



A high AR:ER α or PDEF:ER α ratio predicts a sub-optimal response to tamoxifen therapy in ER α -positive breast cancer

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Abstract

Purpose Approximately 30% oestrogen receptor alpha (ER α)-positive breast cancer (BC) patients exhibit intrinsic or recurrent resistance to adjuvant endocrine therapy with tamoxifen. The androgen receptor (AR) is expressed in about 90% of ER α -positive patients, with particularly high expression in tamoxifen-resistant tumours. Prostate-derived Ets factor (PDEF), which is a co-regulator of AR, plays a role in tamoxifen resistance in ER α -positive BC. The purpose of this research was to analyse the potential roles of AR, PDEF and ER α levels in the response to tamoxifen resistance in ER α -positive BC.

Methods The nuclear AR:ER α and PDEF:ER α ratios were examined immunohistochemically in a cohort of 225 ER α -positive pre-menopausal BC patients who had received adjuvant tamoxifen therapy.

Results For both AR:ER α and PDEF:ER α ratios, the optimal cutoff value was 2.0. Patients receiving adjuvant tamoxifen treatment who had a high AR:ER α (≥ 2.0) (HR = 3.90) or PDEF:ER α ratio (≥ 2.0) (HR = 2.77) had a beyond twofold increased risk of failure. Both the AR:ER α ratio ($P = 0.001$) and PDEF:ER α ratio ($P = 0.002$) were independently associated with the risk of tamoxifen treatment failure. Furthermore, both a high ratio of AR:ER α (≥ 2.0) and PDEF:ER α (≥ 2.0) were associated with shorter disease-free survival (DFS) and shorter disease-specific survival (DSS). In addition, both the AR:ER α ratio and PDEF:ER α ratio were independent predictors of DFS (both $P < 0.0001$) and DSS ($P = 0.001$ and $P < 0.0001$, respectively).

Conclusions AR:ER α and PDEF:ER α ratios are independent predictors of the response to conventional ER α -directed tamoxifen endocrine therapy.

Keywords Prostate-derived Ets factor · Androgen receptor · Oestrogen receptor alpha · Tamoxifen · Breast cancer

Introduction

Tamoxifen is a selective oestrogen receptor modulator (SERM), which selectively prevents or induces oestrogen-like activity in different tissues and affects oestrogen receptors (ERs) [1]. By blocking oestrogen receptors on BC cells, tamoxifen inhibits the development of these cells. Other anti-oestrogen therapies include fulvestrant and aromatase inhibitors. Unlike pure ER agonists and antagonists, such as fulvestrant, which are specific to a particular tissue type, the actions of SERMs differ in various tissues, thereby enabling them to selectively inhibit or stimulate oestrogen-like action in various tissues [2, 3].

Tamoxifen is the most commonly used hormonal treatment for ER alpha (ER α)-positive BC and ER α -positive metastatic BC [4]. Although tamoxifen functions as an antagonist in BC, this SERM also acts as a partial agonist. In the endometrium, tamoxifen therapy has been linked to

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endometrial side effects, including cancer [5, 6]. Over time, the risk of endometrial cancer with tamoxifen therapy may double or quadruple. Thus, tamoxifen treatment typically does not exceed 5 years [7–9].

There are two different identified ERs in BC: ER α and ER beta (ER β). Both of them are ligand-activated transcription factors (TFs) which stimulate target gene expression [10]. About 70% of BCs are ER α positive. ER α promotes tumour cell growth of BC [11]. ER β is expressed in about 80% of normal human mammary epithelial cells, and ER β decrease or even shows no expression in BC cells. ER β inhibits BC cell growth, promotes apoptosis and enhances chemosensitivity [12].

The androgen receptor (AR) is expressed in a large proportion of primary invasive BCs, where it is often co-expressed with ER α and the progesterone receptor [13, 14]. Previous studies revealed AR expression in about 70% of BCs and up to 90% of ER α -positive tumours [15, 16]. The AR can suppress ER α and work as an anti-proliferative factor in ER α -positive BCs [17–19]. The AR exerts these anti-proliferative effects by directly or indirectly inhibiting ER α target genes. However, the mechanisms underlying AR and ER α signalling in anti-oestrogen therapy remain unclear [20, 21]. Some research revealed a high AR expression in tamoxifen-resistant MCF-7 BC cells [22]. Francesca et al. reported elevated AR mRNA and reduced ER α mRNA expression in tamoxifen-resistant breast tumours [23]. Cochrane et al. analysed AR expression in a cohort of 192 BC patients who had received adjuvant tamoxifen therapy. They found a median of 62.5% ER α positivity and 70.0% AR positivity in tamoxifen-insensitive patients. In contrast, ER α positivity was significantly higher in tamoxifen-sensitive patients (92.5%), and AR positivity was lower (57.5%). Furthermore, in their study, patients with a high AR:ER α ratio (≥ 2.0) had reduced disease-free survival (DFS) and disease-specific survival (DSS) [24].

Prostate-derived Ets factor (PDEF) is a TF and belongs to the E26 transformation specific (Ets) family. The Ets TFs share a unique DNA-binding domain, the Ets domain. The Ets proteins bind downstream target genes preferentially to a core 5'-GGAA/T-3' sequence [25]. In addition, PDEF is highly restricted to epithelial-based cells or tissues in the prostate, mammary gland, colon and ovary [26]. It has been reported that PDEF is a co-regulator of AR and act as an oncogene in prostate cancer [27]. Previously, we reported PDEF overexpression in ER α -negative BC tissues, where the TF functioned as an oncogene. In our study, the expression levels of PDEF were strongly, positively correlated with expression of AR, and AR positively regulates PDEF transcription [28, 29]. Gilles et al. reported that high PDEF expression in ER α -positive BCs contributed to tamoxifen resistance in patients with ER α -positive breast tumours [30]. Some authors suggested that PDEF expression, together with

ER α and AR expression, may be useful in the sub-classification of ER α -positive and AR-positive BC. However, the mechanisms underlying the roles of the AR, ER α and PDEF in ER α -positive BC tamoxifen resistance remain unclear.

