Prognostic Significance of HER2 Heterogeneity in Early-stage and Locally advanced HER2-positive Breast Cancer

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Abstract

Background: Breast cancer tumors often have intratumoral heterogeneity (ITH), which has been associated with therapeutic resistance.(Figure1) Tumors with high ITH show human epidermal growth factor receptor 2 (HER2) heterogeneity, impacting the effectiveness of HER2-targeted therapies. In this study, we investigated the association between HER2 ITH and resistance to Anti-HER2 Neoadjuvant Chemotherapy (NAC).
Methods: The study included 97 patients of primary HER2-positive breast cancer treated with Anti-HER2 NAC. Breast tumor samples were obtained from vacuum-assisted breast biopsy before NAC. HER2 gene amplification was assessed by fluorescence in situ hybridization (FISH), and histograms of HER2 gene copy numbers were generated. Using Gaussian mixture model, the histogram data were analyzed and classified into two groups, high HER2 heterogeneity (HH) and low HER2 heterogeneity (LH). The relationship between HER2 ITH and treatment response was evaluated with the pathological complete response (pCR) rate.
Results: Of 97 patients,18 (18.6%) were classified into the HH group, and 79 (81.4%) into the LH group. The pCR rate in the HH group was significantly lower at 28% (5/18) compared to 65% (51/79) in the LH group (P = 0.01). Multivariate analysis of pathological parameters revealed that HER2 ITH was the significant predictor (P = 0.02), following estrogen receptor status.

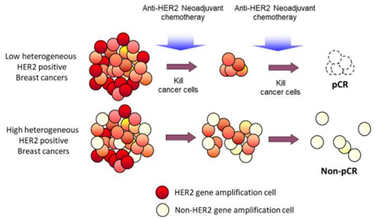


Figure 1.
Schematic representation of anti-HER2 therapy resistance in the context of intratumoral HER2 heterogeneity.

Introduction

ITH represents a pervasive feature of breast malignancies, often undermining therapeutic efficacy. In this investigation, we characterized HER2-specific ITH by examining the morphological profiles of HER2 gene amplification through FISH histogram distributions, derived from intratumoral HER2 copy number variation. We identified a robust correlation between marked HER2 heterogeneity and the attainment of pCR among patients with HER2-positive disease. Our method of analyzing FISH distribution patterns offers a practical and translationally valuable tool for anticipating clinical outcomes in the setting of HER2-targeted adjuvant therapy.

Methods and Materials

HER2 FISH assays were conducted on all tumor tissues of VAB before NAC. FFPE tumor tissue slides were dried, and DAPI counterstain solution was applied. The number of orange-fluorescent HER2/neu signals and green-fluorescent CEP17 signals was counted in more than 20 cell nuclei using a fluorescence microscope. The ratio of HER2/neu to CEP17 signal counts was determined. (Figure 2)
We obtained scanned images from FISH diagnostic reports and extracted the HER2 FISH signal count distribution by applying optical character recognition (OCR) and other image processing techniques to reconstruct the FISH signal histograms. Subsequently, we modeled these distributions using the GMM. From the estimated parameters, we derived an ITH measure that captures the presence of a sub-population with HER2 copy numbers of < 2 while the overall average copy number remains ≥ 2. HER2 ITH was evaluated based on the patterns of HER2 FISH signal distributions. Tumors were classified into two distinct groups according to their distribution patterns as illustrated in Figure 1: a double-peaked distribution indicative of HH, and a single-peaked distribution reflective of LH. The pCR rates after NAC were subsequently compared between these two groups. (Figure 2)

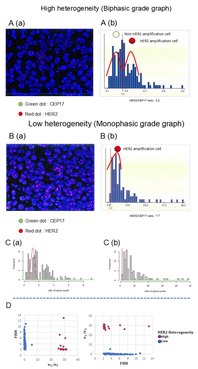


Figure 2.
(a), histogram analysis of HER2 FISH signals
(b), classifying the cases
into the high heterogeneity (A)
and low heterogeneity groups (B),
and fitting with a Gaussian mixture model (C),
HER2 FISH signals and the mixture ratio (π1/π2) in the overall cohort of patients with HER2-positive breast cancer (D)

Table 1.
Association between patient characteristics and HER2 heterogeneity.

