The prognostic value and immune microenvironment association of androgen receptor in HER2 positive early breast invasive ductal carcinoma

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Research article

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Abstract

Background

The prognosis of HER2+ early breast cancer is heterogeneous. AR, as an indicator of prognosis and treatment, is uncertain in HER2+ subtypes. We aimed to investigate the prognostic value of AR and the relationship between AR expression and immune microenvironment in HER2+ early breast invasive ductal carcinoma (IDC).

Methods

HER2+ early breast IDC patients diagnosed by pathology who underwent surgery at Sun Yat-sen University Cancer Center from 2016 to 2017 were the main population. All patients included performed AR test and their clinicopathological data were collected. The disease-free survival (DFS) and overall survival (OS) were evaluated by the Kaplan–Meier method and Cox proportional hazards model. AR+ and AR-breast IDCs were matched 1:1 according to age, T stage and N stage for immune infiltration analysis.

Results

A total of 554 patients with HER2+ early breast cancer were included in this retrospective study, regardless of HR status. Taking 10% as the cutoff values of AR, 81.6% of patients were AR positive and 18.4% were AR negative. ER+ ($P<0.001$) and PR+ ($P<0.001$) had significant relations with the positive expression of AR. Kaplan-Meier survival curves analysis suggested that AR had close links with OS ($P=0.001$), not DFS ($P=0.051$). Eliminating the potential impact caused by HR, AR also predicted a longer OS ($P=0.014$) and AR was an independent impact factor for OS by multivariate analysis ($P=0.036$) in HER2+HR- early breast IDC patients. In AR+ and AR- matched HER2+HR- patients, TILs ($P=0.043$) and PD-L1 ($P=0.027$) is significantly low in AR+ patients. The strongest negative correlation was observed between AR and PD-L1 (Pearson's $r=-0.299$, $P=0.001$). AR+ seemed to trend a favorable clinical survival in HER2+HR- IDCs with low TILs or positive PD-L1.

Conclusions

AR+ were markedly related to the better OS in HER2+HR- early breast cancer, while the negative correlation was observed between AR and PD-L1/TILs. We provided new insights for the prognostic value and immune microenvironment association of AR to optimize treatment strategies in HER2+ early breast IDCs.

Introduction

Breast cancer is a most prevalent cancer that affects women all over the world [1] and is a heterogeneous disease based on the immunohistochemical subtype. Among all invasive breast cancers, human epidermal growth factor receptor 2 (HER2) positive breast cancer accounts for 15-20% [2], which has distinct biological behavior and clinical outcomes. HER2 activation occurs through dimerization after

ligand binding and HER2 signaling activates proliferation, cell survival, metastasis and adhesion through different pathways [3, 4]. In HER2 positive (HER2+) early breast cancer, neoadjuvant/adjuvant chemotherapy together with anti-HER2 therapy has become the standard of care and is correlated with better outcome [5–7]. Although anti-HER2 therapy has significantly improved survival, the prognosis is different in HER2+ early breast cancer patients.

Androgen receptor (AR) is a steroid hormone nuclear receptor and is widely expressed in breast cancer [8, 9]. AR plays inhibitory and stimulatory effect in different types of breast cancer [10, 11]. In estrogen receptor (ER) positive breast cancer, MDV3100 research [12] has shown that AR signaling is required for both androgen- and estrogen-induced tumor cell growth in vitro and in vivo. In triple negative breast cancer (TNBC), luminal androgen receptor (LAR) subtype, characterized by the expression of the androgen receptor, has been associated with better prognosis, less chemotherapy responsiveness and lower pathologic complete response after neoadjuvant treatment [13]. Notwithstanding the role of the AR in several pathways, its impact on a biological and clinical standpoint is still controversial in HER2+ breast cancer. In vivo and in vitro experiments show that inhibition of AR can reduce the growth of HER2+ breast cancer cells, inhibit the phosphorylation of HER2 and the activation of Akt and Erk, but does not affect the expression of HER2 protein [14]. Epidemiological evidence suggests that in HER2+ER- patients with non-metastatic breast cancer, AR+ patients seems to have worse prognosis [15]. On another hand, a study comprising of 304 HER2-enriched metastatic breast cancer cases, considers that AR+ tumors have longer PFS and 5-year OS rate compared with AR-negative subjects [16].

