FGFR1 and *HER1* or *HER2* co-amplification in breast cancer indicate poor prognosis

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Abstract. Human epidermal growth factor receptor 1 or 2 (HER1/2), and fibroblast growth factor receptor 1 (FGFR1) signaling serve critical roles in the progression of breast cancer; however, cross-talk between HER1/2 and FGFR1 signaling has not been extensively studied. In the present study, the copy number variation status of *FGFR1* and *HER1/2*, and the clinical implications and prognostic relevance of this, were evaluated in invasive ductal breast cancer (IDC) tissue samples. Quantitative polymerase chain reaction and fluorescence *in situ* hybridization were used to assess gene copy number variation in IDC samples, and the clinical characteristics and survival curves of patients with IDC were analyzed. The amplification of *FGFR1* was identified in 16.0% of the samples (12 of 75), of *HER1* in 26.7% (20 of 75), of *HER2* in 37.3% (28 of 75), and of *FGFR1* and *HER1/2* simultaneously in 8.0% (6 of 75). *FGFR1* and *HER1/2* co-amplification were significantly correlated with distant metastasis (P=0.035), recurrence (P=0.026) and decreased disease-free survival time (P=0.042). This was the case for patients undergoing endocrine therapy (P=0.002) and chemotherapy (P=0.044). Taken together, the results indicate that patients with *FGFR1* and *HER1/2* co-amplification may exhibit a less favorable prognosis compared with patients with either *FGFR1*, *HER1/2* amplification or without amplification.

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Abbreviations: HER1/2, human epidermal growth factor receptor 1 or 2; FGFR, fibroblast growth factor receptor; IDC, invasive ductal breast cancer; CNV, copy number variation; AKT, Akt serine/threonine kinase; PI3K, phosphoinositide-3-kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase 1; PKC, protein kinase C; PLC, phospholipase C; qPCR, quantitative polymerase chain reaction; FISH, fluorescence *in situ* hybridization; FFPE, formalin-fixed paraffin-embedded

Key words: FGFR1, HER1, HER2, breast cancer, prognosis, copy number variation

Introduction

Based on the 2014 World Health Organization report, breast cancer has the second highest incidence of mortality for females in China (1). Breast cancer is a heterogeneous disease; numerous frequent gene copy number variations (CNVs) have been identified, including gene amplification of fibroblast growth factor receptor 1 (*FGFR1*) (2), human epidermal growth factor receptor 1 (*HER1*), human epidermal growth factor 2 (*HER2*), *GATA3*, *PIK3CA*, *MAP3K1*, *TBX3*, *RUNX1*, *CBFB*, *AFF2*, *PIK3R1*, *PTPN22*, *PTPRD*, *NF1*, *SF3B1* and *CCND3* (3). Preliminary data from the next-generation genome sequencing of primary breast cancer has confirmed that CNVs may occur in a large selection of genes (3), and indicate that these variations may lead to different clinical consequences.

HER1 (also known as EGFR or ErbB1) and HER2 (also known as ErbB2) belong to the ErbB family of signaling proteins, which comprises four members: HER1, HER2, ErbB3 and ErbB4. ErbB receptors are often amplified, mutated and/or overexpressed in breast cancer (4,5). Between 15 and 20% of newly diagnosed invasive breast carcinomas overexpress HER2 or exhibit HER2 gene amplification (6). The frequency of HER1 overexpression in breast cancer is variable, reportedly ranging from 7 to 43% (7-13).

Activation of the ErbB family of receptor tyrosine kinases via their cognate epidermal growth factor-like peptide ligands constitutes a major event in the signaling pathways that control the proliferation, survival, angiogenesis and metastasis of breast cancer cells (14). Therefore, ErbB family member receptors are attractive potential therapeutic targets in breast cancer. At present, numerous tyrosine kinase inhibitors that target ErbBs have been successfully developed and approved to treat cancer patients. Trastuzumab (also known as Herceptin), a humanized monoclonal antibody against the extracellular portion of the HER2 protein, is in widespread clinical use (15). Notably, interactions between HER2 and other ErbB receptors, including HER1 and ErbB3, have been suggested as a possible mechanism for the resistance to trastuzumab. Once activated by the binding of its specific ligands, HER1 is the preferred heterodimerization partner for HER2; HER1/HER2 heterodimers are more stable than HER1 homodimers, and binding of HER1 with HER2 can potentiate and amplify the growth signals from HER1 activation (16-18).

