Effects of cold ischemia and inflammatory tumor microenvironment on detection of PI3K/AKT and MAPK pathway activation patterns in clinical cancer samples.

The accuracy of common markers for PI3K/AKT and MAPK pathway activation in preclinical and clinical cancer biomarker studies depends on phosphoepitope stability and changes of phosphorylation under ischemia. Herein, we define conditions under which phosphoepitope-specific duplex immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tumor tissues reflects pathway activation in situ as accurately as possible, and identify activation patterns linked to mutational status, pathway dependency and tumor microenvironment in clinical tumor samples, cell culture and xenograft tissues. Systematically assessing robustness of pAKT, pERK1/2, pMEK1/2 and pmTOR detection and related markers in xenograft tissues exposed to ischemia, we show that control of preprocessing and ischemia times allows accurate interpretation of staining results. Phosphorylation patterns were then analyzed in 33 xenograft models and in 58 cases with breast cancer, including 21 paired samples of core-needle biopsies with corresponding mastectomy specimens, and 37 mastectomy samples obtained under rigorously controlled conditions minimizing ischemia time. Patterns of pAKT and pERK1/2 staining (predominant PI3K/AKT, predominant MAPK and concomitant activation) were associated with sensitivity to pathway
inhibition and partially with the mutational status in cell lines and corresponding xenograft tumors. In contrast, no clear correlation between mutational status and staining patterns was observed in clinical breast cancer samples, suggesting that interaction with the human tumor microenvironment may interfere with the use of phosphoepitope-specific IHC as potential markers for pathway dependency. In contrast to core needle biopsies, surgically resected breast cancer samples showed evidence of severe signal changes comparable to those effects observed in xenograft tumors exposed to controlled ischemia.