Although breast cancer has been considered as a cancer with poor immunogenicity [17], the immune system still plays a key role in the growth and development of breast cancer [18]. The preclinical and clinical data suggest that response of in HER2-targeted therapies [19, 20] and clinical prognosis [21] can be predicted by immune cell compositions. The study of biologic basis for the interaction between AR and HER2 in breast cancer cell lines indicate that AR signaling regulates direct transcriptional induction of WNT7B and HER3 to result in ligand dependent Wnt and HER2 signaling pathways activation [22]. As for the relation between AR level, HER2 and immune microenvironment, van Rooijen et al finds that in patients with HER2+ metastatic breast cancer and trastuzumab treatment, the infiltration of tumor immune cells with low AR expression increased, while the infiltration of tumor immune cells with high AR expression decreased [23]. What is the relationship between AR expression and immune infiltration in HER2+ early breast cancer? Based on these, we analyzed the prognostic value of AR in all HER2+ and HER2+ hormone receptor negative (HR-) early breast invasive ductal carcinoma (IDC) patients and its correlation with immune microenvironment to provide new insights for biological behavior and reasonable treatment strategies.

Materials And Methods

Patient population

Considering the length of follow-up and the uniformity of treatment strategy, patients with breast cancer at Sun Yat-sen University Cancer Center between June 2016 and December 2017 were retrospectively reviewed. Inclusion criteria were as follows: (1) HER2+ breast invasive ductal carcinoma (IDC) diagnosed by pathology, (2) having received total mastectomy or lumpectomy, (3) patients without distant organ (including distant lymph node) metastasis at initial diagnosis, and (4) sufficient primary tumor pathological tissue for subsequent immune test. If the patient had a second primary malignancy, the patient was excluded from this study. All patients included performed AR test. Clinicopathologic information was retrieved from medical records, including age, TNM stage, pathologic values (including ER, progesterone receptor (PR), HER2, Ki-67 and AR), and treatment strategy. Disease free survival (DFS) was defined as the time from surgery of primary tumor to the disease recurrence or metastasis after curative surgery and overall survival (OS) was the time from surgery of primary tumor to death (all causes). All patients were followed-up until death or study data cutoff (June 2021). The study was approved by the Ethical Committees of Sun Yat-sen University Cancer Center (No. B2021-266-01) and individual consent for this retrospective analysis as part of usual clinical care was waived.

Markers' assessment

ER, PR, HER2, Ki67, and AR status of the primary tumor were assessed by immunohistochemistry staining and collected from the pathological report. Immunostainings for ER, PR, and Ki67 were evaluated according to the ASCO guidelines [24] and HER2 was assessed by immunostaining or in situ hybridization conformed to the ASCO guidelines. ER and PR were considered positive when $\geq 1\%$ of cells were stained. For HER2, it was expressed as the recommended 0–3+ by immunostaining evaluation. When the case scored was 3+, HER2 was considered positive. Fluorescence in situ hybridization was carried out in cases with a score of 2+. HER2 gene/chromosome 17 centromere ratio ≥ 2 or average HER2 gene copy number per cell ≥ 6 was considered HER2-positive. For Ki67, the positive percentage of its immunohistochemical staining was noted and Ki67 was defined as high when the percentage of stained cells was $\geq 20\%$ and low when $< 20\%$. About AR $\geq 10\%$ of the nuclear stained cells were positive.