FGFR1 has also been investigated and may be amplified in 8-15% of all cases of breast cancer (19-21). FGFR1 is a member of the FGFR family, which exhibit a highly conserved structure between members and throughout evolution. FGFRs are receptors for fibroblast growth factors; the interaction between fibroblast growth factors and FGFRs is associated with the regulation of cell proliferation, survival, migration and differentiation during development and adult life. The mutation and amplification of FGFRs causes the aberrant activation of downstream pathways, promoting cell cycle progression and mesenchymal transformation while inhibiting apoptosis. Amplification of the *FGFR1* gene (at 8p11-12) is the most common alteration to *FGFR1* (22-24). *FGFR1* amplification may also drive resistance to endocrine therapy (25).

FGFR and EGFR signaling may mediate the downstream phosphoinositide-3-kinase/Akt serine/threonine kinase (PI3K/AKT) pathway. In this pathway, activated EGFR binds GRB2-associated binding protein 1 together with growth factor receptor-bound protein 2 to recruit PI3K (26). In addition, FGFRs commonly mediate the PI3K/AKT pathway via FGFR substrate 2a and other adaptor molecules (27). Major pathways downstream of activated EGFRs/FGFRs, besides PI3K/AKT, include extracellular signal-regulated kinase/mitogen-activated protein kinase 1 (ERK/MAPK) and protein kinase C/phospholipase C (PKC/PLC) pathways. All of these pathways serve an important role in cell proliferation, migration, differentiation and the inhibition of apoptosis.

In the present study, the gene amplification statuses of *HER1*, *HER2* and *FGFR1* were evaluated in 75 cases of invasive ductal breast cancer (IDC). Quantitative polymerase chain reaction (qPCR) and fluorescence *in situ* hybridization (FISH) were used to assess the gene CNV. A statistical analysis revealed an association between CNVs and the clinical prognosis.

Materials and methods

Patients and tissue samples. The records of the Department of Pathology of West China Hospital (Chengdu, China) were retrospectively examined and 119 records of IDC cases were initially included in the study (Fig. 1). Of the 119 cases, 7 were excluded as there was no tissue specimen available and 18 were excluded due to incomplete information. Of the remaining 94 formalin-fixed paraffin-embedded (FFPE) IDC tissue samples, 75 were suitable for qPCR detection. The samples qualified for qPCR if IDC was >70% of the FFPE sample and if they were acquired prior to systemic treatment. Data regarding clinical characteristics were gathered while preserving patient anonymity. The expression status of ER and PR were obtained from clinical pathology reports. The tumors were regarded as estrogen or progesterone receptor-positive if ≥1% of tumor cells were stained positively, according to previously reported criteria (28). The study was approved by the Ethics Committee of West China Hospital (no. 2013-191) and written informed consent was obtained from all patients.

DNA isolation and qPCR. The tumor areas of each IDC sample were identified on slides stained with hematoxylin and eosin, and matched with corresponding FFPE tumor tissues. DNA was extracted from 4-μm-thick FFPE tissue sections using the QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany). DNA was quantified with the Nanodrop2000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and samples with A260/280 absorbance ratios <1.70, or ≥1.95, were excluded. qPCR was performed with the Bio-Rad CFX96 system with SsoFast Evagreen Supermix (both Bio-Rad, Hercules, CA, USA) and quantified using the $2^{-\Delta\Delta Cq}$ method (29). A total of 100 ng DNA was used per reaction and each reaction was performed at 98˚C for 2 min, 98˚C for 5 sec and 60˚C for 10 sec, for 40 cycles. Primer sequences are included in Table I. Transferrin receptor and GAPDH were used as reference genes. Additionally, 50 normal samples were used as a control group, which were also obtained from Department of Pathology of West China Hospital and reported to possess no tumor cells in the initial pathologist reports. The relative amplification levels of *FGFR1*, *HER1* and *HER2* were normalized to the mean of the reference genes in order to calculate the relative CNV, compared with the control samples, using the $2^{-\Delta\Delta Cq}$ method. A gene was considered to be amplified where the fold-change compared to the control group was ≥2.