The present study aimed to investigate whether AR, PDEF and ER α expression levels affected responses to adjuvant tamoxifen therapy in a group of 225 pre-menopausal female patients diagnosed with ER α -positive BC. The results suggested that an elevated AR:ER α or PDEF:ER α ratio may be indicative of tumours with a sub-optimal response to conventional tamoxifen endocrine therapy.

Materials and methods

Study population

This was a retrospective study about pre-menopausal female BC patients diagnosed with ER α -positive BC at the Cancer Hospital of Tianjin Medical University from 2009 to 2012. Patients who had received adjuvant endocrine therapy with tamoxifen for a period of 6 months to 5 years were recruited. Patients who discontinued tamoxifen treatment for various reasons (i.e., other than relapse) were excluded from the study. Follow-up deadline was December 12, 2017. The range of follow-up time was 60–108 months and corresponding mean follow-up time was 84 months.

Tissue specimens

In total, 225 formalin-fixed paraffin-embedded breast tumour specimens were collected and analysed. Disease recurrence was defined as local or regional recurrence or disease metastasis and BC-related death. Tamoxifen-sensitive patients received standard treatment of adjuvant tamoxifen for 5 years without any signs of disease recurrence. Tamoxifen-resistant patients experienced cancer recurrence or died after receiving the treatment of tamoxifen for at least 6 months.

Immunohistochemistry (IHC)

Immunohistochemical analyses were performed as described previously [28, 29, 31]. Anti-AR (1:200 dilution, ab9474), anti-PDEF (1:200 dilution, ab197375) antibodies were purchased from Abcam. Normal breast tissues were ER α and PR positive controls. Similarly, Ki67, p53, HER2, AR and PDEF-positive BC tissues were regarded as positive controls, respectively. In addition, primary antibodies were substituted by normal goat serum for negative controls. AR and PDEF were considered positive in case the nuclear staining was present beyond 10% of the tumour cells [28, 31]; the staining intensity of AR, PDEF is not within the calculation range.

Calculation of AR:ER α and PDEF:ER α ratios

Two pathologists independently quantified the nuclear AR, PDEF and ER α expression. The AR-positive expression percentage divided by the percentage of ER α -positive expression was the ratio of AR:ER α . The AR, PDEF and ER α staining intensity could not be calculated, as it was outside the calculation range. The AR:ER α and PDEF:ER α ratios were based on the average of the two pathologists' scores. Based on the DFS and DSS of the cohort, the optimal cutoff value for the AR:ER α and PDEF:ER α ratios was 2.0 [24].

Statistical analysis

All statistical analyses in this study used the SPSS 20.0 statistical software. Chi-squared tests were used to evaluate associations between AR:ER α and PDEF:ER α ratios and the status of some clinicopathological factors. Kaplan–Meier analyses were used to calculate AR:ER α and PDEF:ER α ratios and the survival analysis. All independent prognostic effects of the variables with 95% confidence interval were evaluated by multivariate Cox proportional hazard regression model. $P < 0.05$ was considered to reflect significance.

Results

AR, PDEF and ER α staining in tamoxifen-resistant and tamoxifen-sensitive patients

Fifty-two (23.1%) patients failed to respond to the tamoxifen therapy. Those who relapsed while receiving tamoxifen therapy generally had a higher proportion of grade 3 tumours (36.2% vs 22.0%, $P = 0.032$), a higher proportion of lymphatic metastasis (47.7% vs 43.2%, $P = 0.034$) and larger tumours (median 2.4 vs 1.9 cm³, $P = 0.004$) compared with those who did not relapse. There were no differences in the pTNM stage or HER2, Ki-67 and P53 staining percentages between the two groups.

In the tamoxifen-resistant group, a median of 50.0% of the tumours were ER α positive as compared with 75.0% of the tumours in the group of tamoxifen sensitive ($P < 0.0001$). Furthermore, compared with the group of tamoxifen sensitive, tamoxifen-resistant group has a higher percentage of AR-positive cells in the tumours (47.6% vs 77.5%, $P < 0.0001$). In addition, the percentage of PDEF-positive cells in the tumours in the tamoxifen-resistant group was higher than that in the tumours in the tamoxifen-sensitive group (78.3% vs 45.0%, $P < 0.0001$).

Ratios of AR:ER α and PDEF:ER α in tamoxifen-resistant and tamoxifen-sensitive cases

Based on the cutoff value of 2.0, the AR:ER α and PDEF:ER α ratios were significantly different in the tamoxifen-resistant and tamoxifen-sensitive cases. In the cohort, the AR:ER α ratio was ≥ 2.0 in 51.9% (27/52) of tamoxifen-resistant cases, whereas the ratio was ≥ 2.0 in only 1.73% (3/173) of tamoxifen-sensitive cases ($P < 0.0001$). In terms of the PDEF:ER α ratio, this was ≥ 2.0 in 65.4% (34/52) of the tamoxifen-resistant cases and ≥ 2.0 in 1.20% (2/173) of tamoxifen-sensitive cases ($P < 0.0001$) (Table 1).

