Combined Hormone Receptor and Human Epidermal Growth Factor Receptor-2 Expression Reflects Inter-and Intra-tumor Heterogeneity in Hormone Receptor-positive/human Epidermal Growth Factor Receptor-2-positive Breast Cancer

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Abstract

Background

Hormone receptor-positive and human epidermal growth factor receptor-2-positive (HR+/HER2+) breast cancer comprise approximately 5-10% of all invasive breast cancers. However, the lack of knowledge regarding the complexity of tumor heterogeneity in HR+/HER2+ disease remains a barrier to more accurate therapies. This study aimed to describe the tumor heterogeneity of HR+/HER2+ breast cancer and to establish a novel indicator to identify the HER2-enriched-like subtype in patients with HR+/HER2+ breast cancer.

Methods

First, a comprehensive analysis was performed on HR+/HER2+ breast cancer samples from the TCGA (n=141) and METABRIC (n=104) databases. We determined the distribution of PAM50 intrinsic subtypes within the two cohorts and compared the somatic mutational profile and RNA expression features between HER2-enriched and non-HER2-enriched subtypes. We then performed multiplex immunofluorescence to evaluate HER2 and estrogen receptor (ER) expression simultaneously in the third cohort, enrolling 43 cases of early HR+/HER2+ breast cancer from Cancer Hospital, Chinese Academy of Medical Sciences (CAMS).

Results

All four main intrinsic subtypes were identified in HR+/HER2+ breast cancer, of which the luminal-B subtype was the most common, followed by the HER2-enriched and luminal-A subtypes. Significantly increased TP53 and ERBB3 and decreased PIK3CA somatic mutation frequency were observed in the HER2-enriched subtype compared with the non-HER2-enriched subtype. In addition, the HER2-enriched subtype was characterized by significantly higher ERBB2 and lower ESR1 expression. We then constructed a marker termed rH/E to reflect the relative expression of ERBB2 to ESR1 in each patient. rH/E discriminates the HER2-enriched subtype from the non-HER2 subtypes better than the expression of ERBB2 or ESR1 alone. In the CAMS cohort, we observed four subtypes of tumor cells: ER+/HER2-, ER+/HER2+, ER-/HER2+, and ER-/HER2-. Tumor cell diversity was common, with 86% of patients having all four subtypes of tumor cells. Moreover, rH/E showed a significant prognostic association in the CAMS cohort.

Conclusions

This study furthers our understanding of the complexity of tumor heterogeneity in HR+/HER2+ breast cancer, and suggests that the combined analysis of ERBB2 and ESR1 expression may contribute to identifying patients with specific subtypes in this population.

Introduction

Human epidermal growth factor receptor-2 (HER2) is overexpressed in 15-20% of breast cancer cases.[1] Approximately 50% of these HER2+ breast cancers also express hormone receptors (HRs).[2] More treatment options are available for HR+/HER2+ patients than for HR-/HER2+ patients. Currently, the standard first-line treatment for metastatic HR+/HER2+ disease consists of chemotherapy and anti-HER2 therapy.[3, 4] Recently, several studies demonstrated that the combination of endocrine therapy with anti-HER2 blockade agents could provide more benefit to some HR+/HER2+ patients with metastasis.[5, 6] In 2021, the American Society of Clinical Oncology (ASCO) annual meeting reported the results of the SYSUCC-002 study, which showed the non-inferiority of endocrine therapy plus anti-HER2 treatment relative to chemotherapy plus anti-HER2 treatment for HR+/HER2+ metastatic breast cancer as the first-line treatment..[7]

Although significant progress has been made, the impact of tumor heterogeneity in HR+/HER2+ breast cancer has been underestimated. Emerging evidence supports the influence of tumor heterogeneity on clinical outcomes and drug sensitivity. First, all four main PAM50 intrinsic subtypes are represented within HR+/HER2+ disease.[8, 9] Patients with the luminal type were considered to have a better prognosis and stronger response to endocrine treatment.[10, 11] Patients with the HER2-enriched subtype were highly sensitive to anti-HER2 treatment in a neoadjuvant setting.[12, 13]

