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Original Article

The discordance pattern of molecular sub-types between primary and metastatic sites in Chinese breast cancer patients

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Abstract: Objective: Based on estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER-2), and proliferation cell nuclear antigen (Ki-67) status, breast cancer (BC) can be divided into several molecular sub-types. The patterns of these biological receptors may change during the course of progression and metastasis which could lead to new treatment strategies accordingly. Method: The present multi-center-based clinical data investigated the discordance patterns of molecular features in Chinese BC patients between primary tumors and distant metastasis. 151 pathologically confirmed BC patients were enrolled. The comparison of the statuses of ER, PR, HER-2, and the Ki-67 index by the IHC and/or FISH method was performed. Results: The discordance rate in one or more molecular markers was 52.4% and varied between primary and metastatic lesions. The most common transformation pattern was the loss of ER and PR. On the other hand, the ER-positive patients have the longest OS. Patients with ER changing from positive to negative have the shortest OS. The patients with PR changing from negative to positive have the longest OS, while PR-negative patients have the shortest OS. The median DFI (disease-free interval) was 54.93 months in this study. ER, PR, and HER-2 transformation rates are common in patients with DFI < 2 years than in patients with DFI ≥ 5 years. For patients with an ER-positive expression in metastatic lesions, a significantly prolonged PFS was observed ($P < 0.05$) in those receiving endocrine treatment. Conclusion: The transformation of molecular subtyping status was identified between primary and corresponding relapse lesions and was used for determining the treatment strategies and prognosis prediction in advanced BC patients.

Keywords: Breast cancer, relapse, molecular subtyping, re-biopsy

Introduction

Breast cancer (BC) is the most common carcinoma in females, with steadily increasing incidences over the past two decades. Compared to their counterparts in Western countries, Chinese BC patients manifest as youth-oriented, and an increasing proportion of newly diagnosed cases are at advanced stages [1, 2]. BC is well-established as a heterogeneous disease encompassing several distinct entities with remarkably different physiological characteristics and clinical behaviors [3, 4]. Despite the sustained increase in BC incidence, cancer-related deaths have been declining due to two advances in treatment. The first occurred when hormonal therapy was introduced as a treatment for ER/PR-positive BC during the mid-to-late 1980s, and the second occurred when trastuzumab was introduced to treat human epidermal growth factor receptor (HER-2)-positive BC in the late 1990s. These prominent accomplishments in developing novel targeted therapies for BC, along with a better understanding of the disease biology, have improved the prognosis of BC over the past decades [5]. Based on estrogen receptor (ER), progesterone receptor (PR), HER-2, and proliferation cell nuclear antigen (Ki-67) expression statuses, BC can be divided into Luminal A, Luminal B,

HER-2 overexpression, triple-negative breast cancer (TNBC), and other molecular sub-types. These sub-types help doctors choose among cytotoxic chemotherapy, hormonal therapy, and targeted therapy [6].

Recent developments in tumor molecular biology have shed some light on the mechanism of occurrence and transferring of BC, which underlies the development of targeted and individualized therapies [7]. In the past, some studies investigated the changing pattern between primary tumors with metastatic sites [8-10], indicating that physiological characteristics may alter during the course of cancer progression and metastasis, leading to new treatment strategies [11]. In a prospective study, 35 females with suspected new metastases underwent biopsy. Discordance rates between primary tumors and recurrence sites for ER, PR, and HER-2 expression were found in 16%, 40.4%, and 9.6% of the patients, respectively, which led to a changed case management in 20% of the patients [12].

However, the lack of efficacy data on race-based differences for treating BC patients in Eastern countries limits our understanding of this malignancy. Hence, we conducted the present study based on multi-center clinical data to investigate the discordance pattern of molecular features between primary tumors with distant metastatic diseases in Chinese BC patients when altered treatment is contemplated. We collected the data from Chinese BC patients with recurrence and metastasis who accepted re-biopsy pathology, analyzed the transformation profiles of the advanced BC sub-types and their impact on survival and treatment decisions.

Patients and methods

Patients and study design

The present cohort consisted of 151 recurrent or metastatic BC patients from Shanghai Renji Hospital and Shanghai General Hospital, collected between January 2006 and June 2016. The eligibility criteria were as follows: availability of archival primary tumor, recurrent or metastatic BC, measurable or assessable lesions, and written informed consent. The number of prior lines of systemic therapy was not restricted. The exclusion criteria included bilateral breast cancer, male gender, blood coagulation disorders precluding biopsy, and a history of non-breast secondary malignancies.