	Total	HER-2 heterogeneity		P value
		High	Low	
	n = 97, %	n = 18, %	n = 79, %	
Menopause				
pre	47 (48.5%)	12 (66.7%)	35 (44.3%)	0.1178
post	50 (51.5%)	6 (33.3%)	44 (55.7%)	
Histological type				
IDC	95 (97.9%)	17 (94.4%)	78 (98.8%)	0.3383
ILC	2 (2.1%)	1 (5.6%)	1 (1.3%)	
Histological grade				
1	5 (5.2%)	0 (0.0%)	5 (6.3%)	0.2479
2	33 (34.0%)	9 (50.0%)	24 (30.4%)	
3	59 (60.8%)	9 (50.0%)	50 (63.3%)	
Tumor size				
T1	7 (7.2%)	1 (5.6%)	6 (7.6%)	1
T2-T4	90 (92.8%)	17 (94.4%)	73 (92.4%)	
ER				
Positive	52 (53.6%)	13 (72.2%)	40 (50.6%)	0.1198
Negative	45 (46.4%)	5 (27.8%)	39 (49.4%)	
PgR				
Positive	28 (28.9%)	7 (38.9%)	21 (26.6%)	0.3876
Negative	69 (71.1%)	11 (61.1%)	58 (73.4%)	
HER2 IHC				
3+	90 (92.8%)	16 (88.9%)	74 (93.7%)	0.6105
2+	7 (7.2%)	2 (11.1%)	5 (6.3%)	
HER2 ratio				
Mean	4.8 ± 2.2	2.5 ± 0.5	5.3 ± 2.2	1.23×10^{-17}
Stage				
I	4 (5.1%)	0 (0.0%)	4 (5.1%)	1
II	74 (76.3%)	14 (77.8%)	60 (75.9%)	
III	19 (19.6%)	4 (22.2%)	15 (19.0%)	

Results

Among the 97 HER2-positive cases analyzed, 18 (18.6%) were assigned to the HH group, while 79 (81.4%) were designated as LH.(Table1) The average HER2 FISH ratio in the HH group was 2.5, whereas a markedly higher mean ratio of 5.3 was observed in the LH group. The HH group exhibited a tendency toward being estrogen receptor (ER)-positive (p = 0.12), premenopausal (p = 0.11), positive for pretreatment lymph node (LN) metastasis (p = 0.10), and positive for resected LN metastasis (p = 0.12); however, no statistically significant associations were noted. The proportion of HH of HER2-positive tumors following the ER and PgR status is depicted in Figure 2.
The pCR rate to anti-HER2 NAC in patients with HER2-positive breast cancer in the HH group was significantly lower than that in the LH group, indicating that tumors in the HH group exhibited a markedly reduced response to HER2-targeted neoadjuvant therapy and resistance to anti-HER2 agents (univariate analysis, p < 0.01) (Figure 3). Multivariate analysis of the pathological factors revealed that ER status and HER2 ITH were significantly correlated with the pCR rate (multivariate analysis: ER, p = 0.03; HER2 heterogeneity, p = 0.02) (Table 2).

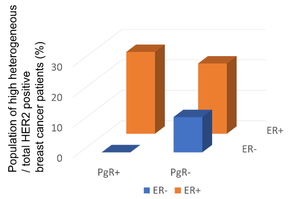


Figure 2. Correlation between the proportion of patients with HER2-positive breast cancer with high heterogeneity (%) and ER/PgR status (n = 97).
The ER+ and PgR+ tumors exhibited the highest HH frequency (ER+ and PgR+, 26.9% [7/26]; ER+ and PgR−, 23.1% [6/26]; ER− and PgR−, 11.6% [5/43]; and ER− and PgR+: 0% [0/2]).