AR+ and AR- patients with HER2+HR- breast IDC were matched 1:1 according to age, T stage and N stage for subsequent analysis. The expression of markers was quantified on formalin-fixed paraffin-embedded (FFPE) tissues and Haematoxylin and Eosin (H&E) and immunohistochemistry (IHC) staining were carried out in one batch per marker to avoid the differences of intensity. PD-L1 expression scores were reported as the percentage of tumor cells with membranous staining as determined and PD-L1 IHC testing was performed using the E1L3N antibody (Cell Signaling) in the biomarker cohort. When $\geq 1\%$ of cells were stained, PD-L1 were considered positive. [Tumor-infiltrating lymphocytes](#) (TILs) were evaluated throughout the whole tumor slides according to a standardized method developed by the international TILs working group [25]. Results are showed as percentage of TILs in the stromal tissue. The percentages of PD-L1 and TILs were averaged by two observers and used as the final score for every sample. If there was a greater than 20% difference, the two observers talked over the results to reach agreement.

Statistical Analysis

The categorical variables were described by percentages and the continuous variables were represented by median and range. Chi-square test's Phi coefficient or Spearman's correlation coefficient was used to evaluate the relation among the studied markers. Propensity score matching served to select matched AR+ and AR- patients. The Wilcoxon signed-rank test was used for the statistical analysis of variation in studies markers between paired tissues. The impact of the extent of clinicopathologic factors on DFS and OS was calculated by Kaplan-Meier curves. The Cox-proportional hazards model was carried out to evaluate the simultaneous influence on DFS and OS of all covariates. *P* values less than 0.05 were considered statistical difference for all tests, and all *P* values were tested two sided. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS), version 25.0.

Results

Clinicopathological characteristics and survival for HER2+ patients

A total of 554 female breast IDC patients were included from 4859 breast cancer patients visited Sun Yat-sen University Cancer Center between June 2016 and December 2017. The median follow-up time after the diagnosis of primary breast cancer was 53.3 months. The median level of AR per tumor was 60.0% (IQR: 20.0-80.0). Taking 10% as the cutoff values of AR, 81.6% of patients were AR positive and 18.4% were AR negative. The positive expression of ER ($P<0.001$) and PR ($P<0.001$) had significant relations with the high expression of AR. There was also a trend that breast IDC patients in the AR negative group may be older (the cutoff value was the median value of 50 years) ($P=0.030$). The clinicopathological data of included patients were summarized in Table 1. The treatment strategy of patients was determined by the physician, taking into account the patient's own wishes and their economic situation, especially medical insurance. There was no significant bias in HER-2 targeted therapy, adjuvant/neoadjuvant therapy and radiotherapy for included patients between AR+ and AR- cohorts. The median DFS and OS of these patients were 48.00 and 66.00 months, respectively. Kaplan-Meier survival curves analysis suggested that AR had close links with OS ($P=0.001$), not DFS ($P=0.051$) (Figure 1), regardless of whether these patients received HER2 targeted treatment or not (Figure 2). However, AR was not an independent impact factor for DFS and OS on multivariate analysis (Supplementary Table 1).

Clinicopathological characteristics and survival for HER2+HR- patients

To eliminate the potential impact caused by HR, we further explored the distribution of AR in clinicopathological values and the clinical outcomes in HER2+HR- breast IDC patients. A total of 266 patients were HR- in all HER2+ breast IDC patients. 75.2% (200/266) of patients were AR positive and 24.8% (66/266) of patients were AR negative and the percentage of AR- in HER2+HR- patients (24.8%) was higher than that in all HER2+ patients (18.4%). The median AR expression was 42.5% (IQR: 8.8-80.0). There was no bias in clinicopathological factors and treatment strategies between AR+ and AR- groups in these patients shown in Table 2. Similarly, HER2+HR- breast IDC patients with AR+ had longer OS ($P=0.014$) (Figure 3). And AR was also an independent impact factor for OS by multivariate analysis ($P=0.036$) in these patients (Table 3).