Biopsy and histopathological procedures

A core-needle biopsy was performed under ultrasound or computed tomography guidance. Samples were fixed in 10% formalin immediately after the biopsy. The malignancy was confirmed, and ER, PR, HER-2, and Ki67 statuses were evaluated from all re-biopsies and compared to the corresponding primary tissues. Two pathologists independently reviewed the pathological samples.

ER and PR expression were examined by immunostaining [13]. The SP1 and 1E2 antibodies to ER and PR were commercially purchased (Roche, Shanghai, China). The procedures were carried out according to the manufacturers' instructions. A positive result was defined as 1% of tumor cell nuclei staining positively with any intensity.

HER-2 staining was carried out using the 1E2 antibody [14]. 0/negative was defined as no staining or ≤ 10% of invasive cancer cells exhibiting incomplete, weak membrane staining. 1+/negative was defined as > 10% invasive cancer cells exhibiting incomplete, weak membrane staining. 2+/uncertain was defined as >10% invasive cancer exhibiting incomplete and/or weak to moderate intensity membrane staining or ≤ 10% of invasive cancer cells with strong and complete membrane staining. A 2+/uncertain result required a further fluorescent in situ hybridization (FISH) test. A 3+/positive result was defined as > 10% invasive cancer cells showing robust, complete, and uniform membrane staining. HER-2 FISH was carried out using the Linked-Biotech Pathology HER-2 DNA Probe Kit (LBP, Guangzhou, China) [15]. HER-2 and CEP17 signals were enumerated from 60 tumor nuclei. In borderline cases, an additional 60 nuclei were counted. $FISH^-$ was defined as HER-2/CEP17 ratio < 2.0 and average HER-2 copies/cells ratio < 4.0. $FISH^+$ was defined as HER-2/CEP17 ratio ≥ 2.0 or HER-2/CEP17 ratio < 2.0 and average HER-2 copies/cells ratio ≥ 6.0. FISH uncertain was defined as HER-2/CEP17 ratio < 2.0 and average HER-2 copies/cells ratio ≥ 4.0 but < 6.0. To allow consistent comparison with FISH performed on primary tumors, HER-2 FISH on the cytology specimens was performed on the paraffin sections of the pelleted cells. To assess the receptor discor-

Table 1. Basic characteristics of patients with breast cancer recurrence

Clinical features	First diagnosis		Re-biopsy	
	No.	%	No.	%
Median age (range)	56.61 (32-82)		60.96 (38-90)	
Pathologic diagnosis				
IDC	137	90.7	137	90.7
ICC	1	0.7	1	0.7
Carcinoma simplex	5	3.3	5	3.3
ILC	4	2.6	4	2.6
Intraductal carcinoma	3	2.0	3	2.0
MA	1	0.7	1	0.7
Size				
≤ 2.0 cm	18	18.9	/	/
> 2 cm; ≤ 5 cm	72	75.8	/	/
> 5.0 cm	5	5.3	/	/
LN				
0	33	27.3	/	/
1-3	36	29.7	/	/
> 4	52	43.0	/	/
Grade				
I	1	1.6	/	/
II	32	51.6	/	/
III	29	46.8	/	/
Lymphatic/vascular invasion				
Positive	27	19.3	/	/
Negative	113	80.7	/	/
ER status				
Positive	80	53.0	74	49.0
Negative	71	47.0	77	51.0
PR status				
Positive	58	38.4	48	31.8
Negative	93	61.6	103	68.2
HER-2 status				
Positive	66	57.4	63	56.8
Negative	49	42.6	48	43.2
Ki-67 index				
≥ 14%	54	85.7	62	78.5
< 14%	9	14.3	17	21.5
ADT				
Yes	51	34.2	35	23.2
No	100	65.8	116	76.8
DFI				
< 2 years	53	35.3	/	/
2-5 years	48	32.0	/	/
≥ 5 years	49	32.7	/	/

Note: IDC, Invasive ductal carcinoma; ILC, Invasive lobular carcinoma; ICC, Invasive cribriform carcinoma; MA, Mucinous adenocarcinoma; LN, Lymph node; ADT, Adjuvant hormonal therapy.

dance, all the results were split into either positive or negative using the methods described previously. The quantitative alterations in receptor expression were analyzed descriptively.

