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Comparison of Immunohistochemical and Western Blot Analyses of Paraffin-embedded Cells and Xenografts for Profiling Cellular Signaling.

Cell Signaling TECHNOLOGY

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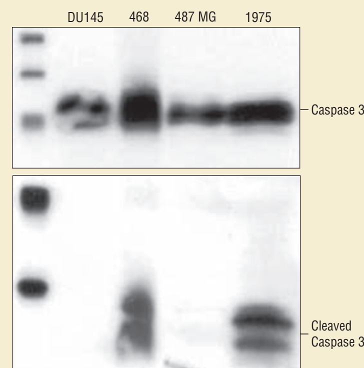
Introduction

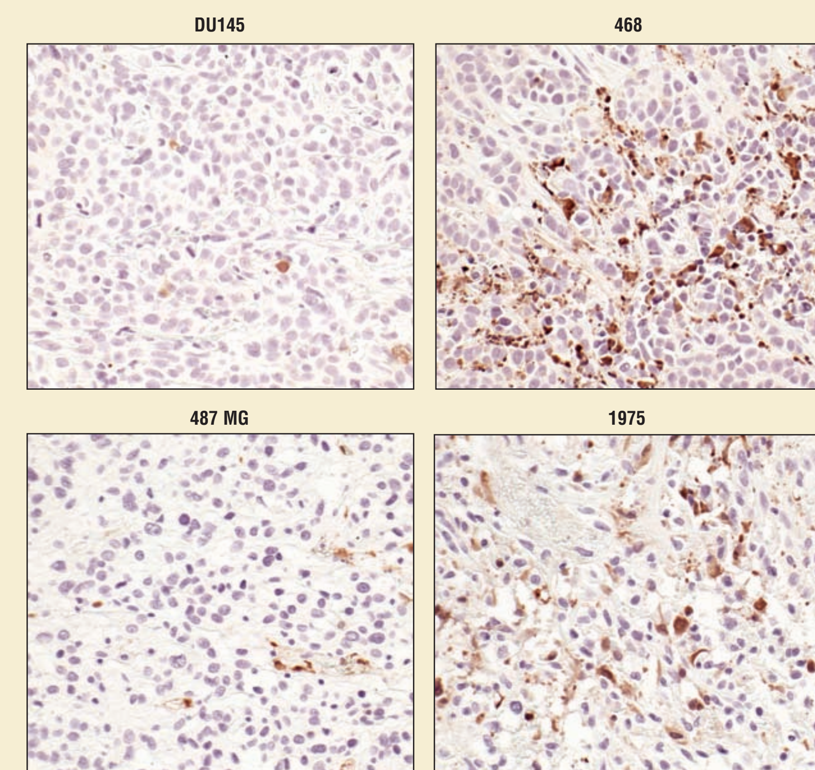
Current development of targeted cancer therapeutics relies on the use of model cell lines and mouse xenografts. These samples are often analyzed by immunohistochemistry and Western blot to profile the activity of cell signaling proteins; however, the relative sensitivity and specificity of these methods for the profiling of cellular signaling has not been established. In this study we compared the results obtained by IHC with those obtained by Western blot analysis using a broad panel of total and phospho-specific antibodies on paraffin-embedded cell lines and xenografts or lysates from the same samples. The results demonstrate that the methods each have limitations in sensitivity but do allow for consistent characterization of protein phosphorylation and pathway activation. In addition, the results suggest the most robust methods for tissue handling for IHC. Staining was observed with phospho-specific erk and Akt antibodies with varied fixatives, times of fixation and time before fixation.

Methods

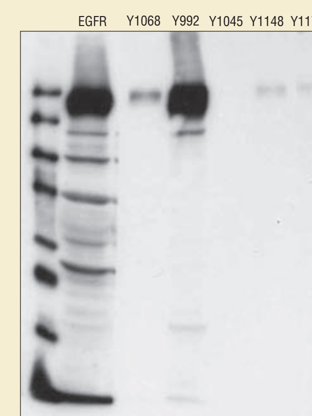
Western blot and Immunohistochemistry were performed according to standard CST protocols, with primary antibody incubations overnight at 4°. Xenografts were initiated using 5-10x106 cells in 50% Matrigel™ and fixed for 24 or 48 hours in 10% NBF or Streck Tissue Fixative (STF). Cell pellets were fixed in NBF, then processed and embedded per standard procedures. All antibodies were from CST.