FISH assays. FISH was performed on the tumor tissue samples with the following commercially available locus-specific and chromosome enumeration probes: *HER1* (EGFR Spectrum Orange) with centromere 7 (CEP 7 Spectrum Green); *HER2* (EGFR Spectrum Orange) with centromere 17 (CEP 17 Spectrum Green; all from LBP Medicine Science & Technology, Co., Ltd., Guangzhou, China); and *FGFR1* (Orange) with centromere 8 (CEP 8 Spectrum Green; Empire Genomics, Buffalo, NY, USA). De-paraffinized 4-mm tumor sections were heated in antigen retrieval solution (sodium citrate, pH 6.0) in the microwave for 16 min, then in pepsin solution (LBP Medicine Science & Technology, Co., Ltd.) for 20 min at 43˚C. The slides were dehydrated with 70, 85 and 100% ethanol. The tissue sections with probes for EGFR/CEP 7 or HER2/CEP 17 were denatured in a ThermoBrite hybridization chamber (IRIS International, Inc., Norwood, MA, USA) at 85˚C for 5 min, followed by 20 h hybridization at 43˚C. The tissue sections with probes for *FGFR1*/CEP 8 were denatured at 85˚C for 5 min, followed by 20 h hybridization at 40˚C. Following hybridization, washes were performed according to the supplier protocols. Slides were counterstained with 0.2 μmol/l 4',6-diamidino-2-phenylindole in an anti-fade solution, and viewed with a fluorescence microscope. From each slide, a total of 60 tumor cells were evaluated; the gene and centromere copy number, and average predominant gene as defined by the mean ratio of the gene copy number vs. centromere copy number of each slide were estimated. A oncogene-to-centromere signal ratio ≥2 was considered to indicate amplification (30,31).

Statistical analysis. Statistical significance was assessed using SPSS software version 22.0 for Windows (IBM SPSS, Armonk, NY, USA). $P<0.05$ was considered to indicated a statistically significant difference. The association between clinical characteristics and gene CNV were analyzed using χ^2 tests. Kaplan-Meier survival curves were plotted, and the significance of differences between survival curves was determined using the log-rank test.

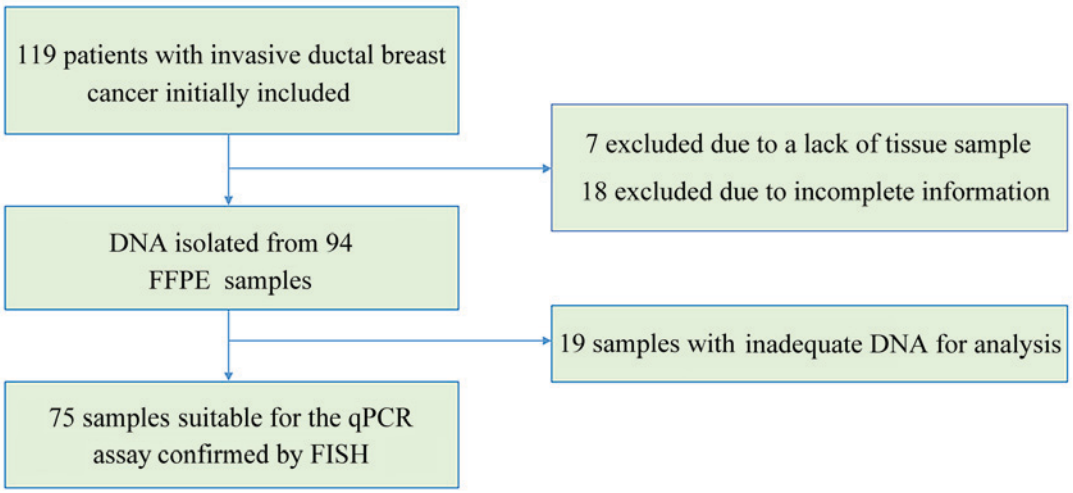

Figure 1. Flowchart showing the inclusion criteria of the study. FFPE, formalin-fixed paraffin-embedded; qPCR, quantitative polymerase chain reaction; FISH, fluorescence *in situ* hybridization

Results

CNV status of FGFR1, HER1 and HER2. The CNV status of *FGFR1*, *HER1* and *HER2* were detected by qPCR and confirmed by FISH analysis for 75 patients with primary IDC (Table II; Fig. 2). It was demonstrated that 16.0% of the samples exhibited *FGFR1* amplification (12 of 75), 26.7% *HER1* amplification (20 of 75), 37.3% *HER2* amplification (28 of 75), 50.7% *HER1/2* amplification (38 of 75), 8.0% *FGFR1* and *HER1/2* co-amplification (6 of 75) and 42.7% samples exhibited no amplification (32 of 75). All samples detected by qPCR were positively confirmed by FISH analysis (100%).