Statistical analysis

The analysis was performed using the SPSS software package (SPSS 22.0, Chicago, Illinois, USA). Statistical data were compared using a chi-square test, and a two-sided test with $P < 0.05$ considered as statistically significant. Survival curves were compared by Kaplan-Meier survival analysis [16] and assessed by a log-rank test [17].

The primary endpoint of the study was the discordance rates of the pathological subtypes (ER, PR, HER-2, and Ki67) between the primary and metastatic lesions, the results of which led to a transformed treatment strategy. The secondary end points consisted of assessing DFI (disease-free interval), PFS (progression-free survival), and OS (overall survival), respectively. In the present study, DFI is defined as the time from BC surgery to the confirmation of recurrence. PFS is defined as the time from the diagnosis of recurrence to the progression of first-line treatment. OS is defined as the time from surgery to death or the end of the study.

Results

Patient clinical characteristics

All the patients were female with a median age of 56.61 years (range: 32-82 years) at

Table 2. Re-biopsy features of recurrent or metastatic lesions

Clinical features	Value	%
Metastasis type		
Visceral	63	43.2
Non-visceral	83	54.8
Biopsy site		
Lung	19	12.5
Ascites	1	0.7
Liver	16	10.5
Bone	5	3.3
Lymph node	33	21.8
Ovary	1	0.7
Skin	6	3.9
Breast	13	8.6
Thoracic wall	25	16.4
Pleural effusion	3	2.0

the initial pathological diagnosis. The basic characteristics of patients included in the study are shown in **Table 1**. Invasive ductal carcinoma (137/151, 90.7%) accounts for the majority of the diagnosed cases. Other pathological sub-types include 1 case of invasive cribriform carcinoma, 5 of carcinoma simplex, 4 of invasive lobular carcinoma, 3 of intraductal carcinoma, and 1 of mucinous adenocarcinoma. Measurement of the BC molecular markers in the primary tumors showed that the ER, PR, and HER-2 positive rates were 53.0%, 38.4%, and 57.4%, respectively. A Ki-67 index > 14% was observed in 85.7% of the patients.

All the 151 patients enrolled in the study underwent a biopsy for the pathological assessment of recurrent or metastatic lesions (**Table 2**). Patients with non-visceral metastasis accounted for 54.8%, including regional lymph nodes, chest wall, and bone metastasis. Patients with visceral metastasis accounted for 43.2%, which primarily comprised lung and liver metastasis (accounting for 12.5% and 10.5%, respectively).

Discordance of ER, PR, HER-2, and Ki-67 status between primary tumors and metastases

There is an individual difference in the inconsistency between ER, PR, HER-2 and Ki-67 statuses between the primary tumor and the metastatic tumor (**Figure 1**). We summarized the similarities and differences in the subtype distribution by comparing the receptors' expression between the primary tumors and the metastatic lesions (**Table 3**). The median DFI from the initial pathological diagnosis of the primary malignancy to the biopsied assessment of the recurrent metastasis was 54.93 months (range, 0-336 months). DFI > 5 years accounted for 32.7% and < 2 years accounted for 35.3%. The status of ER and PR in all the patients was tested. 85 and 47 patients were examined for HER-2 and Ki-67 status, respectively, in both primary and metastatic lesions. The study revealed that the discordance rate in one or more molecular markers was 52.4% in the comparison.

The positive expression of ER and PR in primary diseases was 53.0% and 38.4%, respectively. However, their positive rates in the corresponding recurrent metastatic lesions were 49.0% and 31.8%, respectively. The *p* values for both indicated a statistical significance, thereby suggesting that the expression levels of ER and PR had declined in the process of invasion and metastasis. As a nuclear protein which is associated with cellular proliferation, Ki-67 expression increased in metastatic sites compared to the primary tissues (78.5% of metastatic lesions showed a ≥ 14% Ki-67 labeling index vs. 85.7% of the primary tumors). Although the difference was not statistically significant, it revealed a high proliferative potential in recurrent metastatic lesions.

Together, the discordance rates of ER, PR, HER-2, and Ki-67 statuses between the primary and recurrent or metastatic lesions were 23.18%, 32.35%, 28.24%, and 25.53% respectively, which are shown in **Table 3**. The most common transformation pattern was the loss of ER and PR expression, i.e., 22 (14.6%) patients showed the ER status changing from positive to negative expression, whereas 13 (8.6%) patients changed from negative to positive. With respect to PR expression, 30 (19.9%) patients changed from positive to negative expression, whereas 19 (12.6%) transformed from negative to positive.

