Proximity-Based Assays for the Detection of Activated HER3, HER2/HER3 Heterodimers and HER3/PI3K Complexes in Formalin-Fixed, Paraffin-Embedded Cell Line Controls and Tumors

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Abstract

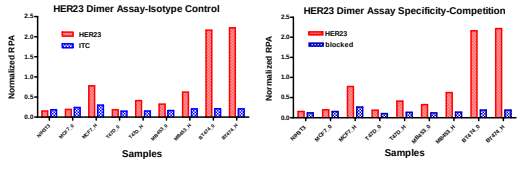
Although kinase-defective, the role of the HER3 receptor in driving tumor growth through dimerization with other HER family members is well recognized. There is increasing interest in developing therapeutic strategies to interfere with signaling through this receptor, either directly or indirectly. At the same time, there is recognition that the activation of HER3 could be an important biomarker of drug response and resistance .In particular the HER2/HER3 heterodimer has been extensively studied and, among all the possible homo and heterodimers formed by HER receptors, is considered the most mitogenic because of the ability of HER3 to effectively couple to the pro-survival PI3K/Akt pathway.

Using the VeraTag™ technology, we have developed a suite of assays designed to detect quantitatively the activation status of the HER3 receptor by measuring HER3-specific phosphorylation and formation of HER3 signaling complexes in formalin-fixed, paraffin-embedded (FFPE) cell line controls and tissue samples. . These assays are dual antibody-based and measure complexes of proteins in close association since signal is generated only when the antibodies are bound to target-specific epitope sites proximal to one another. A capillary electrophoresis instrument equipped with laser-induced fluorescence provides a highly sensitive and quantitative read-out of the assay. We report data on assays which detect a 2-5-fold change in HER2/HER3 heterodimer formation, a >10- fold change in phosphorylated HER3 signal and as high as a >5 fold change in HER3/PI3K complex formation in a series of cell line controls, spanning a wide range of HER2 and HER3 receptor levels, stimulated by the HER3 ligand neuregulin. FFPE data were verified with results from co-immunoprecipitation. The influence of the HER2 and HER3 levels on the formation of HER2/HER3 heterodimers and correlation with HER3 phosphorylation and formation of the PI3K adaptor complex will be discussed.

In addition, we have used these assays to landscape HER3 activation in a number of tissues, including breast and ovarian tumors, focusing particularly on HER2 positive breast cancer, where the HER2/HER3 heterodimer is thought to play a particularly significant role.

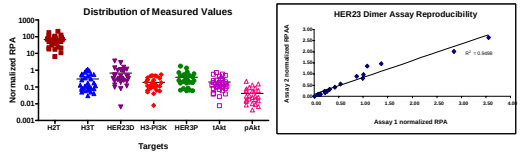
Results

Assay Specifications

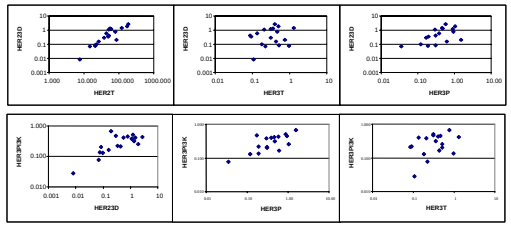


HER23 assay precision was determined by making 20 replicate measurements; sensitivity was determined by the ability to distinguish measurements for negative control from that for low positive control. Reproducibility was measured over four batches. Specificity of the assay was determined by blocking the assay signal with cold antibody and by comparing to signal generated by isotype control.