We compared the correlation between the AR:ER α or PDEF:ER α ratios (< 2.0 or ≥ 2.0) and clinical and pathological variables in the whole cohort. As shown in Table 1, the AR:ER α ratio was associated with tumour size ($P < 0.0001$), tumour grade ($P = 0.034$), pTNM stage ($P = 0.025$), tamoxifen treatment ($P < 0.0001$) and AR expression ($P < 0.0001$). The PDEF:ER α ratio was associated with tumour size ($P < 0.0001$), tumour grade ($P = 0.029$), pTNM stage ($P = 0.002$), tamoxifen treatment ($P < 0.0001$) and PDEF expression ($P < 0.0001$). Furthermore, the correlation analysis showed that a high AR:ER α ratio (≥ 2.0) was positively associated with a high PDEF:ER α ratio (≥ 2.0) ($r = 0.433$, $P = 0.001$) in the tamoxifen-resistant cases ($n = 52$). However, in the tamoxifen-sensitive cases, there was no association between the AR:ER α and PDEF:ER α ratios ($r = -0.014$, $P = 0.851$). Failure to respond to tamoxifen treatment was associated with elevated AR:ER α (≥ 2.0) or PDEF:ER α ratios (≥ 2.0) (Table 2).

Association of high AR:ER α or PDEF:ER α ratios (≥ 2.0) with poor sensitivity to tamoxifen endocrine therapy

Figure 1 shows the survival analyses comparing AR:ER α ratios < 2.0 and ≥ 2.0 . Tumours with a higher AR:ER α ratio (≥ 2.0) were associated with shorter DFS ($P < 0.0001$) and shorter DSS ($P = 0.0005$) in the 225 tamoxifen-treated patients (Fig. 1a, b). In the 52 tamoxifen-treated failure cases, an AR:ER α ratio ≥ 2.0 was related to reduced DFS ($P = 0.0207$), but the same ratio was not associated with DSS ($P = 0.1500$) (Fig. 1c, d). Furthermore, in tamoxifen-treated failure cases, recurrence occurred approximately 10 months earlier in those with a high AR:ER α ratio as compared with the recurrence time in those with a low (< 2.0) ratio. Figure 1e shows representative AR:ER α staining in the < 2.0 and ≥ 2.0 patterns.

Figure 2 shows the survival analyses separated into two groups according to PDEF:ER α ratios < 2.0 and ≥ 2.0 . As shown in Fig. 2a and b, a high PDEF:ER α ratio (≥ 2.0) was associated with poorer DFS ($P < 0.0001$) and shorter DSS

Table 1 Comparison of AR:ER α ratio and PDEF:ER α ratio to clinical and pathologic variables

Variable	AR:ER α (%)		P value	PDEF:ER α (%)		P value
	<2 195 (86.7)	\geq 2 30 (13.3)		<2 189 (84.0)	\geq 2 36 (16.0)	
Age						
\leq 40	49 (81.7)	11 (18.3)	0.135	46 (76.7)	14 (23.3)	0.057
>40	146 (88.5)	19 (11.5)		143 (86.7)	22 (13.3)	
Tumour size (cm)						
< 2	134 (91.8)	12 (8.20)	0.001*	133 (91.1)	13 (8.90)	<0.0001*
2–5	40 (93.8)	8 (16.7)		34 (70.8)	14 (29.2)	
> 5	21 (67.7)	10 (32.3)		22 (71.0)	9 (29.0)	
Grade						
1	36 (90.0)	4 (10.0)	0.034*	37 (92.5)	3 (7.50)	0.029*
2	107 (90.7)	11 (9.30)		102 (86.4)	16 (13.6)	
3	52 (77.6)	15 (22.4)		50 (74.6)	17 (25.4)	
pTNM stage						
TNM I	95 (92.2)	8 (7.80)	0.025*	95 (92.2)	8 (7.80)	0.002*
TNM II	81 (84.4)	15 (15.6)		77 (80.2)	19 (19.8)	
TNM III	19 (73.1)	7 (26.9)		17 (64.4)	9 (34.6)	
Lymph node						
Negative	92 (89.3)	11 (10.7)	0.190	89 (86.4)	14 (13.6)	0.236
Positive	103 (84.4)	19 (15.6)		100 (82.0)	22 (18.0)	
Tamoxifen treatment						
Succeed	170 (98.3)	3 (1.70)	<0.0001*	171 (98.8)	2 (1.20)	<0.0001*
Failed	25 (48.1)	27 (51.9)		18 (34.6)	34 (65.4)	
AR						
Negative	52 (100.0)	0 (0.00)	<0.0001*	44 (84.6)	8 (15.4)	0.541
Positive	143 (82.7)	30 (17.3)		144 (83.8)	28 (16.2)	
PDEF						
Negative	50 (89.3)	6 (10.7)	0.339	56 (100.0)	0 (0.00)	<0.0001*
Positive	145 (85.8)	24 (14.2)		133 (78.7)	36 (21.3)	
HER2						
Negative	151 (88.8)	19 (11.2)	0.078	145 (85.3)	25 (14.7)	0.232
Positive	44 (80.0)	11 (20.0)		44 (80.0)	11 (20.0)	
Ki-67 (%)						
<20	60 (84.5)	11 (15.5)	0.326	59 (83.1)	12 (16.9)	0.472
\geq 20	135 (87.7)	19 (12.3)		130 (84.4)	24 (15.6)	
P53						
Negative	131 (83.4)	26 (16.6)	0.210	124 (79.0)	33 (21.0)	0.100
Positive	64 (94.1)	4 (5.90)		65 (95.6)	3 (4.40)	