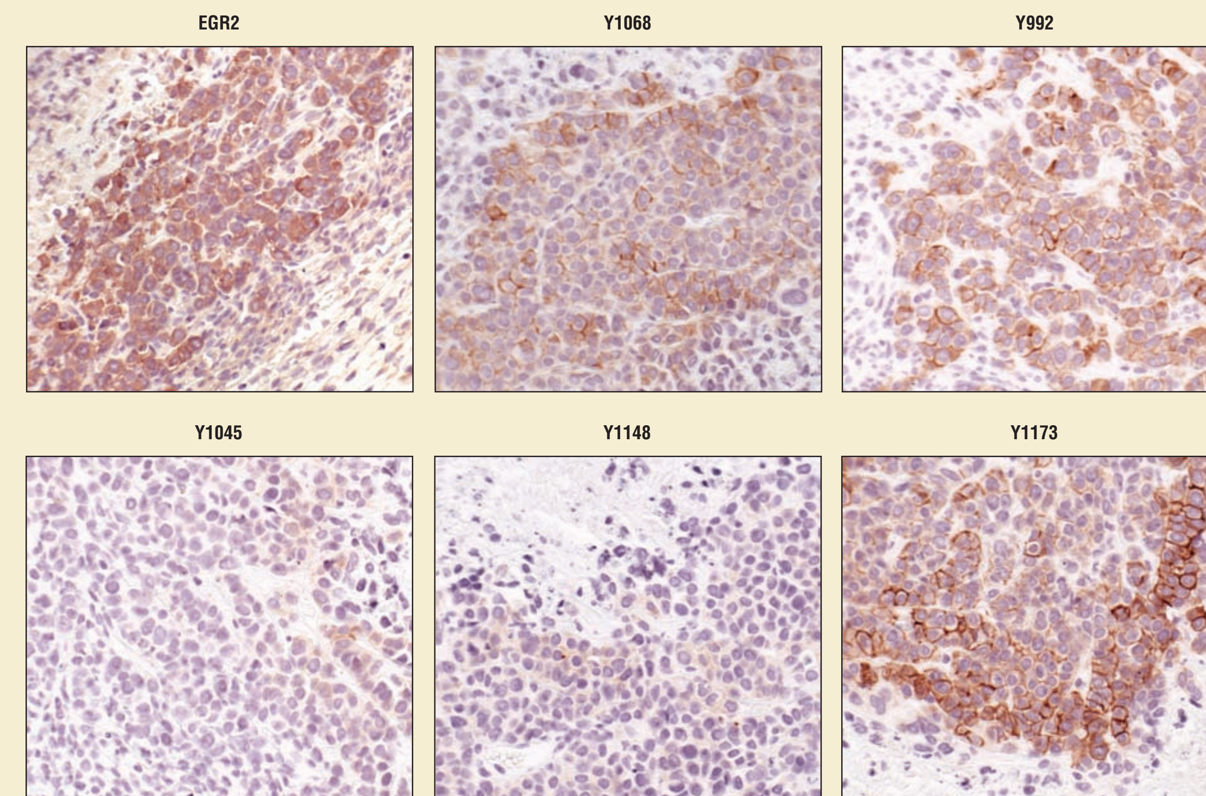
Western blot analysis of various xenograft lysates (60ug), using Caspase-3 or Cleaved Caspase-3 antibodies.