Baseline clinical characteristics. A total of 75 patients with primary IDC were included (Table II). The median age at diagnosis of IDC was 48.4 years (range, 29-72 years). Patients were grouped according to the amplification status of *FGFR1* or *HER1/2*. The amplification of *HER1/2* was significantly associated with estrogen receptor (P=0.007) and HER2 (P<0.001) expression status. The frequency of local recurrence (P=0.026) and distant metastasis (P=0.035) were significantly higher in the subgroup with *FGFR1* and *HER1/2* co-amplification. A total of 5 patients developed a distant metastasis, of which 1 patient had metastasis at the time of diagnosis, and 2 patients had distant metastases and local recurrence simultaneously. There were 5 cases that resulted in mortality in the entire group; 2 were directly associated with breast cancer, whereas the other cases were uncertain.

The mean disease-free survival time was 25.7 months (range, 0-41 months; Table III); the mean overall survival time was 26.4 months (range, 12-41 months). Tumor size was correlated with disease-free survival time (P=0.006). Nodal status was significantly associated with disease-free (P=0.021) and overall (P=0.009) survival times. Additionally, the clinical stage of the cancer was significantly associated with overall survival time (P=0.042).

Prognostic significance of FGFR1 and HER1/2 co-amplification. *FGFR1* and *HER1/2* co-amplification was significantly associated with local recurrence and distant metastasis, as mentioned previously (Table II). To further investigate the association between *FGFR1* and *HER1/2* co-amplification and prognosis, *FGFR1* and *HER1/2* co-amplification status was assessed with a Kaplan-Meier analysis (Fig. 3). Based on this analysis, *FGFR1* and *HER1/2* co-amplification was significantly associated with reduced disease-free survival time (P=0.042; Fig. 3A). This was true for patients receiving chemotherapy (P=0.044) or endocrine therapy (P=0.002; Table IV). However, *FGFR1* amplification (Fig. 3B) and *HER1/2* amplification (Fig. 3C) individually exhibited no significant correlation with disease-free (P=0.375 and P=0.057, respectively) or overall (P=0.334 and P=0.167, respectively) survival time (Table III).

Discussion

The present retrospective study aimed to investigate the association between the CNV of *FGFR1, HER1* and *HER2*, and the prognosis of patients with IDC. In the present study, *FGFR1* was amplified in 15.6% of samples, and the amplification rates of *HER1* and *HER2* were 26.7 and 37.3%, respectively. In other studies, the amplification rates of *FGFR1*, *HER1* and *HER2* have been reported as ~10, ~15 and ~20%, respectively (6,32,33). These differences may be due to differences in the methods for detection, classification standards for amplification, or sample sizes between the studies.

In the present study, the group of patients with co-amplification of *FGFR1* and *HER1/2* was significantly more likely to experience recurrence and distant metastasis. Co-amplification also influenced disease-free survival time, with the co-amplification experiencing less favorable outcomes. No association between prognosis and amplification of *FGFR1* or *HER1/2* alone was identified. The data of the present study contrasted from previous indications that the amplification of *FGFR1* is associated with poor survival time (20), as *FGFR1* amplification did not affect disease-free or overall survival time in the current study. The difference may be due to the constitution of the sample; different types of breast cancer or a different number of patients may have caused the drift. The amplification of *FGFR1* was, however, associated with menopausal

Table I. Primers used for the quantitative polymerase chain reaction detection of *TFRC*, *GAPDH*, *FGFR1*, *HER1* and *HER2*.

Gene	GenBank no.	Oligo type	Oligo sequence	Target size (bp)
TFRC	NC_000003.12	Forward	5'-ACTTCCTCTCTCCCTACGTATC-3'	105
		Reverse	5'-GCAGTTTCAAGTTCTCCAGTAAAG-3'	
GAPDH	NG_007073.2	Forward	5'-CCTCAAGATCATCAGCAATGCCTC-3'	100
		Reverse	5'-GTGGTCATGAGTCCTTCCACGATA-3'	
FGFR1	NC_000008.11	Forward	5'-AGGCTGTGCTGTTGCACCTA-3'	128
		Reverse	5'-ATCCGGGGCAGTTGCTAGTC-3'	
HER1	NG_007726.3	Forward	5'-CGGGACGTTTCGTTCTTCGG-3'	130
		Reverse	5'-GAAAGTTGGGAGCGGTTCGG-3'	
HER2	NG_007503.1	Forward	5'-ATGAGCTACCTGGAGGATGT-3'	103
		Reverse	5'-CCAGCCCGAAGTCTGTAATTT-3'	

TFRC, transferrin receptor; *FGFR1*, fibroblast growth factor receptor 1; *HER1*, human epidermal growth factor receptor 1; *HER2*, human epidermal growth factor receptor 2.