Clinical significance of receptors' transformation in metastasis

Considering the heterogeneity and intricate assignment of tumor evolution, we performed the survival analysis based on molecular sub-

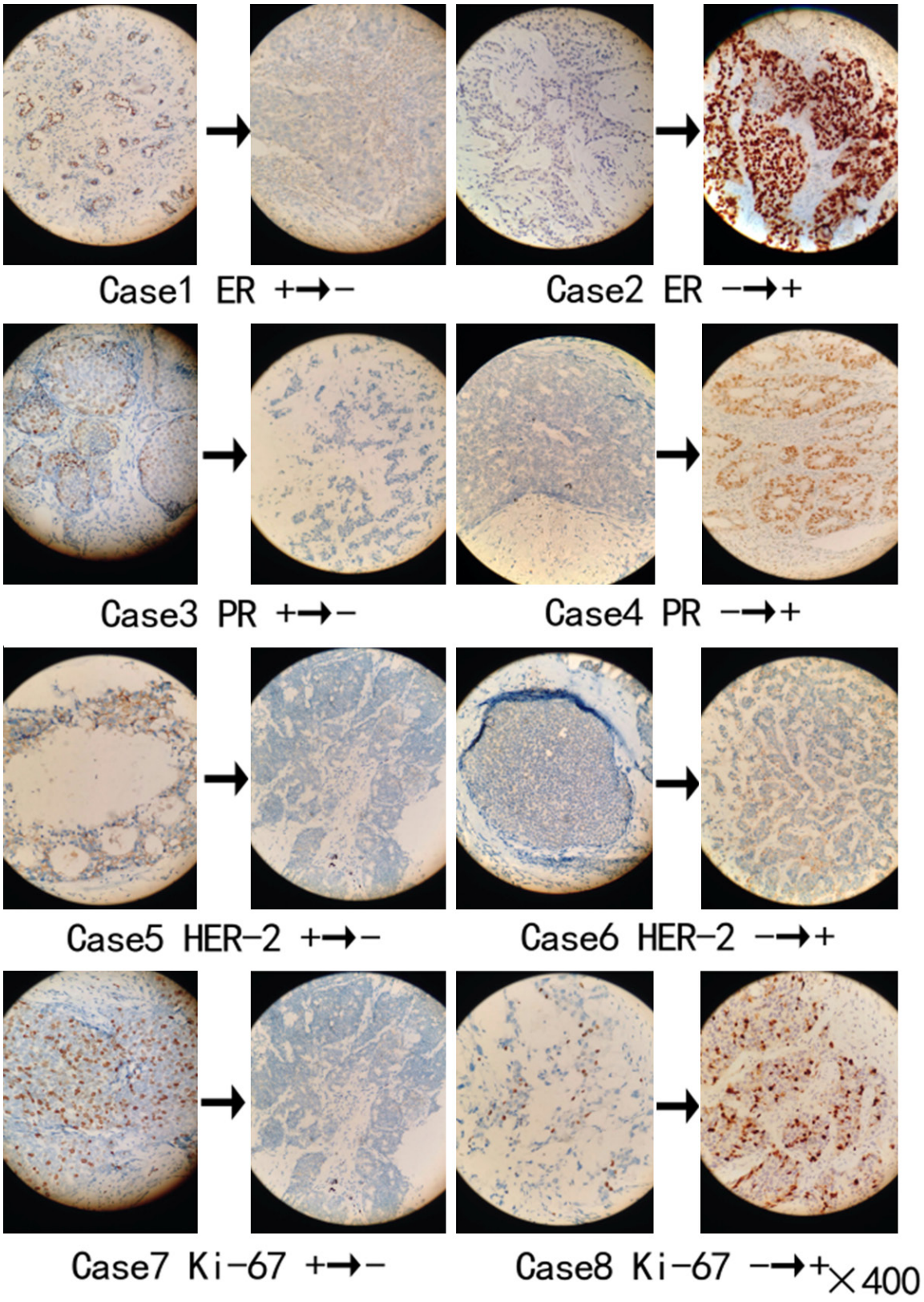

Figure 1. Some cases of biomarkers altered between the primary and metastatic lesions.