In addition, the complexity of tumor heterogeneity in HR+/HER2+ disease is enhanced by HER2 expression, which can be divided into inter- and intra-tumor heterogeneity. It was observed that the levels of HER2 are not homogeneous among the HER2+ population defined by immunohistochemistry. The levels of ERBB2 mRNA and protein progressively increase across samples upon IHC score.[14] Moreover, the intra-tumoral heterogeneity is present in the distinct HER2 status in different areas of the same tumor. [15] Importantly, these heterogeneities showed significant correlation with the therapeutic efficacy of anti-HER2 treatment in both neoadjuvant and metastatic settings.[16, 17]

Currently, our understanding of the complexity of tumor heterogeneity in HR+/HER2+ breast cancer is relatively limited. For example, HR expression is a crucial factor, but has not been considered previously when assessing heterogeneity. Moreover, the lack of feasible and reproducible indicators to describe tumor heterogeneity limits the further clinical applications of this concept.

In this study, we aimed to describe the tumor heterogeneity of HR+/HER2+ breast cancer from multiple aspects by: 1) determining the distribution of PAM50 intrinsic subtypes, 2) comparing the DNA mutational profile and RNA expression features between HER2-enriched and non-HER2-enriched subtypes, and 3) showing the heterogeneity using multiplex immunofluorescence (mIF) to determine the HER2 and estrogen receptor (ER) status simultaneously. In addition, we aimed to establish a novel indicator to identify the HER2-enriched-like subtype in patients with HR+/HER2+ breast cancer.

Materials And Methods

Study cohorts

Our study included three cohorts of patients with HR+/HER2+ breast cancer. The first cohort included 141 patients from The Cancer Genome Atlas (TCGA). The clinical data of the TCGA cohort were extracted from the University of California Santa Cruz (UCSC) Xena (<http://xena.ucsc.edu/>).

The second cohort enrolled 104 patients from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC). The data of the METABRIC cohort were hosted by the European Bioinformatics Institute and deposited in the European Genome-Phenome Archive (<http://www.ebi.ac.uk/ega/>) under accession number EGAS00000000083.

The third cohort was a retrospective observational cohort consisting of 43 patients with early HR+/HER2+ breast cancer treated at the Cancer Hospital of the Chinese Academy of Medical Science (CAMS) between 2012 and 2016. The CAMS cohort was enrolled according to the following criteria: 1) female patients with stage I-III primary unilateral breast cancer who underwent radical surgery and complete 1-year trastuzumab treatment; 2) breast cancer was the first and only malignant cancer diagnosis; 3) all patients had formalin-fixed, paraffin-embedded surgical specimens, clinical data, and follow-up data; 4) patients had invasive ductal carcinoma with an HR+/HER2+ phenotype; and 5) approval was obtained from the Medical Ethics Committee of the Cancer Hospital of the CAMS. The exclusion criteria were as follows: 1) male patients, 2) bilateral primary breast cancer, 3) combined with other malignant tumors, and 4) no corresponding clinical or follow-up data.

The ER, progesterone receptor (PR), and HER2 statuses were determined by IHC analysis or *in situ* hybridization. A cutoff value of $\geq 1\%$ positive tumor cells was used to define ER and PR positivity, while the HER2 status was defined according to the most recent ASCO/College of American Pathologists guidelines. Follow-up was completed on May 8, 2020, with a median follow-up duration of 64 months (interquartile range, 20-101 months). Our study was approved by the independent ethics committee/institutional review board of the CAMS (20/272–2468).

Bioinformatic analysis

Somatic mutation analysis

Somatic mutation data for the TCGA cohort were extracted from UCSC Xena (<http://xena.ucsc.edu/>), and the most commonly mutated cancer-related genes in HER2+ breast cancers were identified. Differences between mutation rates in the HER2- and non-HER2-enriched groups (luminal A, luminal B, basal-like, and normal) were compared using the “maftools” R package.[18]

RNA-seq analysis

RNA-seq data (level 3) of the TCGA cohort were extracted from UCSC Xena (<http://xena.ucsc.edu/>) and the normalized gene expression was measured as fragments per kilobase of transcript per million mapped reads (FPKM). The FPKM values were log₂-transformed after a constant value of 1 was added to all values. Statistically significantly differentially expressed genes were detected using the “limma” R

package. The “genefu” R package was used to assign an intrinsic molecular subtype to the tumor of each patient using the PAM50 classifier.[19]

Multiplex immunofluorescence (mIF)

mIF was used to detect the expression of ER and HER2: red fluorescence in the nucleus indicated ER positivity and green fluorescence in the cell membrane indicated HER2 positivity. The number and proportion of different tumor cell types were analyzed using Image-Pro Plus (Version 7.0.1.658, Media Cybernetics, Rockville, MD, USA) image processing and analysis software. Further details can be found in the Supplementary File.