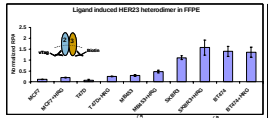
Analysis of HER2 Positive Breast Tumors

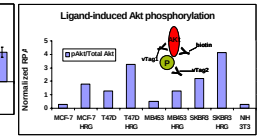


VeraTag FFPE assays performed on 18 tumors to determine correlations between different measurements of HER3 activation

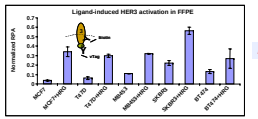


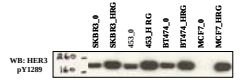
Pathway Profiling in Breast Cancer Cell Lines



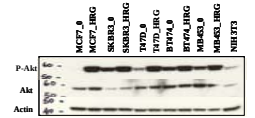


High HER2 cell lines have basal HER23 heterodimers





High HER2 cell lines have basal HER3 activation



High HER2 cell lines have basal Akt phosphorylation

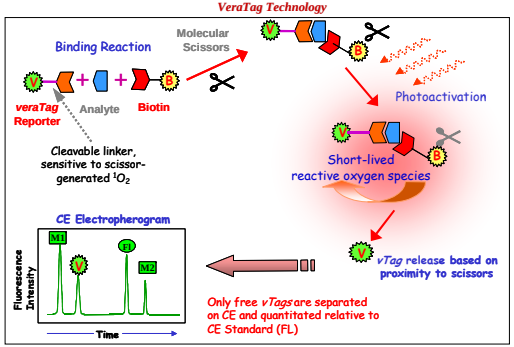
Cell Line	HER2 (Receptors/cell)	HER3 (Receptors/cell)	PIK3CA mutation*
MCF7	35,000	35,000	E545K
T47D	60,000	30,000	H1047R
MB453	300,000	35,000	H1047R
SKOV3	500,000	500	
SKBR3	1,000,000	27,000	None
BT474	1,100,000	35,000	K111N

*Stemke-Hale et al, Cancer Res., 68, 6084- 91 (2008)

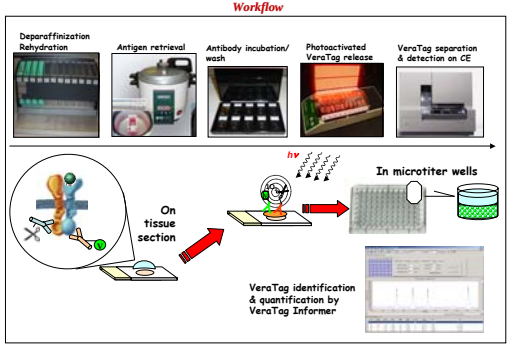
HER2-HER3 heterodimer formation, HER3-phosphorylation and Akt phosphorylation were measured by veraTag assays and the data was confirmed by Co-IP western on lysates prepared from the same cell lines

Background

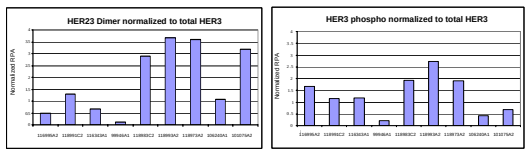
VeraTag Technology

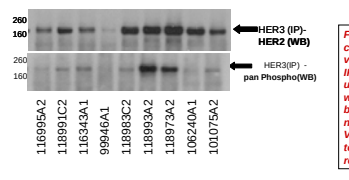


Workflow



VeraTag FFPE assay cross-validation with Co-IP western





Frozen tumors were either converted into FFPE blocks for veraTag analysis or lysed for co-IP WB. 32 Breast tumors were used in this study of which 18 were analyzed for correlation between activated targets and nine chosen for cross-validation. VeraTag analysis was repeated to determine batch-to batch reproducibility.

Summary

- We have developed novel assays to measure HER23 heterodimers, receptor phosphorylation and HER3-PI3K complex formation in FFPE samples
- We have demonstrated reproducibility, specificity and sensitivity of these veraTag assays in cell lines and they have been cross-validated using conventional assays like co-IP western on lysates
- High HER2 cell lines have high basal levels of HER23 heterodimers, HER3 phosphorylation and Akt activation the levels of which are upregulated upon neuregulin stimulation
- We have determined the levels of HER3 heterodimers and complexes in breast and other tumors enabling us to profile the activated HER pathway using veraTag assays
- We have demonstrated batch-to-to reproducibility and correlation expected from pathway activation between the different proteins/complexes measured in breast tumors
- These assays have the potential to measure clinically relevant responses (e.g., Herceptin/Lapatinib/Pertuzumab:breast cancer) in conjunction with our current HERmark assay (HER2T and HER22D)

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