Table 3. Changes in ER, PR, HER-2, and Ki-67 expression between primary and metastatic lesions

Molecular markers	Positive (%)		PL → ML		Transformation rate (%)	χ^2	*P*-value
	PL	ML	P → N	N → P			
ER	80 (53.0)	74 (49.0)	22 (14.6)	13 (8.6)	35/151 (23.18)	28.785	< 0.05
PR	58 (38.4)	48 (31.8)	30 (19.9)	19 (12.6)	49/151 (32.35)	8.824	< 0.05
HER-2	66 (57.4)	63 (56.8)	14 (16.5)	10 (11.8)	24/85 (28.24)	16.290	< 0.05
Ki-67	54 (85.7)	62 (78.5)	5 (10.6)	7 (14.9)	12/47 (25.53)	0.001	> 0.05

Note: Primary lesion, PL; Metastatic lesion, ML; Positive, P; Negative, N.

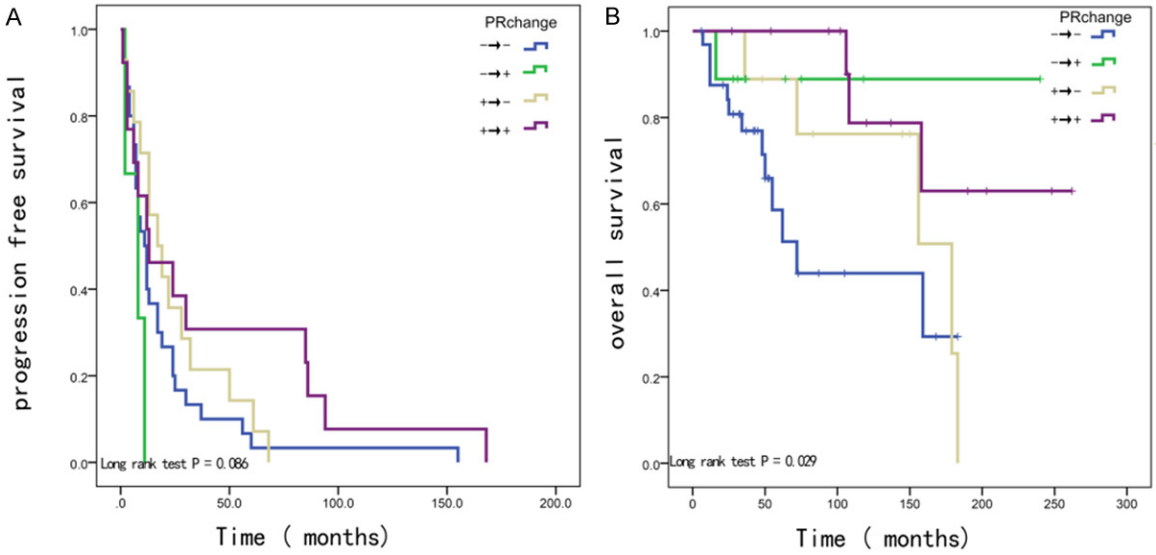

Figure 2. Kaplan-Meier curves for PFS and OS probability according to PR status altered between the primary and metastatic lesions.

types altering among BC patients. We divided the patients into different groups according to status changes in ER, PR, HER-2, and Ki-67 between the primary and metastatic lesions, as well as the changed mode.

The analysis revealed that, in the ER groups, the ER-positive patients have the longest OS (median survival 184.4 months), which is followed by those ER patients changing from negative to positive expression (median survival 166.0 months). However, patients with ER changing from positive to negative have the shortest OS (median survival 94.3 months); none of these alterations exhibit a statistical significance (**Figure 2A**). We also noted that in the PR groups, patients with PR changing from negative to positive expression have the longest OS (median survival 215.1 months) while the PR-negative patients have the shortest OS (100.5 months), which is statistically significant (P=0.029) (**Figure 2B**).

Variation in ER, PR, HER-2, Ki-67 expression and its correlation with DFI

To further analyze the expression differences of biomarkers between primary and metastatic lesions, we analyzed the relationship between the transformation rate of the biomarkers and DFI (DFI < 2 years and ≥ 5 years) (**Table 4**). The results showed that the ER, PR, and HER-2 transformation rates were statistically significant in patients with DFI < 2 years and ≥ 5 years; however, the Ki-67 transformation rate was not significant.