Statistical analysis

Statistical analyses were conducted using SPSS version 24.0. (SPSS Inc., Chicago, IL, USA), GraphPad Prism (Version 8.0, GraphPad Software Inc., La Jolla, CA, USA), X-tile software (Version 3.6.1, Yale University School of Medicine, New Haven, CT, USA), and R software (version 3.6.0). Normally distributed quantitative data are described using the mean and standard deviation, with *t*-tests used for between-group comparisons, whereas quantitative data with a skewed distribution are described using the median and interquartile range, and rank sum tests were used for between-group comparisons. Categorical variables are described using numbers and percentages, and between-group comparisons were performed using Pearson’s chi-square tests. Normally distributed data were analyzed using Pearson’s correlation, and non-normally distributed data were analyzed using Spearman’s correlation. Disease-free survival (DFS) was used as the prognostic evaluation index, defined as the period from surgery to the first local, regional, or distant tumor recurrence or death. X-tile software was used to determine the optimal cut-off value for rH/E, and survival differences between the two groups were compared using Kaplan-Meier survival analysis. Univariate and multivariate Cox regression analyses were performed to identify significant prognostic factors, and the performance of the prognostic model was assessed by calculating Harrell’s concordance index.[20] *P* values of <0.05 were considered to indicate statistical significance.

Results

Distribution of the PAM50 intrinsic subtypes of HR+/HER2+ breast cancer

Both the TCGA and METARIC datasets contained four PAM50 intrinsic subtypes. The luminal-B subtype was the most common, and the HER2-enriched and Luminal-A subtype accounted for approximately one-third of both datasets. Only a very small proportion of patients were classified as basal-like or normal subtypes in the TCGA and METABRIC datasets, respectively. (Fig. 1)

Comparison of the molecular characteristics of the HER2-enriched and non-HER2-enriched subtypes in HR+/HER2+ breast cancers

To better understand inter-tumor heterogeneity, we determined the differences between the HER2-enriched and non-HER2-enriched subtypes by analyzing the somatic mutation and RNA expression data. The HER2-enriched subtype was characterized by significantly higher TP53 (48% vs.24%, $p<0.01$) and ERBB3 (15% vs.1%, $p<0.001$) and significantly lower PIK3CA mutation frequencies (15% vs.42%, $p<0.001$) than the non-HER2-enriched subtypes. (Fig. 2)

Furthermore, RNA-seq data from TCGA showed that the activity of the G2/M cell cycle checkpoint, E2F pathway, and mechanistic target of rapamycin complex 1 signaling was significantly higher, whereas the activity of epithelial mesenchymal transition, estrogen response, and NF- κ B-mediated tumor necrosis factor-alpha signaling was significantly lower in the HER2-enriched subtype than in the non-HER2-enriched subtype.(Fig. 2)

rH/E better predicts the HER2-enriched subtype

In both the TCGA and METABRIC databases, the HER2-enriched subtype was characterized by significantly higher ERBB2 and decreased ESR1 mRNA expression than the non-HER2-enriched subtypes. (Fig. 3) However, the luminal A subtype cannot be readily distinguished from the luminal B subtype by the expression of ERBB2 or ESR1.(Fig. 3)

Based on the above results, we constructed a novel marker termed rH/E, which was calculated as ERBB2 expression quantity/(ESR1 expression quantity + 1). Thus, rH/E reflected the relative expression of ERBB2 to ESR1 in each patient. To determine the optimal predictor for the HER2-enriched subtype, we compared the area under the curve (AUC) values of ERBB2 expression, ESR1 expression, and rH/E. rH/E had the highest AUC value in the TCGA (AUC=0.918, 95% confidence interval [CI]: 0.874-0.963) and METABRIC (AUC=0.746, 95% CI: 0.648-0.845) databases. (Fig. 3)

The existence of four tumor cell subtypes reflects the intra-tumor heterogeneity in HR+/HER2+ breast cancer

To further evaluate the tumor heterogeneity in HR+/HER2+ breast cancer, we tested the expression of HER2 and ER proteins simultaneously using mIF in 43 patients with HR+/HER2+ breast cancer who underwent surgery followed by chemotherapy and 1-year adjuvant trastuzumab treatment in CAMS (CAMS cohort).