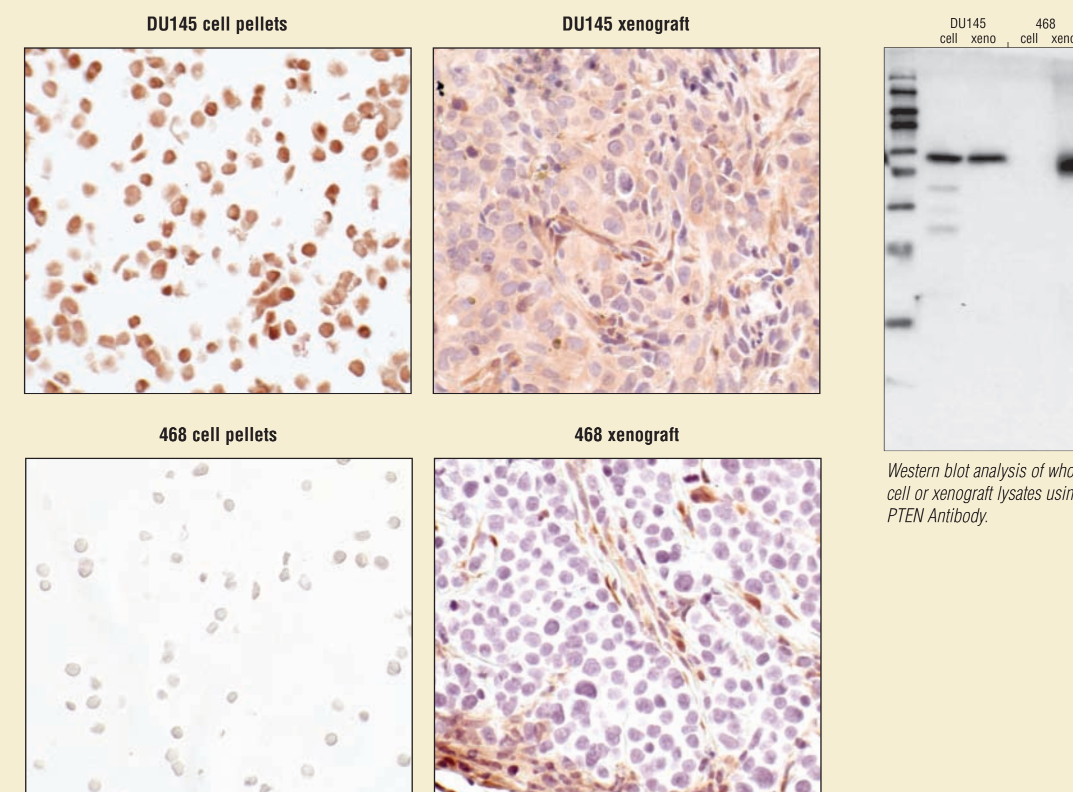
IHC analysis of the corresponding xenografts, using Cleaved Capspase-3 Antibody.



Western blot analysis of MDA-MB-468 xenograft lysate (100ug), using EGFR and p-EGFR antibodies, as indicated.



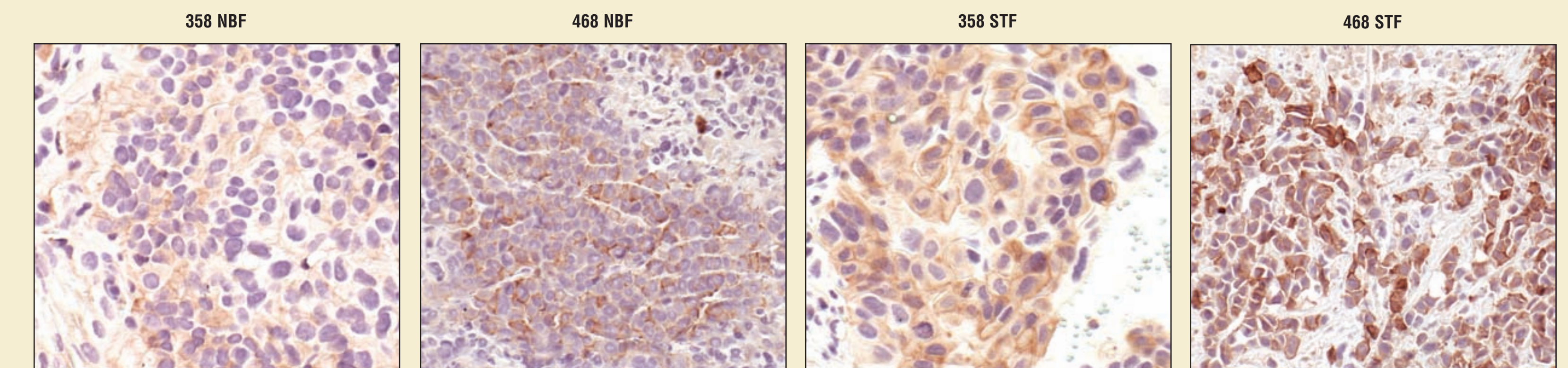
Corresponding IHC analysis of the same xenograft using EGFR and p-EGFR antibodies, as indicated.



