Quantitative measurement of total erbB2 (H2T), p110-erbB2, and erbB2:erbB3 heterodimer (H23D) expression implicates p110-erbB2 in malignant progression from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC)

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Materials and Methods
- **Patient samples:** Using Shanghai JiaoTong University Breast Cancer Biorepositories ( mindset) A57 T1D1 A57 ER, we collected information on consecutive breast cancer patients undergoing breast surgery between January 2010 and December 2013. Only erbB2 IHC3+ and IDC and erbB2 IHC3FISH amplified samples were included. Of 117 erbB2+ DCIS and 71 erbB2+/IDC archived formalin-fixed, paraffin-embedded (FFPE) primary tumor samples were used.

- **Quantitative total erbB2 (H2T) protein expression assay:** H2T expression was measured using the HERmark® IHC2 assay (Monogram Biosciences). Tumors were stained with anti-pH2T antibodies and scanning by an avidin-biotinylated photoprotein molecule (PM) upon illumination with red light (see Figure). Fluorescence, quantified by capillary electrophoresis, was normalized to invasive tumor area on the FFPE tissue section to give units of relative fluorescence (RF) per mm². H2T measurements were compared to pre-established cutoffs for H2T low (H2T <10.5 RF/mm²), H2T intermediate (H2T 10.5-17.8 RF/mm²), and H2T high (H2T >17.8 RF/mm²), derived from the overlap of the lower and upper 5th percentiles of erbB2 positive and negative distributions, respectively, within a reference database of 1,000 breast cancer patient samples (Huang, Am J Pathol 182:2210–2215, 2013).

- **Quantitative p110-erbB2 expression assay:** p110-erbB2 protein expression was measured in FFPE samples using the p110 VeraTag® assay (Monogram Biosciences), specific for the active p110-erbB2-C domain of truncated erbB2 (Spindle Cell Res Res, 16:4226, 2010 and Sperinde, Clin Cancer Res, 16:3), measured using an antibody proximity pair of Pro11 VeraTag conjugated to a novel photosensitizer molecule (PM) upon illumination with red light. Fluorescence, quantified by capillary electrophoresis, was normalized to invasive tumor area on the FFPE tissue section to give units of relative fluorescence (RF) per mm². p110-erbB2 expression was measured using the p110 VeraTag assay.

- **Statistical analysis:** T-test and Mann-Whitney U test were used to assess the differences between the means of two groups. Chi-Square test was applied for categorized variables. Bivariate Pearson correlation test was applied to investigate the relation between two categorized variables.

Results

- **Characteristics of 67 erbB2 IHC 3+ DCIS patients**

- **Characteristics of 71 erbB2 IHC 3+ IDC patients**

- **Expression frequency and level of total erbB2, p110-erbB2 and erbB2:erbB3 heterodimers in all patients.**

- **Expression frequency and level of total erbB2, p110-erbB2 and erbB2:erbB3 heterodimers in high H2T patients.**

Conclusion

This study is the first quantitative assessment to reveal total erbB2 expression, truncated p110-erbB2 expression as well as erbB2:erbB3 heterodimerization level in both DCIS and IDC breast human tissue via a new measuring approach. Our findings supported a unique pathophysiological mechanism that presence of truncated erbB2 species could drive progression of erbB2-overexpressed DCIS to invade erbB2-positive disease.