Quantitative measurements reveal elevated levels of HER2-HER3 heterodimers in brain metastases compared to matched primary breast cancers

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ABSTRACT

Context: The HER2-HER3 heterodimer (H23D) is recognized as the most potent initiator of HER2 mediated cell signaling. Limited data exists on H23D levels in primary or metastatic tumor tissues, primarily due to a lack of quantitative assays. Using a novel dual-antibody, proximity-binding immunoassay platform (VeraTag®, Monogram Biosciences, South San Francisco, California), we describe the first characterization of H23D levels in primary breast tumors and matched brain metastases.

Design: H23D levels were measured in 75 formalin-fixed, paraffin-embedded (FFPE) primary breast cancers and matched brain metastases using the VeraTag H23D assay. HER2 levels were measured by conventional immunohistochemistry and the HERmark® Breast Cancer Assay (Monogram Biosciences).

Results: H23D levels spanned nearly a 2-log10 dynamic range. H23D levels were significantly higher in matched brain metastases than in primary breast tumors (Wilcoxon p=0.0065), implicating H23D signaling in brain metastasis. H23D levels correlated significantly with HER2 levels as measured by HERmark in both primary breast tumors (Spearman r=0.6724, p<0.0001) and brain metastases (Spearman r=0.5841, p<0.0001). The correlation between H23D and HER2 based on immunohistochemistry category was significant in brain metastases (Jonckheere p<0.0001), and trended toward significance in primary breast tumors (Jonckheere p=0.1744).

Conclusions: Significantly higher levels of H23D in brain metastases implicate H23D signaling in these lesions, and suggest that H23D levels may serve as a clinically useful biomarker for HER2-targeted therapies. The evaluation of H23D in additional cohorts and its clinical significance is warranted.
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I. Abstract

Context: The HER2-HER3 heterodimer (H23D) is recognized as the most potent initiator of HER2 mediated cell signaling. Using a novel dual-antibody, proximity-dimming immunoassay platform (VeraTag®, Monogram Biosciences, San Francisco, California), we describe the first H23D assay. HER2 levels were measured by conventional immunohistochemistry (FFPE) primary breast cancers and matched brain metastases using the VeraTag H23D assay. HER2 levels were measured by conventional immunohistochemistry and the HERmark® Breast Cancer Assay (Monogram Biosciences). Results: H23D levels spanned nearly a 2-log10 dynamic range. H23D levels were significantly higher in matched brain metastases than in primary breast tumors (Wilcoxon p<0.005), implicating H23D signaling in brain metastasis. H23D levels correlated significantly with HER2 levels as measured by HERmark in both primary breast tumors (Spearman r = 0.5841, p<0.0001) and brain metastases (Spearman r = 0.6724, p<0.0001). The correlation between H23D and HER2 based on immunohistochemistry was significant in brain metastases (Jonckheere test p=0.1744). Conclusions: Significantly higher levels of H23D in brain metastases implicate H23D signaling in these lesions, and suggest that H23D levels may serve as a clinically useful biomarker for HER2-targeted therapies. The evaluation of H23D in additional cohorts and its clinical significance is warranted.

II. VeraTag® Assay Workflow

Figure 1: VeraTag® Assay Workflow

III. Quantitative VeraTag Assays

Figure 2A: HER2-HER3 heterodimer (H23D) assay

Figure 2B: HER2-HER3 heterodimer (H23D) assay

IV. HER2 Classification by HERmark

Table 1. Seventy five (75) formalin-fixed, paraffin-embedded primary breast cancers and matched brain metastases were included in the study. Histologic type, histologic grade, stage, age at diagnosis, number of primary tumors, central HER2 status (positive, negative, equivocal), and number of brain metastases were determined. Table 1: Seventy five (75) formalin-fixed, paraffin-embedded primary breast cancers and matched brain metastases were included in the study. Histologic type, histologic grade, stage, age at diagnosis, number of primary tumors, central HER2 status (positive, negative, equivocal), and number of brain metastases were determined.

V. Patient characteristics

Table 2. The HER2-HER3 heterodimer (H23D) was quantified using an antibody proximity pair of VeraTag conjugated to HER3 and biotinylated HER2 monoclonal antibodies against cancer cells. Significantly higher levels of H23D in brain metastases suggest that H23D signaling may play an important role in brain metastasis of breast cancer. Clinical studies on H23D expression as a clinically useful biomarker for HER2-targeted therapies are currently going.

VI. VeraTag assays vs. HER2 IHC

Figure 3A: Quantitative VeraTag Assays. Total HER2 protein expression (H2T) was measured using the HERmark assay (Fig. 2A) as previously described (Am J Clin Pathol 134:303, 2010). H2T was quantified through the release of a fluorescent tag (VeraTag) conjugated to a HER2 monoclonal antibody (Ab-8, Thermo Fisher). The antibody was paired with a biotinylated second HER2 monoclonal antibody (Ab15, Thermo Fisher). VV reporters were cleaved by singlet O2 (1O2), produced by an avidin-linked photosensitizer molecule upon illumination with red light. The cleaved reporters were imaged using a fluorescent scanning confocal microscope equipped with a high-dimensional analysis software suite. Similarly, the HER2-HER3 heterodimer (H23D) was quantified on an antibody-proximally cleaved VeraTag conjugated to HER2 (VeraTag H23D) as previously described (Cell Cancer J. 2011;1:112-123). HER2-HER3 heterodimers were measured using an avidin-linked photosensitizer molecule upon illumination with red light. The cleaved reporters were imaged using a fluorescent scanning confocal microscope equipped with a high-dimensional analysis software suite.

VII. HER2-HER3 heterodimer vs. quantitative HER2

Figure 4: Quantitative HER2 levels (H2T) were measured in 75 formalin-fixed, paraffin-embedded primary breast cancers using the HERmark assay. HER2 levels were measured by conventional immunohistochemistry (FFPE) primary breast cancers and matched brain metastases using the VeraTag H23D assay. HER2 levels were measured by conventional immunohistochemistry and the HERmark® Breast Cancer Assay (Monogram Biosciences). Results: H23D levels spanned nearly a 2-log10 dynamic range. H23D levels were significantly higher in matched brain metastases than in primary breast tumors (Wilcoxon p<0.005), implicating H23D signaling in brain metastasis. H23D levels correlated significantly with HER2 levels as measured by HERmark in both primary breast tumors (Spearman r = 0.5841, p<0.0001) and brain metastases (Spearman r = 0.6724, p<0.0001). The correlation between H23D and HER2 based on immunohistochemistry was significant in brain metastases (Jonckheere test p=0.1744). Conclusions: Significantly higher levels of H23D in brain metastases implicate H23D signaling in these lesions, and suggest that H23D levels may serve as a clinically useful biomarker for HER2-targeted therapies. The evaluation of H23D in additional cohorts and its clinical significance is warranted.

VIII. Brain metastasis vs. matched primary

Table 3. The H23D VeraTag assay quantified HER2-HER3 heterodimer (H23D) levels in FFPE tissues of primary breast cancer and matched brain metastasis with nearly a 2-log10 dynamic range. Significantly higher levels of H23D in brain metastases suggest that H23D signaling may play an important role in brain metastasis of breast cancer. Clinical studies on H23D expression as a clinically useful biomarker for HER2-targeted therapies are currently going.

IX. Conclusions

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