Correlation between AR and PD-L1, TILs and Ki-67

AR+ and AR- samples were matched 1:1 according to age, T stage and N stage in HER2+HR- breast IDC patients. A total of 59 pairs HER2+HR- patients were selected for pathological staining, but 3 pairs patients were excluded in subsequent analysis due to the absence of pathological specimens. In 56.3% (63 of 112) of the samples, PD-L1 was expressed by tumor cells. And 25 tumors expressed PD-L1 in AR+ group and 38 in AR- group. The median percentages of TILs in AR+ group and AR- group were 30% (IQR: 20-45) and 37.5% (IQR: 20-67.5), respectively. Representative images of TILs and PD-L1 staining in AR+ and AR- cohorts were shown in Figure 4. TILs ($P=0.043$) and PD-L1 ($P=0.027$) is significantly low in AR+ group in HER2+HR- breast cancer patients shown in Figure 5. The strongest inverse relation was found between AR and PD-L1 (Pearson's $r = -0.299$, $P=0.001$), followed by AR and TILs (Pearson's $r = -0.248$, $P=0.009$). There was no obvious correlation between AR and Ki-67 in HER-2+HR- breast IDC patients ($P=0.793$). In patients with low TILs (the cutoff value was the median value of 30%), high levels of AR in primary tumors were more likely to have longer DFS ($P=0.168$) and OS ($P=0.140$) than patients with low level of AR, although there was no statistical value (Figure 6A). There was also a trend that high expression of AR seemed to predict longer DFS and OS than patients with low AR in HER2+HR- breast IDC patients with positive PD-L1 (Figure 6B).

Discussion

Androgen receptor (AR) is frequently expressed in breast cancer and might be a predictive or prognostic factor and a drug target. However, controversial inferences on the prognostic value of AR and the relation between AR and immune microenvironment were observed in HER2+ breast cancer from present researches. Our study not only confirmed the prognostic role of AR in all HER2+ early breast IDCs, but also confirmed the favorable prognostic value of AR in HR-HER2+ early breast cancers. We found for the first time that AR was negatively correlated with immune microenvironment in patients with early breast cancer in HR-HER2+. We explored the association between AR and immune infiltration in HER2+ early breast cancer, in order to expand the current understanding of this disease and increase the stratification of prognosis, and hope to provide a reference for individualized treatment.

AR, as a potentially biomarker, is given more attention recently. The prognostic relevance of AR may be different among different BC subtypes. It has been reported that the relationships between AR and HER2 seems to be associated with ER status in all types of HER2+ breast cancer, as a statistically significant association between AR and HER2 was observed in ER-negative tumors but not in ER-positive tumors [26]. Ligand-bound AR binds to estrogen-related element, leading to cell apoptosis by binding to estrogen-related element in ER-positive/AR positive cell lines, while AR binds to androgen-related element in the nucleus, leading to proliferation in ER negative/AR-positive cell lines [27]. In present study, AR expression predicted a longer OS not only in all HER2+, but also in HER2+HR- early breast IDCs. This indicated that there was no documented association between the good prognosis of AR+ tumors and other favorable prognostic factors, such as ER and PR positivity. Consistent with our results, another cohort [16], including 304 HER2+HR- metastatic breast cancer patients, demonstrated that AR positivity had

favorable survival and predicted the efficacy of first-line Trastuzumab treatment in these patients. Moreover, we also found that survival of AR+ patients was better than that of AR- patients, regardless of whether AR- patients received HER2 targeted treatment or not. That further showed that positive AR was a good prognostic factor in HER2+ breast cancer. As our results and previous studies demonstrate that AR expression carries prognostic value, we recommend that patients with HER2-enriched breast cancer should have AR status evaluated on initial diagnosis in order to obtain comprehensive information. Interestingly, a meta-analysis [28] revealed that the positive expression of AR had the advantage of DFS and OS in all types of breast cancer patients, while the worse clinical outcome conferred by AR expression in patients with HER2+ER- (HER2-enriched) breast cancer. Gene analysis performed simultaneously in this study found that in HER2-enriched breast cancers, OS with AR mRNA expression was significantly better.