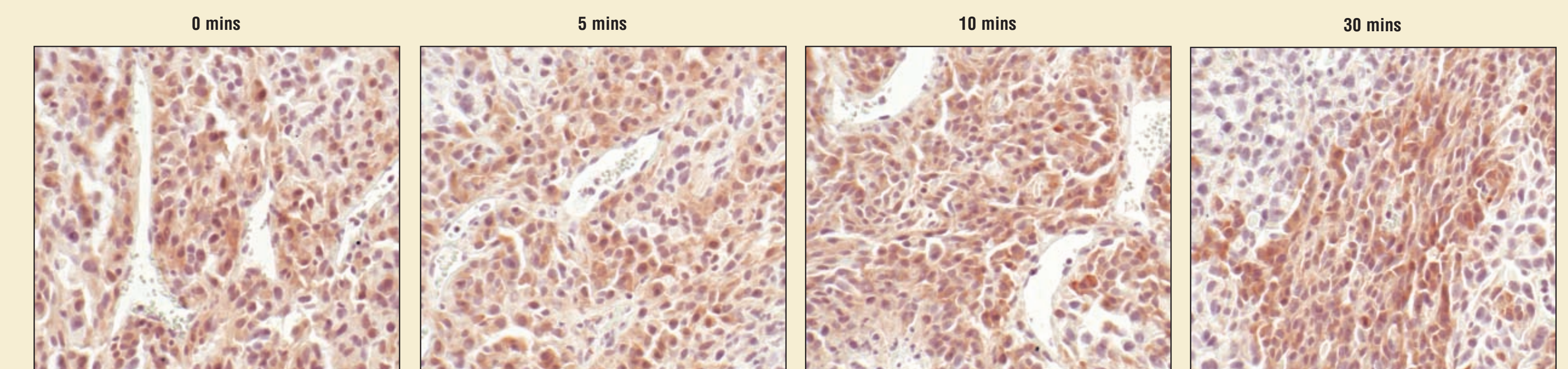
IHC on the corresponding cell pellets or xenografts using the same antibody.

DU145 cell xeno 468 cell xeno

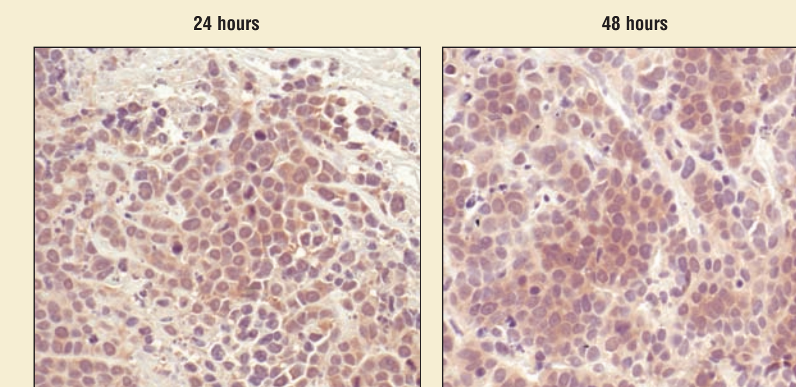
Western blot analysis of whole cell or xenograft lysates using PTEN Antibody.



IHC of xenografts fixed with NBF or Streck Tissue Fixative (STF), using Phospho-Tyrosine Antibody (P-Tyr-100).



IHC of U-87 MG xenografts using p-Akt (Ser473) held in PBS for indicated time (minutes) prior to fixation in STF.



IHC of MDA-MB-468 xenografts fixed in NBF for 24 or 48 hours, using p-Akt (Thr308) Antibody.

Summary

- Western blot and IHC analyses can correlate and enable for pathway profiling in cell and tissue samples.
- Streck Tissue Fixative may be superior to NBF for preserving phospho-tyrosine epitopes.
- In our experiments neither the time of fixation nor the time before fixation negatively affected IHC results with two Popsho-Akt antibodies.

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