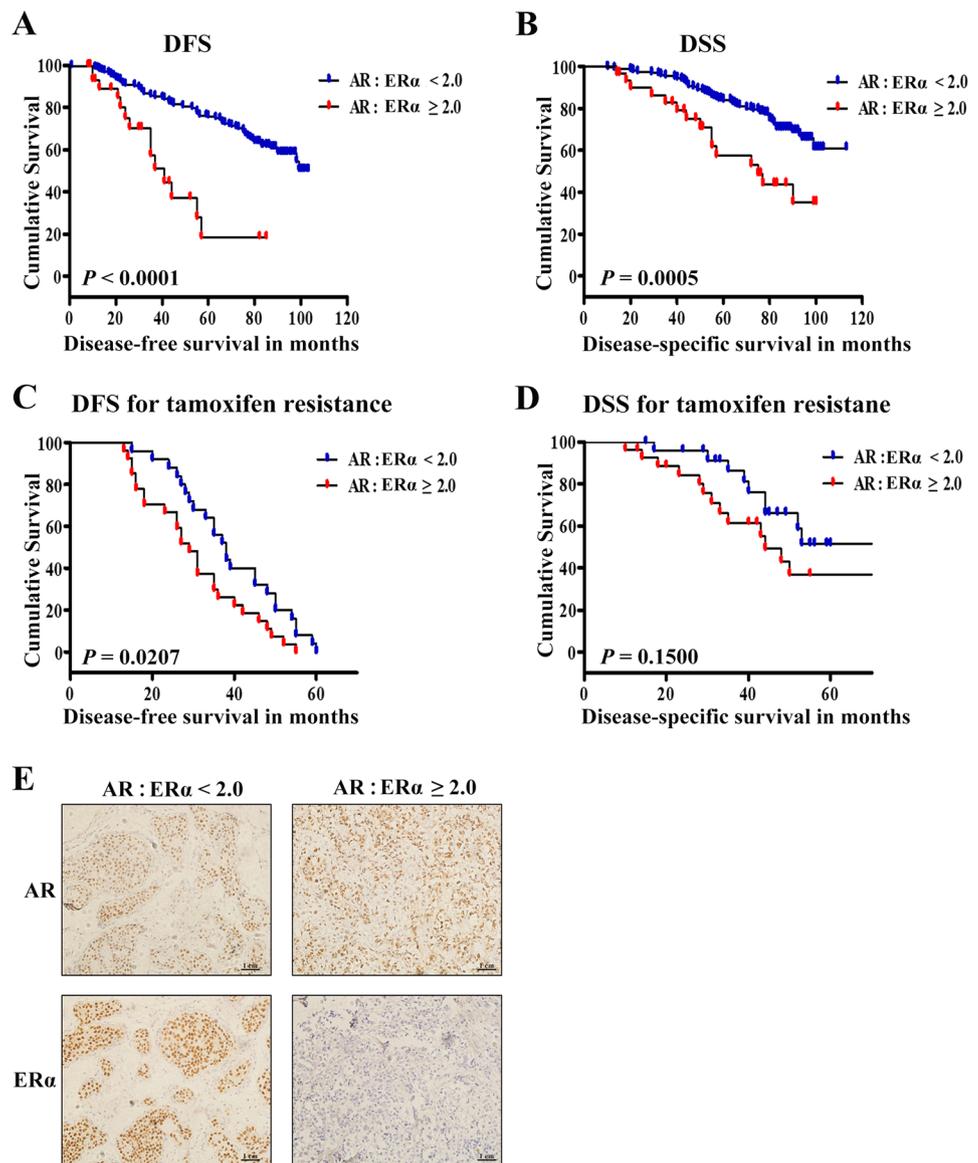
*Significantly different

Table 2 Relationship of AR:ER α and PDEF:ER α in the tamoxifen treatment failed cases and tamoxifen treatment succeeded cases

Variable	TAM treatment failure	AR:ER α				TAM treatment success	AR:ER α			
		<2	\geq 2	R	P		<2	\geq 2	R	P
PDEF:ER α										
<2	18	14	4	0.433	0.001*	171	168	3	-0.014	0.851
\geq 2	34	11	23			2	2	0		

*Significantly different

Fig. 1 Disease-free survival (DFS) and disease-specific survival (DSS) in patients according to the AR:ER α ratios. The patients were divided into two groups: those with AR:ER α ratios <2.0 (blue squares) and those with AR:ER α ratios \geq 2.0 (red squares). Kaplan–Meier survival curves for: **a** DFS of all the patients, **b** DSS of all the patients, **c** DFS of the tamoxifen-resistant patients, **d** DSS of the tamoxifen-resistant patients and **e** representative images of AR and ER α staining in the two groups (\times 200 magnification)



($P < 0.0001$) in the whole cohort. As depicted in Fig. 2c and d, in the 52 tamoxifen-treated failure cases, a high PDEF:ER α ratio (≥ 2.0) was associated with shorter DFS ($P = 0.0372$) but not with shorter DSS ($P = 0.2227$). Representative PDEF:ER α staining in the <2.0 and ≥ 2.0 patterns is shown in Fig. 2e.

