC patients

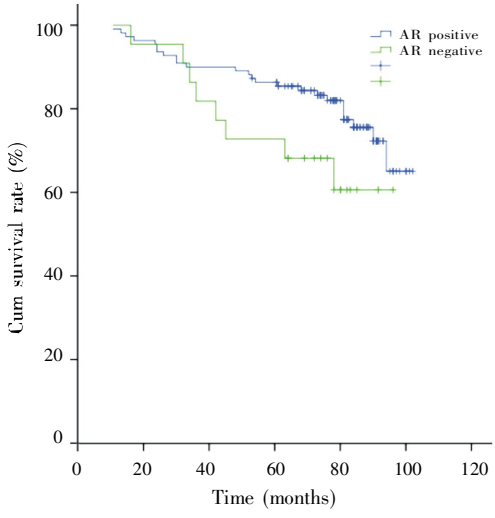

Figure 3 Correlation between AR expression and OS of 132 non-TNBC patients

correlated with the OS of TNBC patients; m ultivariate analysis showed that AR expression, primary tumor size and lymph node metastasis were correlated with the OS; univariate analysis showed that AR expression was not correlated with the DFS and OS of non-TNBC patients. The effect of AR expression on the DFS of breast cancer patients in our study is consistent with the results of Agoff *et al.*[7], but its effect on OS was inconsistent between the two studies. The possible reasons of the inconsistence are as follows: both ER-negative breast cancer and TNBC are ER-negative, therefore there are some similarities between these two types; but both PR and HER2 are negative in TNBC, therefore, its biological behaviors and prognosis are worse. In addition, the small sample size in the latter study may also contribute to the inconsistence. The effect of AR expression on the OS of breast cancer patients in our study is consistent with the results of Gonzalez *et al.*[8], however, our results showed that AR expression was mainly correlated with the DFS and OS of TNBC patients, but not non-TNBC patients.

It has been reported that AR expression is correlated with the prognosis of breast cancer. Kuenen-Boummeester *et al.*[9] analyzed the correlation between AR expression and the clinicopathologic features through detecting AR expression in 153 breast cancer patients. Univariate analysis showed that AR expression was an important prognostic factor for breast cancer, while multivariate analysis showed that only lymph node metastasis, tumor size and ER status were the independent prognostic factors for breast cancer[9], which was consistent with our results. Differing from the results of Kuenen-Boummeester *et al.*[9], we did not find the significant correlation between AR status and Ki67 status, although a correlation trend was found. Isola *et al.*[10] found that AR expression was significantly correlated with tumor grade. Narita *et al.*[11] studied AR expression and its significance in 156 female breast cancers, and found that AR expression was correlated with the pathologic type (P = 0.001), histological grade (P = 0.007) and lymph node metastasis (P = 0.002) of breast cancer. Our results showed that AR expression was correlated with the histological grade and lymph node metastasis of breast cancer, which is consistent with the literature. Bryan *et al.*[6] found that AR expression was not correlated with menorrheal status, while we found that AR expression was significantly correlated with menorrheal status. These differences may be due to that most patients in our study were TBNC patients.

Our study mainly focused on TBNC because TNBC has poor prognosis as compared with non-TNBC, and TNBC can not benefit from anti-estrogen therapy and HER2-targeted therapy. Our study showed that TNBC could be divided into good prognosis subtype and poor prognosis subtype according to AR status. Poor prognosis subtype may need more active postoperative treatment. The shortcomings of the present study are as follows: this was a retrospective study; some TNBC patients were lost to follow-up, and not all non-TNBC patients in the same period were included in our study.

In summary, the positive rate of AR in TNBC patients is about 30%, and AR expression is significantly correlated with the clinicopathologic features and prognosis of TNBC. TNBC could be further divided into good and poor prognosis subtypes according to AR expression. AR-positive patients could benefit from endocrine therapy. However, AR expression has no correlation with the clinicopathologic features and prognosis of non-TNBC. Whether AR expression is an important prognostic factor and whether it is correlated with the clinicopathologic features and prognosis of non-TNBC still require prospective studies with large sample sizes.

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