Critical roles of specimen type and temperature before and during fixation in the detection of phosphoproteins in breast cancer tissues.

The most efficient approach for therapy selection to inhibit the deregulated kinases in cancer tissues is to measure their phosphorylation status prior to the treatment. The aim of our study was to evaluate the influence of pre-analytical parameters (cold ischemia time, temperature before and during tissue fixation, and sample type) on the levels of proteins and phosphoproteins in breast cancer tissues, focusing on the PI3 kinase/AKT pathway. The BALB-neuT mouse breast cancer model expressing HER2 and pAKT proteins and human biopsy and resection specimens were analyzed. By using quantitative reverse phase protein arrays (RPPA), 9 proteins and 16 phosphoproteins relevant to breast cancer biology were assessed. Cold temperatures before and during fixation resulted in a marked improvement in the preservation of the reactivity of biological markers (eg, ER, HER2) in general and, specifically, pHER2 and pAKT. Some phosphoproteins, eg, pHER2 and pAKT, were more sensitive to prolonged cold ischemia times than others (eg, pS6RP and pSTAT5). By comparing the phosphoprotein levels in core needle biopsies with those in resection specimens, we found a marked decrease in many phosphoproteins in the latter. Cold conditions can improve the
preservation of proteins and phosphoproteins in breast cancer tissues. Biopsies≤1 mm in size are
the preferred sample type for assessing the activity of deregulated kinases for personalized cancer
treatments because the phosphoprotein levels are better preserved compared with resection
specimens. Each potential new (phospho)protein biomarker should be tested for its sensitivity to
pre-analytical processing prior to the development of a diagnostic assay.