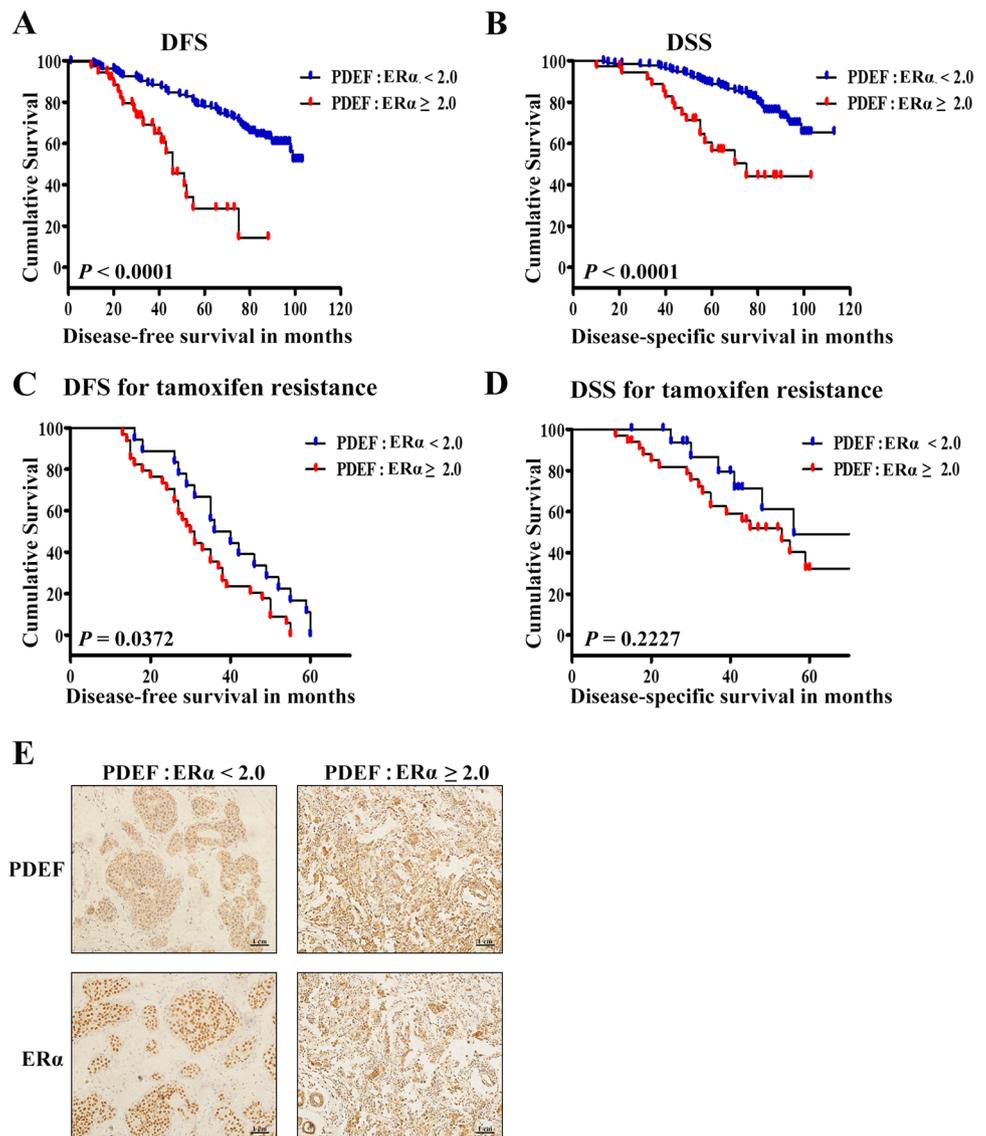
Based on these results, the survival data were separated into the following four groups: AR:ER α ratios <2.0 and PDEF:ER α ratios <2.0, AR:ER α ratios ≥ 2.0 and PDEF:ER α ratios <2.0, AR:ER α ratios <2.0 and PDEF:ER α ratios ≥ 2.0 and AR:ER α ratios ≥ 2.0 and PDEF:ER α ratios ≥ 2.0 . In all 225 cases, tumours with a high AR:ER α ratio (≥ 2.0) and high PDEF:ER α ratio (≥ 2.0) were associated with shorter DFS ($P < 0.0001$) and shorter DSS ($P < 0.0001$) (Fig. 3a, b). In the 52 tamoxifen-treated failure cases, tumours with a high AR:ER α ratio (≥ 2.0) and high PDEF:ER α ratio

(≥ 2.0) were associated with shorter DFS ($P = 0.0454$) but not with shorter DSS ($P = 0.2834$) (Fig. 3c, d). These data showed that as compared with patients with low AR:ER α /PDEF:ER α ratios, those with high AR:ER α (≥ 2.0) and PDEF:ER α ratios (≥ 2.0) tended to have shorter DFS and DSS and treatment failure. Representative staining patterns in the four groups are shown in Fig. 3e.

Roles of high AR:ER α and PDEF:ER α ratios as independent predictors of poor survival

As shown in Table 3, in the 52 tamoxifen-resistant cases, a univariate survival analysis revealed that younger age ($P = 0.027$), higher tumour grade ($P = 0.024$), higher AR:ER α ratio ($P = 0.001$) and higher PDEF:ER α ratio ($P = 0.001$) were significantly associated with tamoxifen

Fig. 2 Disease-free survival (DFS) and disease-specific survival (DSS) in patients according to the PDEF:ER α ratios. The patients were divided into two groups: those with PDEF:ER α ratios < 2.0 (blue squares) and those with PDEF:ER α ratios ≥ 2 (red squares). Kaplan–Meier survival curves for: **a** DFS of all the patients, **b** DSS of all the patients, **c** DFS of the tamoxifen-resistant patients, **d** DSS of the tamoxifen-resistant patients and **e** representative images of PDEF and ER α staining in the two groups ($\times 200$ magnification)



failure at 5 years. A multivariate analysis confirmed that a higher tumour grade ($P = 0.003$), higher AR:ER α ratio ($P = 0.001$) and higher PDEF:ER α ratio ($P = 0.002$) were significant independent prognostic predictors of tamoxifen failure at 5 years.

