Quantitative HER2 measurement and PI3K mutation profile in matched primary and metastatic breast cancer tissues

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Abstract

Background: HER2 status of primary breast cancer (PBC) is routinely used to determine systemic treatment for metastatic breast cancer (MBC) patients. Discordance rates of HER2 status between PBC and MBC range from 5.5% to 29% based on published meta-analyses. The clinical benefit of re-assessing HER2 in MBC tissues remains controversial. In this study, we measured quantitative HER2 expression in matched PBC and MBC tissues and correlated changes of HER2 with mutations in the catalytic domain of PI3 kinase (PIK3CA).

Methods: Total HER2 protein expression (H2T) was quantified by the HERmark® assay in 41 matched PBC and MBC formalin-fixed, paraffin-embedded specimens. PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R) was determined using a validated pyrosequencing assay.

Results: MBC samples included 5 lymph node, 13 viscera, 6 brain, and 17 soft tissue lesions (N=41). 27 (66%) cases showed higher H2T in MBC than in matched PBC; and 14 (34%) cases had higher H2T in PBC than in matched MBC, indicating an overall increase of H2T in matched MBC lesions (fold change 0.25-17.57; p=0.005, paired Wilcoxon rank sum test). HER2 positive conversion (HERmark negative/equivocal in PBC, but positive in matched MBC) was found in 6 (15%) cases, while HER2 negative conversion (HERmark positive in PBC, but negative/equivocal in matched MBC) was seen in 2 (5%) cases. HER2 status was unchanged in 33 (80%) cases. PIK3CA mutations were detected in 13 (32%) of PBC and 19 (46%) of MBC samples. Among the HER2 positive conversion cases, PIK3CA mutation was identified in 50% (3/6) PBC and 67% (4/6) MBC, compared to 0% (0/2, PBC or MBC) in the HER2 negative conversion cases. Among cases with unchanged HER2 status, PIK3CA mutation was observed in 30% (10/33) PBC and 42% (14/33) MBC.

Conclusions: Quantitative HER2 assessment revealed a 20% discordance in HER2 status between matched PBC and MBC tissues, with more frequent conversion from low HER2 in PBC to high HER2 in MBC. PIK3CA mutation was observed more frequently in patients who converted from HER2 negative PBC to HER2 positive MBC. These results suggest that reassessment of biomarkers in MBC tissues may better inform the selection of therapeutic options for patients with MBC.
Accurate assessment of HER2 status is critical in determining appropriate therapy for patients with invasive breast cancer. HER2 status of primary breast cancer (PBC) is routinely used to determine systemic treatment for metastatic breast cancer (MBC) patients. Discordance rates of HER2 status between PBC and MBC range from 5.5% to 29% based on published meta-analyses (N. Housami et al, Breast Cancer Res Treat. 2011; 129:659; S. Richter, 2011 SABCS abstract PD05-05). The clinical benefit of re-assessing HER2 status in MBC tissues remains controversial. In this study, we measured quantitative HER2 protein expression in matched PBC and MBC tissues and correlated changes of HER2 expression with mutations in the catalytic domain of Phosphoinositide 3-kinase (PIK3CA).

Methods

Tissue Samples
66 pairs of matched primary-metastatic breast cancer tissues were provided by University of Modena, Modena, Italy. Tissue samples with inadequate amount of invasive tumor for either HERmark® or PI3KCA mutation testing were excluded. Cases of bone metastasis were also excluded due to uncertain impact of fixation of bone samples on HER2 testing. A total of 41 pairs of matched primary-metastatic, formalin-fixed, paraffin-embedded (FFPE) samples had valid results for both HERmark® and PI3KCA mutation testing and were included in the final analysis (Figure 2).

The HERmark® Breast Cancer Assay
Total HER2 protein expression (H2T) was quantified using the HERmark assay (Monogram Biosciences, SO, San Francisco, CA) as previously described (Huang et al. Am J Clin Pathol 2010;134:303). H2T was detected through the release of a fluorescent ligand (V) for a VeraTag reporter. Figure 1a) conjugated to a monoclonal antibody directed against the cytoplasmic domain of HER2 (Ab-15, LabVision, part of Thermo Fisher Scientific). The antibody is paired with a biotinylated second antibody directed against the Carboxinim of HER2 (Ab15, LabVision). The photosensitizer molecule (PM) liberates singlet O2 (1O2) upon illumination with red light. Signal (V) quantified by capillary electrophoresis is normalized to invasive tumor area (mm²). The continuous H2T results are categorized as HERmark negative, HERmark equivocal, and HERmark positive with predefined H2T cutoff values (Huang et al. Am J Clin Pathol 2010;134:303).

Two cases of primary breast cancer (PBC) were excluded from an individual patient.

Summary & Discussion

• Quantitative HER2 assessment by HERmark revealed a 20% discordance in HER2 status between matched PBC and MBC tissues, with more frequent conversion (66%) from low HER2 in PBC to high HER2 in matched MBC.

• A significant overall increase of H2T in matched MBC lesions (H2T change 0.25-17.57; p=0.005, paired Wilcoxon rank sum test) was observed.

• HER2 positive conversion (HER2 negative/equivocal in PBC, but positive in matched MBC) was found in 15% cases, while HER2 negative conversion (HER2 positive in PBC, but negative/equivocal in matched MBC) was seen in 5% cases. HER2 status was unchanged in 80% cases.

• PIK3CA mutations were detected in 32% of PBC and 46% of MBC samples.

• PIK3CA mutation was observed more frequently in patients who converted from HER2 negative PBC to HER2 positive MBC. Among the HER2 positive conversion cases, PIK3CA mutation was identified in 50% PBC and 67% MBC, compared to 0% (PBC or MBC) in the HER2 negative conversion cases. Among cases with unchanged HER2 status, PIK3CA mutation was observed in 30% PBC and 45% MBC.

• MBC cases with positive HER2 conversion and PIK3CA mutation (~10% in this study) may present a significant clinical challenge because of more aggressive phenotype associated with acquired HER2 overexpression and resistant to therapy due to PIK3CA mutations.

• These results suggest that re-assessment of biomarkers in MBC tissues may be informative in the selection of therapeutic options for patients with metastatic breast cancer.