The characteristic feature of the CAMS cohort was shown in **Supplementary Table 1**. Nineteen patients had lymph node metastasis. Only two patients did not receive adjuvant endocrine treatment. At the final follow-up, six patients had experienced tumor recurrence or progression. No significant differences in clinicopathological features were detected between patients with and without recurrence.

Interestingly, mIF revealed that the tumor cells of HR+/HER2+ breast cancer can be classified into four categories based on HER2 and ER expression: ER+HER2+, ER+HER2-, ER-HER2+, and ER-HER2-. Fig. 4 shows the mIF images of a patient with ER 80%+, PR 10%, and HER2 3+ breast cancer.

In addition, we found that the distribution of the four tumor cell subtypes was distinct among patients. A total of 7%, 7%, and 86% of patients presented with two, three, and four types of tumor cells, respectively. (Fig. 5). The proportion of ER-HER2+ tumor cells was negatively correlated with that of ER+HER2- and ER-HER2- tumor cells, and the proportion of ER-HER2- tumor cells was positively correlated with that of ER+HER2- tumor cells. (Fig. 5)

The potential clinical significance of rH/E in the CAMS cohort

When using mIF, the calculation of rH/E was modified as the proportion of HER2 positive cells (ER+HER2+ and ER-HER2+)/((the proportion of ER-positive cells [ER+HER2+ and ER+HER2-]+1).

In the CAMS cohort, we evaluated the clinical relevance of these four types of tumor cells. Interestingly, rH/E, but not the tumor cell phenotype, showed a significant prognostic correlation. TNM staging and rH/E were independent risk factors for DFS.(Table 1) Furthermore, the combination of rH/E and TNM staging significantly increased the prognostic predictive efficacy compared to TNM staging alone. (Table 2) X-tile software determined the optimal cut-off value of rH/E at 1.5. According to the level of rH/E, we classified the 43 patients into a HER2-enriched-like subgroup (n=9) and a non-HER2-enriched-like subgroup (n=34).The HER2-enriched-like subgroup with higher rH/E showed significantly reduced 5-year DFS than those in the non-HER2-enriched-like subgroup (67% vs.91%, log-rank p=0.046) (Fig. 6)

Table 1
Univariate and multivariate Cox regression analyses.

Univariate Cox regression				Multivariate Cox regression		
Variables	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value
TNM	3.83	1.04-14.05	0.04	4.60	1.29-16.38	0.02
Age	0.93	0.85-1.02	0.14			
ER+HER2-	0.96	0.90-1.04	0.33			
ER-HER2+	1.04	1.00-1.08	0.07			
ER+HER2+	1.00	0.96-1.04	0.96			
ER-HER2-	0.96	0.88-1.05	0.37			
rH/E	2.09	1.04-4.21	0.04	2.63	1.15-6.01	0.02
Abbreviations: HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; HR: hormone receptor or hazard ratio; CI: confidence interval.						

Table 2
Prognostic predictive efficacy of TNM staging with
or without rH/E.

Variables	C-index	95% CI	<i>p</i> value
TNM	0.74	0.60-0.89	0.022
TNM and rH/E	0.80	0.63-0.96	
Abbreviations: CI: confidence interval.			

Discussion

The present study demonstrated considerable inter- and intra-tumoral heterogeneity in HR+/HER2+ breast cancer. We observed significant differences in the patterns of DNA mutations and gene expression profiles between HER2-enriched and non-HER2-enriched subtypes. In addition to ERBB2 expression, the diversity of ESR1 expression also contributed to tumor heterogeneity. Interestingly, the level of rH/E, a marker reflecting the relative expression of ERBB2 to ESR1, may help to identify patients in the HER2-enriched-like subgroup. Finally, the HER2 and HR status as determined by mIF in the CAMS cohort not only suggested the potential clinical relevance of rH/E, but also showed substantial intra-tumoral heterogeneity. This study furthers our understanding of the complexity of tumor heterogeneity in HR+/HER2+ breast cancer, and suggests that combined the analysis of ERBB2 and ESR1 expression may provide a simpler and more cost-effective method of identifying patients with specific subtypes in this population.