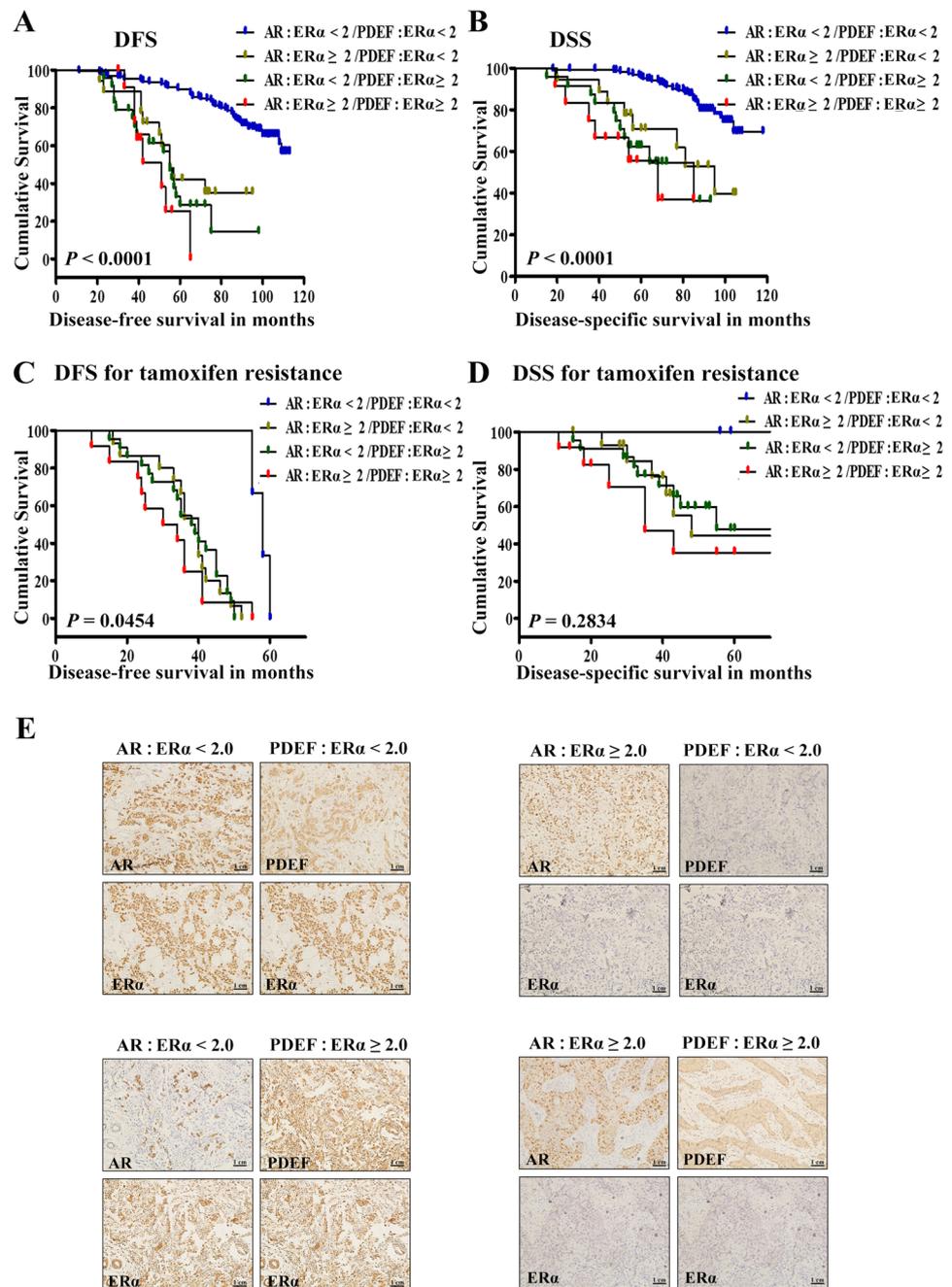
We then compared the variables (tumour size, pTNM stage and AR:ER α /PDEF:ER α ratios) with overall DFS and overall DSS. As shown in Table 4, in the univariate analysis, the tumour size, pTNM stage, AR:ER α ratio and PDEF:ER α ratio were significantly associated with DFS and DSS. Notably, a higher AR:ER α ratio ($P < 0.0001$ for DFS; $P = 0.001$ for DSS) and higher PDEF:ER α ratio ($P < 0.0001$ for DFS; $P < 0.0001$ for DSS) were the most significant markers of poor survival. The multivariate analysis demonstrated that an AR:ER α ratio ≥ 2.0 ($P < 0.0001$), a PDEF:ER α ratio ≥ 2.0 ($P < 0.0001$) and a higher pTNM stage ($P = 0.005$) were independent predictors of poor DFS. In addition, an AR:ER α

ratio ≥ 2.0 ($P = 0.001$), a PDEF:ER α ratio ≥ 2.0 ($P < 0.0001$) and a higher pTNM stage ($P = 0.012$) were independent predictors of poor DSS.

Discussion

In this study, we analysed AR, PDEF and ER α expression in tumours of 225 pre-menopausal women with ER α -positive BCs treated with tamoxifen. The data revealed that a high AR:ER α or PDEF:ER α ratio showed a shorter time to relapse in tamoxifen-treated patients. In the study cohort, an AR:ER α ratio ≥ 2 and PDEF:ER α ratio ≥ 2 were independent predictors of tamoxifen treatment failure after 5 years and poor DFS and DSS. Although the results of the present study need to be studied in more researches, they indicate

Fig. 3 Disease-free survival (DFS) and disease-specific survival (DSS) in patients according to the AR:ER α ratios and PDEF:ER α ratios. The patients were divided into four groups: those with AR:ER α ratios <2.0 and PDEF:ER α ratios <2.0 (blue squares), those with AR:ER α ratios \geq 2.0 and PDEF:ER α ratios <2.0 (grey squares), those with AR:ER α ratios <2.0 and PDEF:ER α ratios \geq 2.0 (green squares) and those with AR:ER α ratios \geq 2.0 and PDEF:ER α ratios \geq 2.0 (red squares). Kaplan–Meier survival curves for: **a** DFS of all the patients, **b** DSS of all the patients, **c** DFS of the tamoxifen-resistant patients, **d** DSS of the tamoxifen-resistant patients and **e** representative images of AR, PDEF and ER α staining in the two groups ($\times 200$ magnification)



that AR:ER α and PDEF:ER α ratios may be new predictors of the response to routine ER α -directed endocrine therapies.

