Common protein biomarkers assessed by reverse phase protein arrays show considerable intratumoral heterogeneity in breast cancer tissues.

Proteins are used as prognostic and predictive biomarkers in breast cancer. However, the variability of protein expression within the same tumor is not well studied. The aim of this study was to assess intratumoral heterogeneity in protein expression levels by reverse-phase-protein-arrays (RPPA) (i) within primary breast cancers and (ii) between axillary lymph node metastases from the same patient. Protein was extracted from 106 paraffin-embedded samples from 15 large (\( \geq 3 \text{ cm} \)) primary invasive breast cancers, including different zones within the primary tumor (peripheral, intermediate, central) as well as 2-5 axillary lymph node metastases in 8 cases. Expression of 35 proteins including 15 phosphorylated proteins representing the HER2, EGFR, and uPA/PAI-1 signaling pathways was assessed using reverse-phase-protein-arrays. All 35 proteins showed considerable intratumoral heterogeneity within primary breast cancers with a mean coefficient of variation (CV) of 31% (range 22-43%). There were no significant differences between phosphorylated (CV 32%) and non-phosphorylated proteins (CV 31%) and in the extent of intratumoral heterogeneity within a defined tumor zone (CV 28%, range 18-38%) or between different tumor zones (CV 24%, range 17-38%). Lymph node metastases from the same patient showed a similar heterogeneity in
protein expression (CV 27%, range 18-34%). In comparison, the variation amongst different patients was higher in primary tumors (CV 51%, range 29-98%) and lymph node metastases (CV 65%, range 40-146%). Several proteins showed significant differential expression between different tumor stages, grades, histological subtypes and hormone receptor status. Commonly used protein biomarkers of breast cancer, including proteins from HER2, uPA/PAI-1 and EGFR signaling pathways showed higher than previously reported intratumoral heterogeneity of expression levels both within primary breast cancers and between lymph node metastases from the same patient. Assessment of proteins as diagnostic or prognostic markers may require tumor sampling in several distinct locations to avoid sampling bias.