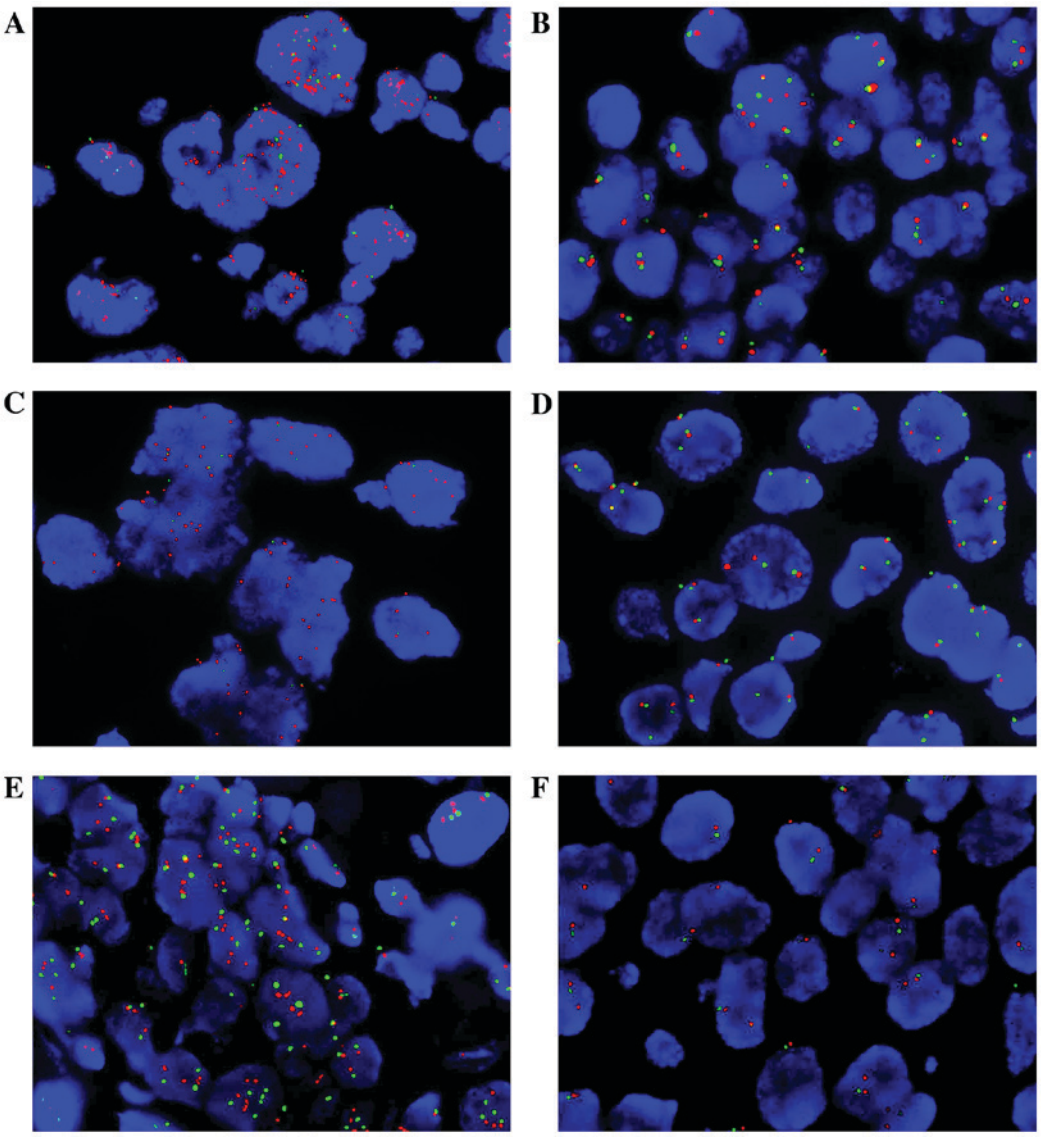

Figure 2. Fluorescence *in situ* hybridization assays confirmed the quantitative polymerase chain reaction-detected *FGFR1*, *HER1* and *HER2* copy number variation in invasive ductal breast cancer tumor tissue samples. The detected signal for gene probes is displayed as red and for centromere probes as green. (A) *FGFR1* amplification and (B) non-amplification. (C) *HER1* amplification and (D) non-amplification. (E) *HER2* amplification and (F) non-amplification. Magnification, x100. FGFR, fibroblast growth factor receptor; HER1, human epidermal growth factor receptor 1; HER2, human epidermal growth factor 2.