Androgen receptor is expressed in about 90% of ER α -positive BC [32]. The physiological interplay of AR and ER (ER α and ER β) signalling is complex. In ER α -positive BC, the AR inhibits ER α transactivation activity and cell proliferation. Several potential mechanisms have been proposed to explain these effects: (1) the AR directly inhibits ER α target genes; (2) the AR binds and sequesters cofactors that are no longer available for ER α gene transcription; (3) the AR competes for binding ER α target genes; and (4), the AR induces

upregulation of ER β , which then exerts growth and invasion inhibitory actions [33–36] (Fig. 4a). There is an added level of complexity in the interplay between AR and ER α signalling in the context of anti-oestrogen therapy. Some studies showed that exogenous overexpression of AR rendered ER α -positive MCF-7 BC cells resistant to tamoxifen [23]. Potential mechanisms by which the AR may interact with ER α signalling include: (1) tumorigenesis and progression from oestrogen dependence to androgen dependence and AR acting as a coactivator to promote cell proliferation, (2) displacement of corepressor proteins, (3) or recruitment of

Table 3 Univariate and multivariate analyses with respect to tamoxifen failure at 5 years in the 52 tamoxifen-resistant cases

Variable	Tamoxifen failure at 5 years			
	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.00			
(≤ 40 vs > 40)	1.068 (0.491, 2.321)	0.027*	–	
Tumour size	1.00			
(< 2 vs 2–5 cm)	0.949 (0.434, 2.074)	0.896	–	
(< 2 vs > 5 cm)	0.571 (0.254, 1.285)	0.176	–	
Tumour grade	1.00			
(1 vs 2)	1.408 (0.562, 3.525)	0.465	–	
(1 vs 3)	2.266 (1.116, 4.604)	0.024*	2.830 (1.419, 5.645)	0.003*
pTNM stage	1.00			
(I vs II)	0.663 (0.261, 1.686)	0.388	–	
(I vs III)	1.208 (0.562, 2.593)	0.628	–	
Lymph node	1.00			
(Negative vs positive)	0.735 (0.415, 1.302)	0.291	–	
AR	1.00			
(Negative vs positive)	0.705 (0.481, 1.031)	0.071	–	
AR/ER α	1.00			
(< 2 vs ≥ 2)	3.903 (2.326, 6.549)	0.001*	2.784 (1.506, 5.147)	0.001*
PDEF	1.00			
(Negative vs positive)	0.688 (0.292, 1.620)	0.392	–	
PDEF/ER α	1.00			
(< 2 vs ≥ 2)	2.776 (0.973, 6.244)	0.001*	2.802 (1.459, 5.381)	0.002*
HER2	1.00			
(Negative vs positive)	1.370 (0.761, 2.467)	0.294	–	
Ki-67	1.00			
(< 20% vs $\geq 20\%$)	1.202 (0.662, 2.182)	0.546	–	
P53	1.00			
(Negative vs positive)	1.297 (0.845, 1.991)	0.234	–	

*Significantly different

activators, such as PDEF. In this research, AR upregulated PDEF expression, and PDEF enhanced the growth and invasion of ER α -positive BC [21, 29, 33, 37] (Fig. 4b).

PDEF, as an Ets factor, is involved in a variety of tumorigenesis processes, such as tumour proliferation, invasion and apoptosis. As compared with other Ets TFs, PDEF has some unique characteristics, the most important of which is its epithelial-specific distribution in breast tissue [38–40]. Therefore, PDEF is closely related to the expression of AR and ER α . Given its localization, PDEF is closely related to both AR and ER α expression. For example, in ER α -positive BC, researches shown PDEF drove luminal differentiation of basal mammary epithelial cells and contributed to tamoxifen resistance [30]. Previous studies, including research by our own group, demonstrated AR and PDEF overexpression in ER α -negative BC, with overexpression associated with

worse survival [28, 41]. These findings suggested that the AR, ER α and PDEF played an important role in tamoxifen resistance in BC, together with ER α positivity. In the present study, the patients with a PDEF:ER α ratio ≥ 2 had a beyond twofold increased risk of tamoxifen treatment failure. We previously demonstrated that PDEF was a direct transcriptional target of AR and AR-induced upregulation of PDEF. Thus, we speculated that PDEF upregulated by AR may act as an activator and contribute to tamoxifen resistance. Further research is needed to determine whether PDEF plays an important role in the biological stratification of tamoxifen resistance in ER α -positive BCs and to identify therapeutic strategies.