The existence of all four main PAM50 intrinsic molecular subtypes clearly demonstrated significant inter-tumoral heterogeneity in HR+/HER2+ breast cancer. Interestingly, the reported distribution of the four subtypes varies across studies. Consistent with some studies[21], our analysis showed that the luminal-B subtype accounted for the highest proportion, while the proportions of HER2-enriched and luminal-A subtypes were similar. In contrast, other studies have reported that the HER2-enriched subtype was predominant in HR+/HER2+ tumors.[22, 23] Possible explanations for this discrepancy include different methodologies of RNA profile evaluation or different numbers of patients included.

This study suggests the potential role of ESR1 expression in tumor heterogeneity in HR+/HER2+ breast cancer. ESR1 is an important marker and driver of the luminal subtype, which is highly expressed in patients with HR+/HER2+ disease. However, the effect of ESR1 expression on tumor heterogeneity remains unclear. Previous studies predominantly focused on the intensity and location of HER2 expression, which had significant prognostic and predictive effects.[15, 16, 24, 25] In this study, we found that the level of ERBB2 relative to ESR1 expression could predict the HER2-enriched subtype more accurately than ERBB2 expression alone. Moreover, we observed a subgroup of tumor cells with ER+/HER2- status in patients with HR+/HER2+ breast cancer. A proportion of patients have a high content of this type of tumor cell, which may be primarily driven by the ER signaling pathway. Taken

together, these data provide evidence indicating an important role of ESR1 expression in HR+/HER2+ breast cancer.

The novel marker, rH/E, also has potential clinical implications. A strong correlation exists between molecular subtyping and clinical outcomes, as well as drug sensitivity. Patients with the luminal-A subtype had a better outcome than non-luminal subtype patients.[10] In addition, HER2-enriched tumors are the most sensitive to anti-HER2-based treatments.[12] However, as molecular testing has not become a routine diagnostic process, there may be clinical value in developing a technically easy and cost-effective method to identify specific subgroups of patients. In the present study, the best AUC value was achieved using the rH/E marker. Furthermore, we detected HER2 and ER by mIF in 43 HR+/HER2+ patients from the CAMS cohort and identified a HER2-enriched-like subgroup. Patients in this subgroup had a poorer prognosis, suggesting that it might be possible for some patients with HER2-enriched-like disease to be treated with intensive anti-HER2 therapy. Further studies are necessary to validate the clinical significance of rH/E in larger samples.

Another interesting finding of this study is that a high degree of intratumoral heterogeneity may be a characteristic feature of HR+/HER2+ breast cancer. We determined the expression and localization of HER2 and ER using mIF, and four types of tumor cells with distinct ER and HER2 status were found in the same patients, which directly reflected substantial intratumoral heterogeneity in this cohort. Although we did not find a correlation between the clinical features and any type of tumor cell in this study, we believe that it is necessary to explore the biological and clinical role of intratumoral heterogeneity in subsequent studies.

Abbreviations

TCGA: The Cancer Genome Atlas; METABRIC: Molecular Taxonomy of Breast Cancer International Consortium; ERBB2/HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; PR: progesterone receptor; ERBB3/HER3: human epidermal receptor 3; PI3KCA: phosphatidylinositol 3-kinase; TP53: tumor protein p53; mTORC1: mechanistic target of rapamycin complex 1; TNF- α : tumor necrosis factor-alpha; EMT: epithelial mesenchymal transition; RNA-seq: RNA sequencing; DEGs: differentially expressed genes. IHC: immunohistochemistry; IF: immunofluorescence; HE: hematoxylin-eosin; ROC: receiver operating characteristic; AUC: area under the receiver operating characteristic curve; HR: hormone receptor or hazard ratio; CI: confidence interval; DFS: disease-free survival;

Declarations

Ethics approval and consent to participate

The study was approved by the independent Ethics Committee/Institutional Review Board of CAMS (20/272-2468).

Consent for publication

All authors agree to publish this article

Availability of data and materials

Data available from the authors upon reasonable request and with permission of National Cancer Centre/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College in China.

