PamChip® arrays to support mechanistic studies to understand why PTEN inactivation leads to resistance to EGFR kinase inhibitors.

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Study Design

PTEN-deficient cancers show increased EGFR activation as tested in an EGFR Western (figure 1). The authors turned towards PamChip® arrays to profile activity of EGFR in more detail. EGFR kinase activity in cell lysates of three different PTEN knockdown lines (A431, HCC4006, and NHA-EGFR) was studied using lysates from the PTEN knowdown lines and their matching parental cell lines. The results shown here are part of the studies performed by Vivanco et al.

Key Findings

PTEN inactivation by shRNA knockdown shows enhanced and significant phosphorylation in 8/10 EGFR-regulated peptides as shown on PamChip® arrays. These peptides are marked with asterisk ($P < 0.05$) and are found in all tested EGFR expressing cell lines (figure 2). The other two peptides (DDR2_733_745_Y740 and VEGFR2_944_956_Y951) showed increased phosphorylation in 2/3 PTEN knockdown lines. None of the other detectable peptides showed a similarly consistent change in phosphorylation rates in response to PTEN knockdown ($P = 0.0173$).
These studies have confirmed that PTEN loss directly affects EGFR activity.

"Author Quote"

Our study identifies a critical role for PTEN in EGFR signal termination and suggests that more potent EGFR inhibition should overcome resistance caused by PI3K pathway activation.

Background

The PTEN tumor suppressor gene is frequently mutated and associated to many cancers. Loss of PTEN leads to resistance to kinase inhibitors. How loss of the tumor suppressor gene PTEN leads to resistance to kinase inhibitors targeting EGFR is currently unkown.

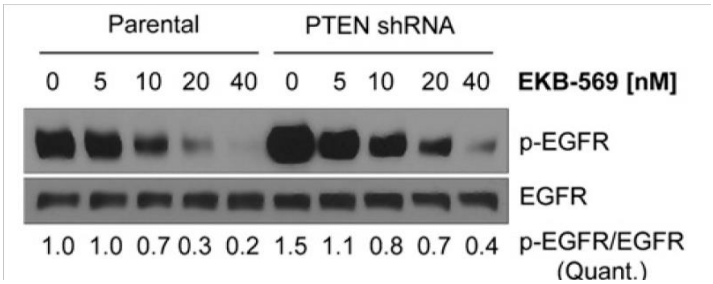

Figure 1: PTEN inactivation raises EGFR phosphorylation in A431 cells and enhances residual EGFR phosphorylation during treatment with EKB-569. Cells were treated with the indicated concentrations of EKB-569 (EGFR inhibitor, Wyeth) for 6 h and whole cell lysates immunoblotted with the indicated antibodies.

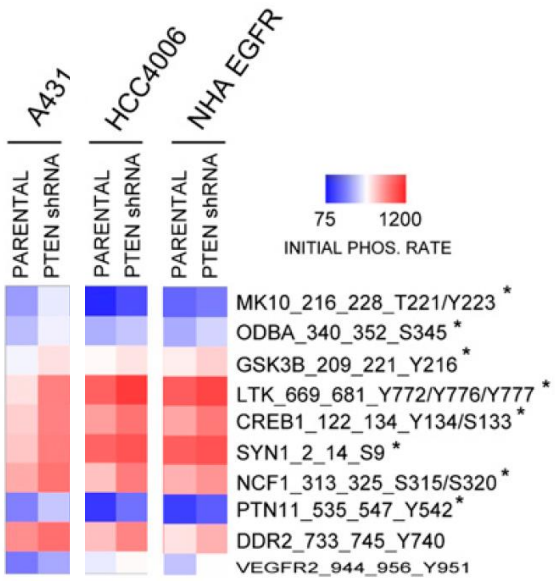

Figure 2: PTEN inactivation enhances phosphorylation of 10 peptides as tested on PamChip® arrays. These 10 peptides are associated with EGFR (Vivanco et al 2010). Whole cell lysates from three different cell lines and their matching PTEN knockdown cell lines were incubated on PamChip® arrays. Peptides that are significantly ($P < 0.05$) up-regulated in all three EGFR overexpressing cell line pairs (A431, HCC4006, and NHA-EGFR) are marked with an asterisk. The heat map shows initial phosphorylation velocities for each peptide.

References:

Vivanco I. et al. PNAS 2010 Apr 6;107(14):6459-64.

Conclusion

PamChip® arrays have confimed functional hypothesis of the involvement of PTEN loss in EGFR activation. This serves as an example of how arrays can be used in understanding the biology of cancer.

Application note: #201004 (9-2010)

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