In conclusion, results of this research based on clinical specimens suggested that the ratios of nuclear AR:ER α and

Table 4 Univariate and multivariate analyses with respect to DFS and DSS in the whole series

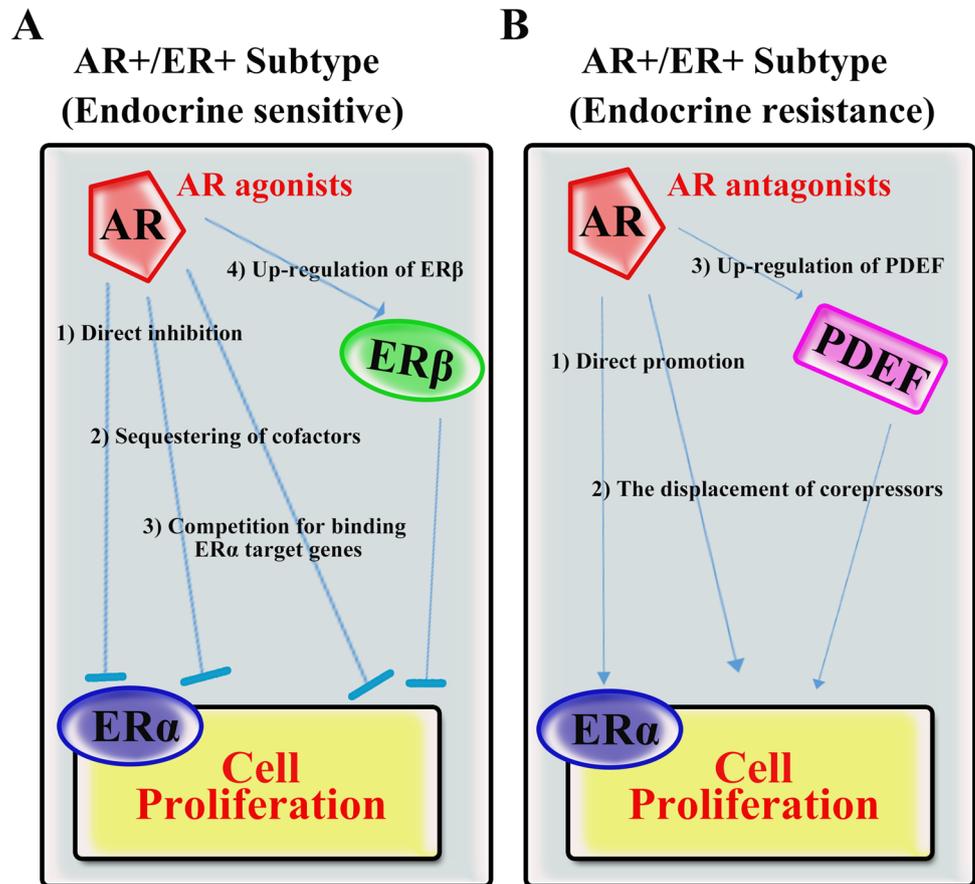
Variable	DFS overall				DSS overall			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age								
(≤ 40 vs > 40)	0.926 (0.596, 1.438)	0.731	–	–	1.041 (0.632, 1.715)	0.875	–	–
Tumour size								
(<2 vs 2–5 cm)	0.416 (0.243, 0.711)	0.001*	–	–	0.322 (0.175, 0.592)	0.001*	–	–
(<2 vs > 5 cm)	0.693 (0.377, 1.273)	0.237	–	–	0.554 (0.276, 1.113)	0.097	–	–
Tumour grade								
(1 vs 2)	0.974 (0.537, 1.766)	0.931	–	–	0.877 (0.416, 1.849)	0.731	–	–
(1 vs 3)	0.860 (0.550, 1.343)	0.507	–	–	0.842 (0.491, 1.445)	0.533	–	–
pTNM stage								
(I vs II)	0.336 (0.188, 0.601)	0.001*	0.442 (0.250, 0.780)	0.005*	0.393 (0.196, 0.786)	0.008*	–	–
(I vs III)	0.601 (0.341, 1.060)	0.078	–	–	0.472 (0.231, 0.965)	0.040*	0.423 (0.217, 0.826)	0.012*
LN								
(Negative vs positive)	0.737 (0.416, 1.402)	0.301	–	–	0.914 (0.571, 1.465)	0.710	–	–
AR								
(Negative vs positive)	0.845 (0.662, 1.078)	0.175	–	–	0.794 (0.582, 1.084)	0.146	–	–
AR/ERα								
(<2 vs ≥ 2)	7.912 (4.965, 12.610)	<0.0001*	5.647 (3.475, 9.175)	<0.0001*	2.599 (1.451, 4.655)	0.001*	2.601 (1.452, 4.660)	0.001*
PDEF								
(Negative vs positive)	0.846 (0.496, 1.443)	0.540	–	–	1.238 (0.623, 2.458)	0.543	–	–
PDEF/ERα								
(<2 vs ≥ 2)	2.745 (0.819, 9.208)	<0.0001*	6.948 (4.325, 11.163)	<0.0001*	5.873 (3.267, 10.557)	<0.0001*	6.222 (3.551, 10.902)	<0.0001*
HER2								
(Negative vs positive)	1.210 (0.771, 1.899)	0.407	–	–	0.808 (0.477, 1.368)	0.427	–	–
Ki-67								
(<20% vs $\geq 20\%$)	0.888 (0.582, 1.353)	0.580	–	–	1.052 (0.639, 1.733)	0.842	–	–
P53								
(Negative vs positive)	1.00 0.485 (0.300, 0.784)	0.137	–	–	1.402 (1.042, 1.886)	0.260	–	–

*Significantly different

PDEF:ER α play a critical role in tumorigenesis and the response to tamoxifen endocrine therapy. A high AR:ER α or PDEF:ER α ratio appeared to be predictive of a sub-optimal

response to ER α -directed tamoxifen therapy. Targeting the AR and PDEF may prove useful in patients with recurrent ER α -positive BC in the future.

Fig. 4 A schematic showing the interaction between the androgen receptor (AR) oestrogen receptor alpha (ER α) and prostate-derived Ets factor (PDEF) in ER α -positive BC. **a** The interaction of the ER α and AR in ER α -positive BC. **b** AR and PDEF overexpression may be a mechanism of tamoxifen resistance



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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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