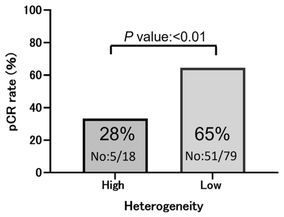


Figure 3. Pathological complete response (pCR) rate to anti-HER2 neoadjuvant chemotherapy (NAC) in patients with HER2-positive breast cancer in both groups.

Discussion

This strategy provides a straightforward cost-effective method that merely involves interpreting the FISH diagnostic report histograms and performing image analysis.
This study has several limitations. First, the sample size is relatively small, which may limit the generalizability of our findings. Second, our study is retrospective in nature, which may introduce selection bias. Third, while we assessed key biomarkers and treatment responses, other potential confounding factors. Fourth, this study was limited to FFPE specimens.
Fifth, treatment resistance may not be solely attributable to intratumoral heterogeneity. Alternative mechanisms may contribute to tumor progression [1–3].
Treatment with trastuzumab deruxtecan significantly improves the PFS and OS compared with chemotherapy in patients with HER2-low metastatic breast cancer [4]. Our method may facilitate precise ITH evaluation for predicting therapeutic responses in HER2-low breast cancers.

			Univariate analysis			Multivariate analysis		
Characteristic	N	pCR(N)	OR	95% CI	P value	OR	95% CI	P value
ER								
Positive	52	21						
Negative	45	35	0.194	0.0791 - 0.474	0.0003	0.321	0.113-0.912	0.03
PgR								
Positive	28	9						
Negative	69	47	0.222	0.0865 - 0.568	0.001	0.38	0.123-1.17	0.09
Histological grade								
1 or 2	38	17						
3	59	39	1.79	0.897 – 3.59	0.09	1.84	0.856-3.97	0.11
HER2 heterogeneity								
Low	79	51						
High	18	5	0.211	0.0682 - 0.654	0.006	0.241	0.0699-0.828	0.02

Table 2. Univariate and multivariate analyses of pathological factors on the pCR rate to anti-HER2 NAC in HER2-positive patients.
Multivariate analysis of the pathological factors revealed that ER status and HER2 ITH were significantly correlated with the pCR rate (multivariate analysis: ER, p = 0.03; HER2 heterogeneity, p = 0.02

Conclusions

The assessment of HER2 ITH may be valuable in predicting therapeutic outcomes in HER2-positive breast cancer. Our novel approach of HER2 ITH method using FISH histograms could potentially serve as a useful tool in predicting resistance to anti-HER2 NAC.

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References

1. Zhang, D.; Tai, L.K.; Wong, L.L.; Chiu, L.L.; Sethi, S.K.; Koay, E.S. Proteomic study reveals that proteins involved in metabolic and detoxification pathways are highly expressed in HER-2/neu-positive breast cancer. Mol. Cell. Proteom. 2005, 4, 1686–1696.
2. Steiner, C.; Tille, J.C.; Lamerz, J.; Kux van Geijtenbeek, S.; McKee, T.A.; Venturi, M.; Rubbia-Brandt, L.; Hochstrasser, D.; Cutler, P.; Lescuyer, P.; Ducret, A. Quantification of HER2 by Targeted Mass Spectrometry in Formalin-Fixed Par-affin-Embedded (FFPE) Breast Cancer Tissues. Mol. Cell. Proteom. 2015, 14, 2786–2799.
3. Debets, D.O.; Stecker, K.E.; Piskopou, A.; Liefaard, M.C.; Wesseling, J.; Sonke, G.S.; E. Lips, E.H.; Altelaar, M. Deep phosphoproteomics profiling of pre- treatment needle biopsies identifies signatures of treatment resistance in HER2+ breast cancer. Cell Rep. Med. 2023, 4, 101203
4. Modi, S.; Jacot, W.; Yamashita, T.; Sohn, J.; Vidal, M.; Tokunaga, E.; Tsurutani, J.; Ueno, N.T.; Prat, A.; Chae, Y.S.; et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N. Engl. J. Med. 2022, 387, 9–20.

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