Adjustment of treatment strategy based on molecular markers transformation in metastasis

We further investigated the influence of ER-positive expression in metastatic lesions based on receiving endocrine treatment according to metastatic assessment. A significantly pro-

Table 4. Transformation pattern of ER, PR, HER-2, and Ki-67 between primary and metastatic lesions and its relationship with DFI

Biomarker/DFI	PL → ML		Transformation rate (%)	χ^2	*P*-value
	P → N	N → P			
ER	11	6	17/68 (25.00)	5.630	< 0.05
DFI < 2 years	7	3	10/68 (14.71)		
DFI ≥ 5 years	4	3	7/68 (10.29)		
PR	11	9	20/66 (30.30)	16.619	< 0.05
DFI < 2 years	6	6	12/66 (18.18)		
DFI ≥ 5 years	5	3	8/66 (12.12)		
HER-2	9	6	15/55 (27.27)	9.016	< 0.05
DFI < 2 years	2	4	6/55 (10.91)		
DFI ≥ 5 years	7	2	9/55 (16.36)		
Ki-67	3	6	9/30 (30.00)	0.844	> 0.05
DFI < 2 years	2	4	6/30 (20.00)		
DFI ≥ 5 years	1	2	3/30 (10.00)		

Note: Primary lesion, PL; Metastatic lesion, ML; Positive, P; Negative, N.

longed PFS was observed (P < 0.05) in patients who received endocrine treatment compared to those who did not receive the treatment. However, the OS was not statistically significant (**Figure 3B**). With regard to patients with ER expression changing from negative to positive, the median PFS was 61.3 months for those who received endocrine treatment and 11.5 months for those who not receive the treatment, which was statistically significant (P= 0.004) (**Figure 3A**).

We also divided the patients into two groups to investigate the influence of HER-2-targeted therapy on patients with HER-2-positive expression in their metastatic lesions. Among the metastatic HER-2-positive patients with targeted therapy, none died until the end of our study, whereas among the metastatic HER-2-positive patients without targeted therapy, the median survival was 77.4 months. The results showed that OS was longer in patients receiving trastuzumab treatment compared to those without targeted therapy, although the *P*-value was not statistically significant.

Discussion

The St. Gallen International Breast Cancer Consensus Conference proposed molecular subtype definitions according to the expression and/or proliferation rates of ER, PR, HER-2, and Ki-67 status [18]. It is instructive not only for the adjuvant therapy of early BC but also for advanced BC palliative treatment strategies. A series of studies indicated that the BC molecular subtype is likely to change between the primary and metastatic lesions, which is a major concern in guiding the treatment decisions for advanced stage patients [19]. Such tumor heterogeneity characterized by molecular differential expression was first reported 30 years ago [20]. However, it was considered as such due to the differences in the detection method at that time, and its reliability remains yet to be verified.

It was not until recently that Lindström et al. [21] reported BC cancer recurrence and puncture lesions in over 400 cases showing discordance rates of ER, PR, and HER-2 expression statuses as 32.4%, 40.7%, and 14.5%, respectively, compared to the primary tumor. The death risk was high in patients with ER changing from positive to negative status, suggesting the prognostic value of re-biopsy in advanced metastatic BC patients. However, the discordance rate of hormone receptors between primary and metastatic BC varied based on different studies [22-24]. In a prospective study conducted by Amir et al. [25], the discordance rates of ER, PR, and HER-2 were 16%, 40%, and 10% respectively, resulting in 14% of patients changing their treatment strategies. Thus, the *ESMO Clinical Practice Guidelines* emphasized the importance of the re-evaluation of hormone receptors and HER-2 status in metastatic lesions [26]. However, with regard to Chinese BC patients, the efficacy data over race-based differences is still lacking, which limits our understanding of this malignancy and clinical practice in Eastern countries.

In the present study, the discordance rates of ER, PR, HER-2, and Ki-67 index were 23.18%, 32.35%, 28.24%, and 25.53%, respectively. ER, PR, and HER-2 transformation rates were statistically significant when comparing the primary tumor and the metastatic lesions (P < 0.05). Ki-67 was reported to have a potential prognostic and predictive ability [27, 28], but its detection had limited technical reproducibility,