Table II. Clinicopathological characteristics of invasive ductal breast cancer with *FGFR1* amplification, *HER1/2* amplification, *FGFR1* and *HER1/2* co-amplification or no amplification.

	FGFR1 amplification, n (%)			HER1/2 amplification[a], n (%)			FGFR1 and HER1/2 co-amplification, n (%)			FGFR1 or HER1/2 amplification, n (%)		
Parameter	+	-	P-value	+	-	P-value	+	-	P-value	+	-	P-value
Total	12 (16.0)	63 (84.0)		38 (50.7)	37 (49.3)		6 (8.0)	69 (92.0)	-	43 (57.3)	32 (42.7)	
Age, years			0.223			0.688			0.411			0.304
≤50	6 (50.0)	43 (68.3)		24 (63.2)	25 (67.6)		3 (50.0)	46 (66.7)		17 (39.5)	9 (28.1)	
>50	6 (50.0)	20 (31.7)		14 (36.8)	12 (32.4)		3 (50.0)	23 (33.3)		26 (60.5)	23 (71.9)	
Tumor size			0.312			0.174			0.495			0.289
T0-T2	12 (100.0)	58 (92.1)		34 (89.5)	36 (97.3)		6 (100.0)	64 (92.8)		39 (90.7)	31 (96.9)	
T3-T4	0 (0.0)	5 (7.9)		4 (10.5)	1 (2.7)		0 (0.0)	5 (7.2)		4 (9.3)	1 (3.1)	
Nodal status			0.324			0.569			0.274			0.486
N0	5 (41.7)	36 (57.1)		22 (53.7)	19 (46.3)		2 (33.3)	39 (56.5)		25 (58.1)	16 (50.0)	
N1-N3	7 (58.3)	27 (42.9)		16 (47.1)	18 (52.9)		4 (66.7)	30 (43.5)		18 (41.9)	16 (50.0)	
Menopausal status			0.084			0.289			0.244			0.147
Menopausal	8 (66.7)	25 (39.7)		19 (50.0)	14 (37.8)		4 (66.7)	29 (42.0)		22 (51.2)	11 (34.4)	
Premenopausal	4 (33.3)	38 (60.3)		19 (50.0)	23 (62.2)		2 (33.3)	40 (58.0)		21 (48.8)	21 (65.6)	
Clinical stage			0.588			0.188			0.714			0.485
I-II	10 (83.3)	48 (76.2)		27 (71.1)	31 (83.8)		5 (83.3)	53 (76.8)		32 (74.4)	26 (81.3)	
III-IV	2 (16.7)	15 (23.8)		11 (28.9)	6 (16.2)		1 (16.7)	16 (23.2)		11 (25.6)	6 (18.7)	
ER Status			0.642			0.007[b]			0.438			0.003[b]
ER+	9 (75.0)	43 (68.3)		21 (55.3)	31 (83.8)		5 (83.3)	47 (68.1)		19 (44.2)	4 (12.5)	
ER-	3 (25.0)	20 (31.7)		17 (44.7)	6 (16.2)		1 (16.7)	22 (31.9)		24 (55.8)	28 (87.5)	
PR Status			0.223			0.063			0.086			0.045[b]
PR+	6 (50.0)	43 (68.3)		21 (44.7)	28 (75.7)		2 (33.3)	47 (68.1)		19 (44.2)	7 (21.9)	
PR-	6 (50.0)	20 (31.7)		17 (55.3)	9 (24.3)		4 (66.7)	22 (31.9)		24 (55.8)	25 (78.1)	
HER2			0.538			<0.001[b]			0.769			0.001[b]
0-1+	8 (66.7)	31 (49.2)		15 (39.5)	24 (64.9)		3 (50.0)	36 (52.2)		20 (46.5)	19 (59.4)	
2+	2 (16.7)	17 (27.0)		6 (15.8)	13 (35.1)		1 (16.7)	18 (26.1)		7 (16.3)	12 (37.5)	
3+	2 (16.7)	15 (23.8)		17 (44.7)	0 (0.0)		2 (33.3)	15 (21.7)		16 (37.2)	1 (3.1)	
Recurrence			0.184			0.157			0.026[b]			0.216
Yes	1 (8.3)	1 (98.4)		2 (5.3)	0 (0.0)		1 (16.7)	1 (1.4)		41 (95.3)	32 (100.0)	
No	11 (91.7)	62 (1.6)		36 (94.7)	37 (100.0)		5 (83.3)	68 (98.6)		2 (4.7)	0 (0.0)	