Since only a fraction of HER2+ breast cancer patients are responsive to a combination of anti-HER2 therapies and immune checkpoint inhibitors, therefore, a more comprehensive idea of the composition of immune infiltration in tumor microenvironment seems to be helpful in predicting treatment response and optimizing treatment strategy. This was the first research to demonstrate that AR level was related inversely to TILs and PD-L1 in primary tumors for HER2+HR- early breast cancer. One research suggested that the level of AR was negatively associated to immune infiltration (M2 tumor-associated macrophages, CD3+ and CD8+ T-cell) in HER2+ metastatic breast cancer [23], which was consistent with our results in HER2+ early breast cancer. At the same time, this study also found that in patients with high immune infiltration, high levels of AR in primary tumors have longer OS than patients with low level of AR [23]. Another study considering AR and immune microenvironment was carried out in 107 patients with early and locally advanced TNBC and found that AR expression was also associated with a low immune response from cluster analysis of RNA Gene expression profiling of primary tumors [29]. AR expression was consistent with an immune deserted environment based on our findings and previous researches on HER2+ metastatic breast tumors and TNBCs. This indicated that immunotherapy may have potential implications for tumors with low AR expression and rich immune cells. In addition, low level of AR was correlated to decreased sensitivity to anti-HER2 therapy in preclinical evidence focusing on the impact of AR signaling in HER2+ER- breast cancer cells. The most probable cause was HER2/HER3 signaling activation mediated impaired androgen, stimulating tumor cell growth [22]. Studies on the correlation between AR and PD-L1 were all focused on TNBC. AR+/FOXA1+ tumors exhibited less frequently PD-L1 expression in TNBC [30]. TNBC lacking AR expression are considered Quadruple negative Breast Cancers, where immune checkpoint inhibitors PD-L1 were significantly upregulated [31].

It is apparent that androgens and AR have many interactions on the biology of HER2+ breast cancer and deserve more clinical and translational research attention. Currently, AR is being actively investigated as therapeutic target in HER2+ breast cancer. A novel single-arm phase II clinical trial (NCT02091960) exploiting anti-AR in combination with anti-HER2 for AR-positive/HER2-positive/HR-negative MBC is underway. Although this study does not involve the exploration of immune infiltration, the findings of this study may also provide additional implications for clinical trials.

The limitations of our research are as follows. The optimal cut-off value for AR status still remains in dispute, varying from >1% to >75%, but the threshold of 10% was recommended commonly and showed independent prognostic and predictive significance. Also, limitations of the study lie in its retrospective feature and modest sample size of AR-negative cases. Therefore, further prospective studies are needed to confirm these results. In addition, unveiling the complicated interactions between AR and the HER2 signaling pathway will further help utilizing AR as an attractive therapeutic target in HER2-enriched early breast cancer.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethical Committees of Sun Yat-sen University Cancer Center (No. B2021-266-01). Individual consent for this retrospective analysis as part of usual clinical care was waived.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DY Z: Conceptualization, formal analysis, data curation, data interpretation, manuscript drafting, and manuscript editing.

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Abbreviations

AR	androgen receptor
DFS	disease free survival
ER	estrogen receptor
FFPE	formalin-fixed paraffin-embedded
HER2	human epidermal growth factor receptor 2
HER2+	HER2 positive
H&E	Haematoxylin and Eosin
HR-	hormone receptor negative
IDC	invasive ductal carcinoma
IHC	immunohistochemistry
LAR	luminal androgen receptor
OS	overall survival
PR	progesterone receptor
SPSS	Statistical Package for the Social Sciences
TILs	tumor-infiltrating lymphocytes
TNBC	triple negative breast cancer

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Tables

Table 1. Clinicopathological characteristics of 554 HER2+ breast IDC patients.