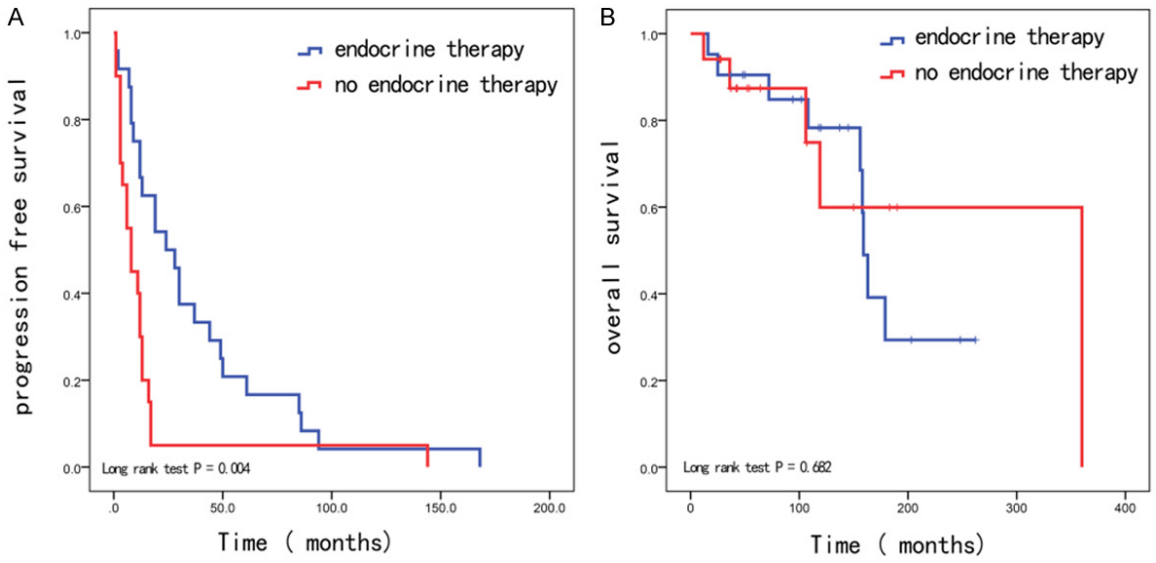

Figure 3. Kaplan-Meier curves for PFS and OS probability based on whether patients with metastatic ER-positive received endocrine therapy.

a subjective interpretation, and variable diagnostic thresholds [29]. The uncertain status and discordance pattern for ER or HER-2 in the process of BC progression/metastasis were shown to be clinically significant and correlated with prognosis [30]. In the ER groups in our study, the patients with ER maintaining a positive rate have the longest OS compared to the other subgroups, which is followed by patients with ER changing from negative to positive. Although none of these analyses were statistically significant, patients with ER changing from a positive to a negative expression have the shortest OS. We also noticed that in the PR subgroups, patients with PR status changing from negative to positive have the longest OS while those with PR maintaining a negative rate have the shortest OS, which is statistically significant. Altogether, patients with negative hormone receptors in the metastatic lesions have poorer survival than those with hormone positive receptors in the metastatic lesions, which is in agreement with previous reports [10, 18]. This phenomenon indicates that when molecular markers are altered, doctors may change the treatment strategies of those patients based on the new molecular subtyping. These patients lose an effective therapeutic response to endocrine treatment due to a lack of hormone receptor expression. This change also reflects heterogeneity, and the extent of the malignancy of the tumors increases, thereby resulting in a poor prognosis. For these patients, the treatment strategies should be transformed from endocrine therapy to chemotherapy as soon as possible. In the present study, PFS was significantly ($P < 0.05$) longer in patients receiving endocrine treatment than it was in those without the treatment, indicating the importance of modifying treatment strategies according to the hormone receptors' expression in metastatic lesions. To further analyze the expression differences of the biomarkers between the primary and metastatic lesions, we assessed the relationship between the transformation rate of the biomarkers and DFI (DFI < 2 years and ≥ 5 years). This analysis showed that the ER, PR, and HER-2 transformation rates were statistically significant in patients with DFI < 2 years and ≥ 5 years; however, the Ki-67 transformation rate was not significant. Thus, patients with ER, PR, or HER-2 changes between the primary and metastatic lesions have a shorter DFI than those without any changes.

Notably, the subjectivity of the immunohistochemistry-based detection of hormone receptors, HER-2 and the Ki-67 index, the selection bias of re-biopsy, lesions, and the essential characteristics of retrospective study may influence the reliability of our results. The success rate of re-biopsy and the accuracy of its influence on treatment decisions should be further investigated.

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Disclosure of conflict of interest

None.

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