Table II. Continued.

Parameter	FGFR1 amplification, n (%)			HER1/2 amplification[a], n (%)			FGFR1 and HER1/2 co-amplification, n (%)			FGFR1 or HER1/2 amplification, n (%)		
	+	-	P-value	+	-	P-value	+	-	P-value	+	-	P-value
Distant metastasis			0.341			0.051			0.035[b]			0.249
Yes	2 (16.7)	5 (7.9)		6 (15.8)	1 (2.7)		2 (33.3)	5 (7.2)		24 (55.8)	16 (50.0)	
No	10 (83.3)	58 (92.1)		32 (84.2)	36 (97.3)		4 (66.7)	64 (92.8)		19 (44.2)	16 (50.0)	

[a]Including 20 HER1$^+$ patients, 28 HER2$^+$ patients, and 10 HER1$^+$ and HER2$^+$ patients. [b]Statistically significant (P<0.05). FGFR1, fibroblast growth factor receptor 1; HER1, human epidermal growth factor receptor 1; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor.

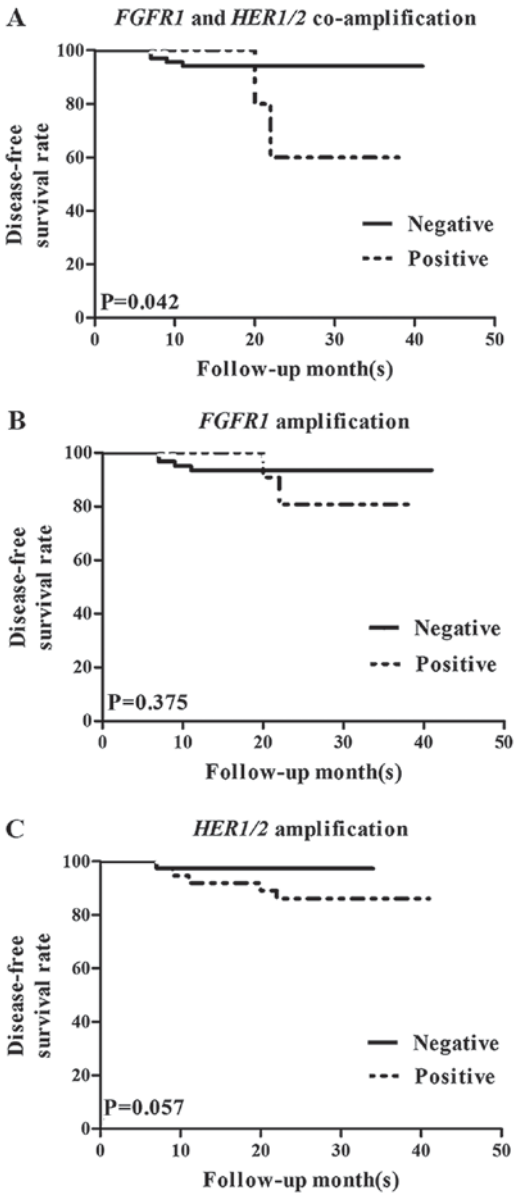

Figure 3. Kaplan-Meier survival analysis of disease-free survival time in association with *FGFR1* and *HER1/2* amplification in invasive ductal breast cancer. (A) The co-amplification of *FGFR1* and *HER1/2* was significantly associated with decreased disease-free survival time, whereas (B) *FGFR1* and (C) *HER1/2* amplification were not individually correlated with disease-free survival time. FGFR, fibroblast growth factor receptor; HER1/2, human epidermal growth factor receptor 1 or 2.

status; the majority of patients with *FGFR1* amplification were menopausal, in contrast to the patients without *FGFR1* amplification. *HER1/2* amplification status was associated with the ER and HER2 protein expression statuses. This result was consistent with the view that *HER2* amplification is highly associated with its protein overexpression (6).