Competing interests

All authors confirm and declare that no conflicts of interest.

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Author contributions

Study design: Jie Ju, Feng Du, Dong-Xu Liu and Peng Yuan. Experiment: Jie Ju and Feng Du. Data collection: Yi-Ran Si, Nan-Lin Hu, Dong-Xu Liu, Xue Wang, Jian Yue, Fang-Chao Zheng, Yi-Kun Kang, Zi-Xuan Yang, Fei Ma and Bing-He Xu. Data analysis: Jie Ju, Feng Du and Song-Lin Gao. Draft writing: Jie Ju, Feng Du and Peng Yuan. Final revision: Peng Yuan. All authors read and approved the final version of the manuscript, and agreed with the order of presentation of the authors.

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Figures

Figure 1

The distribution of PAM50 intrinsic subtypes of patients with HR+/HER2+ breast cancer in TCGA cohort (A) and METABRIC cohort (B)

Figure 2

Molecular characteristics of HER2 enriched subtype and non-HER2 enriched subtype of HR+/HER2+ breast cancer in the TCGA cohort

(A) Waterfall plot highlighting the hot somatic mutations in HER2 enriched subtype and non-HER2 enriched subtype in the TCGA cohort

(B) Heat map of the RNA-seq data for the selected pathway in HER2 enriched subtype and non-HER2 enriched subtype in the TCGA cohort.

TCGA: The Cancer Genome Atlas; mTORC1: mechanistic target of rapamycin complex 1; TNF- α : tumor necrosis factor-alpha; EMT: epithelial mesenchymal transition; HER2-E: HER2-enriched subtype; non-HER2-E: non-HER2 enriched subtype; *** $p < 0.001$; ** $p < 0.01$.

Figure 3

Combined analysis of ERBB2 and ESR1 was a better marker predicting the HER2-enriched subtype

(A) The expression level of ERBB2 according to PAM50 intrinsic subtypes in the TCGA cohort

(B) The expression level of ESR1 according to PAM50 intrinsic subtypes in the TCGA cohort

(C) ROC curves of different markers for the discrimination of HER2-enriched subtype and non-HER2-enriched subtype in the TCGA cohort

(D) The expression level of ERBB2 according to PAM50 intrinsic subtypes in the METABRIC cohort

(E) The expression level of ESR1 according to PAM50 intrinsic subtypes in the METABRIC cohort

(F) ROC curves of different markers for the discrimination of HER2-enriched subtype and non-HER2-enriched subtype in the METABRIC cohort

TCGA: The Cancer Genome Atlas; METABRIC: Molecular Taxonomy of Breast Cancer International Consortium; AUC: area under the curve; ROC: receiver operating characteristic curve; HER2-E: HER2-enriched.

Figure 4

Multiplex immunofluorescence showed four subtypes of tumor cell in a patient with HR+/HER2+breast cancer in the CAMS cohort

IHC results: ER = 80 %, PR = 10 %, HER2 = 3+, and FISH = positive. mIF results: ER+HER2+ cells = 64.4 %, ER+HER2- cells = 5.2 %, ER-HER2+ cells = 16.4 %, and ER-HER2- cells = 14 %. **(A)** H&E image. **(B)** ER IHC image. **(C)** PR IHC image. **(D)** HER2 IHC image. **(E)** Cell nuclei IF image. **(F)** HER2 IF image. **(G)** ER IF image. **(H)** HER2 and ER mIF image (1 $\times$ ER+HER2-; 2 $\times$ ER-HER2+; 3 $\times$ ER-HER2-; 4 $\times$ ER+HER2+). H&E: hematoxylin and eosin; IHC: immunohistochemistry; IF: immunofluorescence; HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; PR: progesterone receptor.

Figure 5

Four subtypes of tumor cell reflect the intra-tumour heterogeneity in HR+/HER2+ breast cancer

(A) Distribution of the four types of tumor cell in each patient in the CAMS cohort.

(B) Proportion of patients containing 2, 3, or 4 types of tumor cell.

(C) Spearman's correlation analysis between the four types of tumor cell;

**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. NS: Not shown; HER2: human epidermal growth factor receptor 2; ER: estrogen receptor.

Figure 6

HER2-enriched like patients showed significantly reduced disease free survival compared with non-HER2-enriched like patients.