Factor	AR+ (n=452)	AR- (n=102)	<i>P</i>
Age			0.030
<50	231	40	
>=50	221	62	
TNM stage			0.259
I-II	312	65	
III	129	35	
ER			<0.001
<1%	204	66	
>=1%	248	36	
PR			<0.001
<1%	242	80	
>=1%	210	22	
Ki-67			0.125
<20%	55	7	
>=20%	397	95	
Adjuvant/neoadjuvant therapy			0.406
Yes	391	85	
No	61	17	
HER2 targeted therapy			0.924
Yes	266	60	
No	143	33	
Radiotherapy			0.380
Yes	128	27	
No	286	75	

Table 2. Clinicopathological characteristics of 266 HER2+HR- breast IDC patients.

Factor	AR+ (n=200)	AR- (n=66)	<i>P</i>
Age			0.555
<50	84	25	
>=50	116	41	
TNM stage			0.821
I-II	129	42	
III	66	23	
Ki-67			0.334
<20%	3	3	
>=20%	197	63	
Adjuvant/neoadjuvant therapy			0.057
Yes	181	54	
No	19	12	
HER-2 targeted therapy			0.073
Yes	140	39	
No	40	20	
Radiotherapy			0.640
Yes	59	19	
No	126	47	

Table 3. Cox proportional hazard regression analysis of DFS (A) and OS (B) in HER2+HR- breast IDC patients.

A

Faction	Univariable		Multivariable	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age (< 50 vs >= 50)	0.634 (0.365-1.100)	0.105		
TNM stage (I-II vs III)	4.125 (2.313-7.358)	<0.001	4.312 (2.180-8.529)	<0.001
AR (<10% vs >= 10%)	0.681 (0.380-1.223)	0.198		
Ki-67 (<20% vs >= 20%)	0.327 (0.102-1.053)	0.061		
Adjuvant/neoadjuvant therapy (no vs yes)	1.181 (0.425-3.280)	0.750		
HER-2 targeted therapy (no vs yes)	0.639 (0.348-1.174)	0.149		
Radiotherapy (no vs yes)	1.896 (1.076-3.341)	0.027	0.907 (0.471-1.747)	0.771

B

Faction	Univariable		Multivariable	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age (< 50 vs >=50)	1.858 (0.823-4.196)	0.136		
TNM stage (I-II vs III)	6.106 (2.699-13.817)	<0.001	10.211 (3.484-29.929)	<0.001
AR (<10% vs >= 10%)	0.410 (0.196-0.856)	0.018	0.408 (0.176-0.944)	0.036
Ki-67 (<20% vs >= 20%)	0.709 (0.096-5.215)	0.736		
Adjuvant/neoadjuvant therapy (no vs yes)	0.567 (0.216-1.487)	0.249		
HER-2 targeted therapy (no vs yes)	0.413 (0.183-0.930)	0.033	0.504 (0.216-1.176)	0.113
Radiotherapy (no vs yes)	1.628 (0.747-3.548)	0.220		

Figures

Figure 1

Kaplan–Meier curve for DFS (A) and OS (B) of breast cancer patients stratified by AR status.

Figure 2

Kaplan–Meier curve for DFS (A) and OS (B) of breast cancer patients stratified by AR status and HER2 targeted therapy.

Figure 3

Kaplan–Meier curve for DFS (A) and OS (B) of HER2+HR- breast cancer patients stratified by AR status.

Figure 4

Representative images of TILs and PD-L1 staining in AR+ and AR- cohorts (100×magnification). In AR+ cohort (A), the percentages of TILs (B) and the expression of PD-L1 (C) were low, and in AR- cohort (D), the percentage of TILs (E) and the expression of PD-L1 (F) were high.

Figure 5

Differential distribution of TILs and PD-L1 in matched AR+ and AR- group.

Figure 6

Kaplan–Meier curve for DFS and OS of HER2+HR- breast cancer patients stratified by AR status, TILs and PD-L1. A. Kaplan–Meier curve for DFS and OS of HER2+HR- breast cancer patients with low TILs stratified by AR status. B. Kaplan–Meier curve for DFS and OS of HER2+HR- breast cancer patients with PD-L1+ stratified by AR status.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [HER2ARSM.docx](#)