In addition, the response to therapy in the *FGFR1* and *HER1/2* co-amplification group was investigated. The data included patients who underwent chemotherapy and endocrine therapy. The 6 patients who harbored *FGFR1* and *HER1/2* co-amplification had poor outcomes following chemotherapy or endocrine therapy. Insensitivity to therapy may have caused the group of patients to have unfavorable outcomes. Accordingly, further study is required to investigate whether,

Table III. Analysis of clinicopathological characteristics with disease-free and overall survival.

		Disease-free survival		Overall survival	
Parameter	n (%)	Log-rank	P-value	Log-rank	P-value
Age, years		0.111	0.739	1.675	0.196
≤50	49 (65.3)				
>50	26 (34.7)				
Tumor size		7.672	0.006[a]	1.967	0.160
T0-T2	70 (93.3)				
T3-T4	5 (6.7)				
Nodal status		5.353	0.021[a]	6.738	0.009[a]
N0	41 (54.7)				
N1-N3	34 (45.3)				
Menopausal status		0.008	0.927	0.614	0.433
Menopausal	33 (44.0)				
Premenopausal	42 (56.0)				
Clinical stage		1.900	0.168	4.151	0.042[a]
I-II	58 (77.3)				
III-IV	17 (22.7)				
ER status		0.906	0.341	0.221	0.638
ER+	52 (69.3)				
ER-	23 (30.7)				
PR status		1.666	0.197	1.436	0.231
PR+	49 (65.3)				
PR-	26 (34.7)				
HER2		1.827	0.401	0.883	0.643
0-1+	39 (52.0)				
2+	19 (25.3)				
3+	17 (22.7)				
FGFR1 amplification		0.786	0.375	0.934	0.334
+	12 (16.0)				
-	63 (84.0)				
HER1/2 amplification		3.628	0.057	1.908	0.167
+	38 (50.7)				
-	37 (49.3)				
FGFR1 and *HER1/2* co-amplification		4.136	0.042[a]	0.394	0.530
+	6 (8.0)				
-	69 (92.0)				

[a]Statistically significant (P<0.05). ER, estrogen receptor; PR, progesterone receptor; FGFR1, fibroblast growth factor receptor 1; HER1, human epidermal growth factor receptor 1; HER2, human epidermal growth factor receptor 2.

Table IV. Association between treatment response and *FGFR1* and *HER1/HER2* co-amplification.

		FGFR1 and *HER1/2* co-amplification, n (%)		Disease-free survival time	
Treatment	n	Positive	Negative	Log-rank	P-value
Chemotherapy	74	6 (8.1)	68 (91.9)	4.038	0.044[a]
Endocrine therapy	50	6 (12.0)	44 (88.0)	9.730	0.002[a]

[a]Statistically significant (P<0.05). *FGFR1*, fibroblast growth factor receptor 1; *HER1/2*, human epidermal growth factor receptor 1 or 2.

and how, *FGFR1* and *HER1/2* co-amplification can influence tumor resistance to drug therapy.

The amplification of *HER1* is not as common in breast cancer as *HER2* amplification, and the effect of *HER1* amplification on patients with breast cancer remains unclear (34,35). By contrast, HER2 is already a therapeutic target, and the effect of *HER2* amplification for patients with breast cancer has been confirmed (14,36). Previously the amplification of *FGFR1* has been regarded as an independent prognostic factor and a potential therapeutic target in breast cancer, and it may be associated with resistance in endocrine therapy (25). *FGFR1* amplification is rarely exhibited in *HER2*-amplified tumors (25); the genes are mutually exclusive methods for activating similar downstream pathways, including PI3K/AKT, ERK/MAPK and PKC/PLC (29). Further investigation is required to study how the co-activation of FGFR and HER1/2 could affect downstream pathways.

In conclusion, the expression levels of *FGFR1*, *HER1*, *HER2* were detected using qPCR and FISH. A specific group of patients with co-amplification of *FGFR1* and *HER1/2* was identified to be associated with worse prognosis, and correlated with resistance to endocrine therapy and chemotherapy. This finding suggests that the gene statuses of *FGFR1*, *HER1* and *HER2* can be predictive of clinical outcome, and that different therapeutic strategies should be implemented for these patients in order to improve the prognosis.

Competing interests

The authors declare that they have no competing interests.

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