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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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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 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

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 Figure) conjugated to a HER2 monoclonal antibody (mAb). The antibody is paired with a biotinylated second HER2 mAb. An avidin-linked photosensitizer molecule (*PM*) produces singlet O_2 ($^1O_2$) upon illumination with red light. Signal (*V*) quantified by capillary electrophoresis is normalized to invasive tumor area on the formalin-fixed, paraffin-embedded (FFPE) tissue section. The continuous H2T results are categorized as HERmark Negative, HERmark Equivocal or HERmark Positive using pre-defined H2T cutoff values (*Huang et al. Am J Clin Pathol 134:303, 2010).*

Quantitative HER3 Assay

Total HER3 protein expression (H3T) 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.

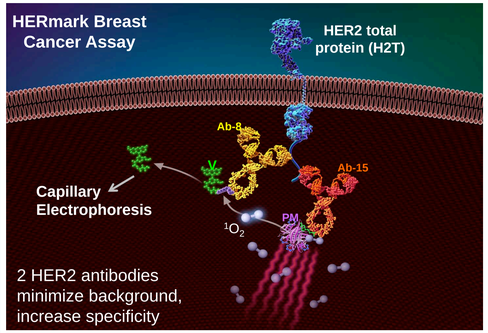
Mutations of the catalytic domain of PI3 kinase (PIK3CA)

PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R) was determined using a pyrosequencing method as previously described (*Cook J, 2011 ASCO, abstract #582*) 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 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 44 pairs of matched FFPE samples had valid results for both HERmark and PI3KCA mutation testing and were included in the final analysis.

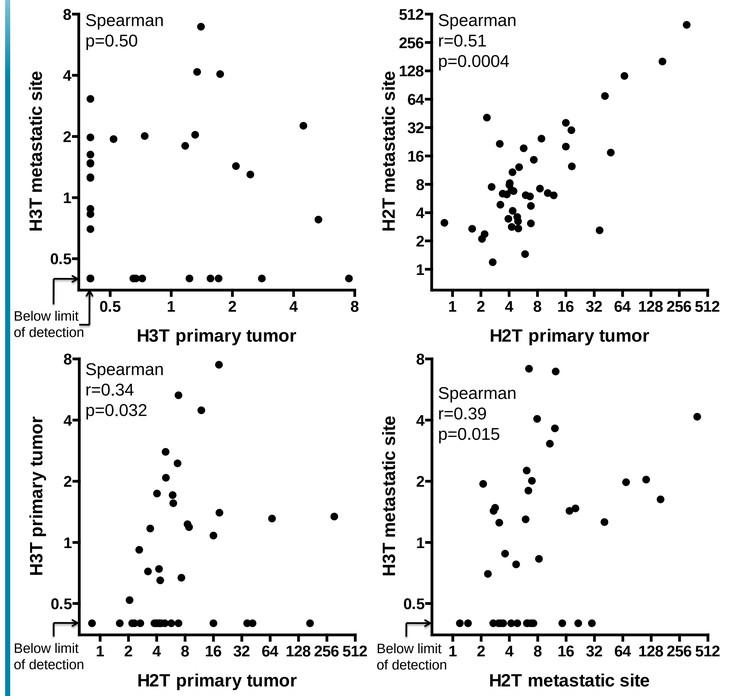
Quantitative HER2 Assay



Patient/Tissue Characteristics

Characteristic	No. (range or %)
Median age at diagnosis	53 (35–82 yr)
Median months from primary to metastasis	61 (1.3–137 mo)
HERmark HER2 status for primary tumor	
Negative (H2T < 10.5)	33 (75%)
Equivocal (10.5 ≤ H2T ≤ 17.8)	3 (6.8%)
Positive (H2T > 17.8)	8 (18%)
Tumor histological type	
Invasive ductal carcinoma	19 (70%)
Invasive lobular carcinoma	6 (22%)
Other tumor types	2 (7.4%)
N/A (data not available)	*17*
Tumor grade	
Grade 1 (well differentiated)	1 (2.8%)
Grade 2 (moderately differentiated)	14 (39%)
Grade 3 (poorly differentiated)	21 (58%)
N/A (data not available)	*8*
Estrogen receptor (ER)	
Negative	12 (29%)
Positive	29 (71%)
N/A (data not available)	3
Metastatic site	
Soft tissue	20 (45%)
Lymph node	5 (11%)
Viscera	13 (30%)
Brain	6 (14%)

Quantitative HER3 and HER2 in matched primary and metastatic sites



PIK3CA mutations independent of quantitative HER3

Correlation			p-value
primary H3T	α	PIK3CA mutation in primary	0.8
primary H3T	α	PIK3CA mutation in met	0.29
primary H3T	α	PIK3CA mutation gain from primary to met	0.11
met H3T	α	PIK3CA mutation in primary	0.5
met H3T	α	PIK3CA mutation in met	1.0
met H3T	α	PIK3CA mutation gain from primary to met	0.9

PIK3CA mutations and ER status

PIK3CA mutations associated with ER-positive tumors

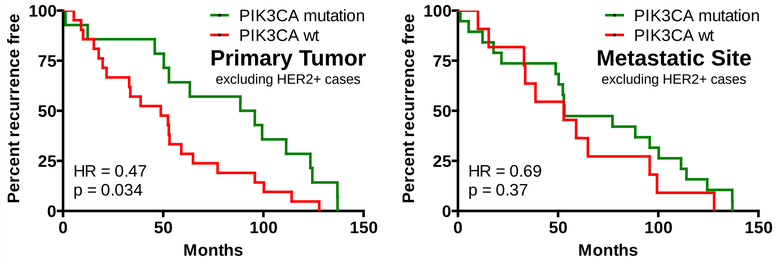
PIK3CA mutation status		ER status: negative	ER status: positive
	wild type (primary *and* met)	8	9
	mutant (primary *or* met)	2	19

Fisher p = 0.023

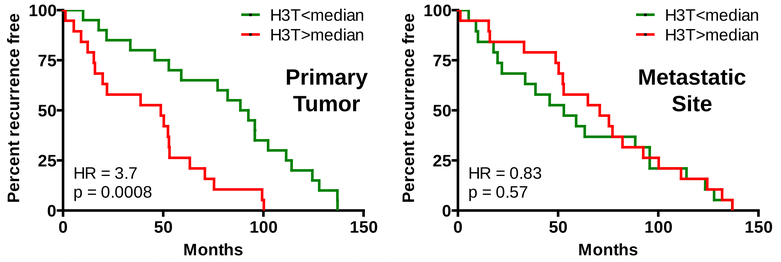
Time to Recurrence (TTR) by quantitative HER3 and PIK3CA mutation status

TTR correlated with PIK3CA mutations only in the primary tumor

- In metastases, all mutations detected in the primary tumor plus new mutations were observed.
- A gain of exon 9 mutation correlated with shorter TTR (p=0.043).



TTR correlated with quantitative HER3 only in the primary tumor



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

Continuous variable	TTR HR	TTR p-value
log_2(H3T primary)	2.0	3e-5
log_2(H3T met)	0.92	0.55

Summary

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