Quantitative HER3 protein expression and PIK3CA mutation status in matched samples from primary and metastatic breast cancer tissues and correlation with time to recurrence

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Abstract

Background: HER3 is thought to play a prominent role in resistance to HER2-directed breast cancer therapies. Recent data suggest that HER3 levels also influence HER2-normal breast tumor biology. HER3 and PI3K signaling are linked in that in HER3 signaling activates PI3K and inhibition of PI3K activity can upregulate HER3 expression. Here, we measured quantitative HER3 protein expression levels and PIK3CA mutation status in matched tissues from the primary tumor and site of metastasis to assess correlations with time to recurrence.

Methods: 44 pairs (8 HER2+ by HERmark®) of matched tissues from the primary tumor and the site of metastasis were evaluated for HER3 protein expression using a sensitive, quantitative assay for HER3 protein expression in FFPE tissue sections (VeraTag®). Matched samples were also evaluated for quantitative HER2 expression (HERmark) and for PIK3CA mutations at exon 9 (E542K and E545K) and exon 20 (H1047R).

Results: HER3 protein expression at the metastatic site was largely independent of HER3 levels at the primary site (Spearman p=0.50) in contrast to HER2 expression (Spearman p=0.0004). HER3 expression in the primary tumor correlated with time to recurrence (TTR) (HR=2.0 per 2-fold increase in HER3; p=0.0001). Conversely, HER3 expression measured at the site of metastasis was not correlated with TTR (p=0.55). Estrogen receptor negative tumors were less likely to have PIK3CA mutations (p=0.023). In cases of primary tumors with PIK3CA mutations, no reversions to wild-type PIK3CA were observed in the metastatic sites. In metastatic tumors, mutations detected in the primary tumor as well as new mutations were observed. A gain of an exon 9 mutation at the metastatic site correlated with shorter TTR (HR=2.5; p=0.043). Excluding the 8 samples that were HER2+ by HERmark, longer TTR was observed for patients with PIK3CA mutations in the primary tumor (HR=0.47; p=0.042), which is consistent with previous reports. Interestingly, the longer TTR for those with PIK3CA mutations appeared to be dependent on quantitative HER3 protein level (interaction p=0.065).

Conclusions: HER3 protein expression in matched primary and metastatic breast cancer tissues were unrelated. This may indicate that HER3 protein is influenced by the different tumor microenvironments of the primary and metastatic sites. PIK3CA mutations were either maintained or acquired at metastatic sites. Both low HER3 protein expression and the presence of PIK3CA mutations in the primary tumor but not the metastatic tumor were associated with longer TTR. These observations suggest that HER3 protein expression may be an important prognostic factor for breast cancer progression.
Background

HER3 is thought to play a prominent role in resistance to HER2-directed breast cancer therapies. Recent data suggest that HER3 levels also influence HER2-normal breast tumor biology. HER3 and PIK3CA signaling are linked in that HER3 signaling activates PIK3 and inhibition of PIK3CA can upregulate HER3 expression. Here, we measured quantitative HER3 protein expression levels and PIK3CA mutation status in matched tissues from the primary tumor and site of metastasis to assess correlations with time to recurrence.

Methods

Quantitative HER2 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 134:303, 2010). H2T was quantified through the release of a fluorescent tag (V for “VeraTag” reporter), see Figures) conjugated to a HER2 monoclonal antibody (mAb). The antibody is paired with a biotinylated second HER2 mAb. An avidin-linked photosensitizer (H1047R) was determined using a pyrosequencing method as PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R) was determined using a proprietary HER3 mAb (“B9A11,” Monogram) and a Quantitative HER3 Assay (H1047R) was determined using a pyrosequencing method as PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R).

Quantitative HER2 protein expression (H2T) was quantified using the same dual antibody VeraTag platform as described above, but using a proprietary HER3 mAb (“B9A11,” Monogram) and a commercial HER3 mAb.

Mutations of the catalytic domain of PI3 kinase (PIK3CA) PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R) were determined using a pyrosequencing method as previously described (Cook J. 2011 ASCO, abstract #502) and performed at the Center for Molecular Biology and Pathology (Laboratory Corporation of America, Research Triangle Park, NC).

Tissue Samples

66 and 34 pairs of matched primary-metastatic breast cancer tissues were provided by Saint Barnabas Medical Center, Livingston, NJ and University of Modena, Modena, Italy, respectively. Tissue samples with inadequate amount of invasive tumor for either HERMark or PIK3CA 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 44 pairs of matched FFPE samples had valid results for both HERMark and PIK3CA mutation testing and were included in the final analysis.

Results

2 HER2 antibodies minimize background, increase specificity

Quantitative HER3 and HER2 in matched primary and metastatic sites

Spearman

p=0.0004

Spearman

p=0.0004

Below limit of detection

Below limit of detection

HER2 total protein (H2T)

HER3 total protein (H3T)

H2T primary tumor

H3T metastatic site

H2T metastatic site

H2T primary tumor

Continuous H2T in the primary tumor correlated with continuous H2T in the metastasis (p=0.043). There was a possible interaction of PIK3CA mutation with continuous H2T (p=0.006) where PIK3CA mutations in the primary tumor were correlated with TTR only in low-H3T cases (p=0.015).

• PIK3CA mutation status and H3T measured in the primary tumor correlated with TTR.
• PIK3CA mutation status correlated with ER status but not with H3T.
• H2T in the primary tumor was correlated with H2T measured at the metastatic site.
• H3T in the primary tumor was unrelated to H3T measured at the metastatic site.

TTR correlated with PIK3CA mutations only in the primary tumor

• PIK3CA mutations associated with ER-positive tumors
• A gain of exon 9 mutation correlated with shorter TTR (p=0.043).

• Continuous H3T in the primary tumor correlated with shorter TTR (p=0.0008).
• There was a possible interaction of PIK3CA mutation with continuous H2T (p=0.065) where PIK3CA mutations in the primary tumor were correlated with TTR only in low-H3T cases.

• There was a possible interaction of PIK3CA mutation with continuous H2T (p=0.065) where PIK3CA mutations in the primary tumor were correlated with TTR only in low-H3T cases.

• H3T in the primary tumor was unrelated to H3T measured at the metastatic site.

Summary

PIK3CA mutations associated with ER-positive tumors

PIK3CA mutations with ER-negative positive tumors

PIK3CA mutation status

Wild type (wt) 8

Mutant (mut) 2

Fisher’s p = 0.023

PIK3CA mutation status correlated with ER status but not with H3T.

• H2T in the primary tumor was correlated with H2T measured at the metastatic site.
• PIK3CA mutation status correlated with ER status but not with H3T.
• PIK3CA mutation status and H3T measured in the primary tumor correlated with TTR.
• The correlation of longer TTR with PIK3CA mutations was largely restricted to cases with low H3T.