

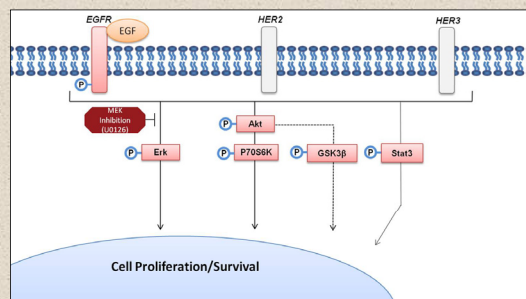
Functional Stratification of Breast Carcinoma Cells Enables Predictive Therapeutic Strategies (#3144)

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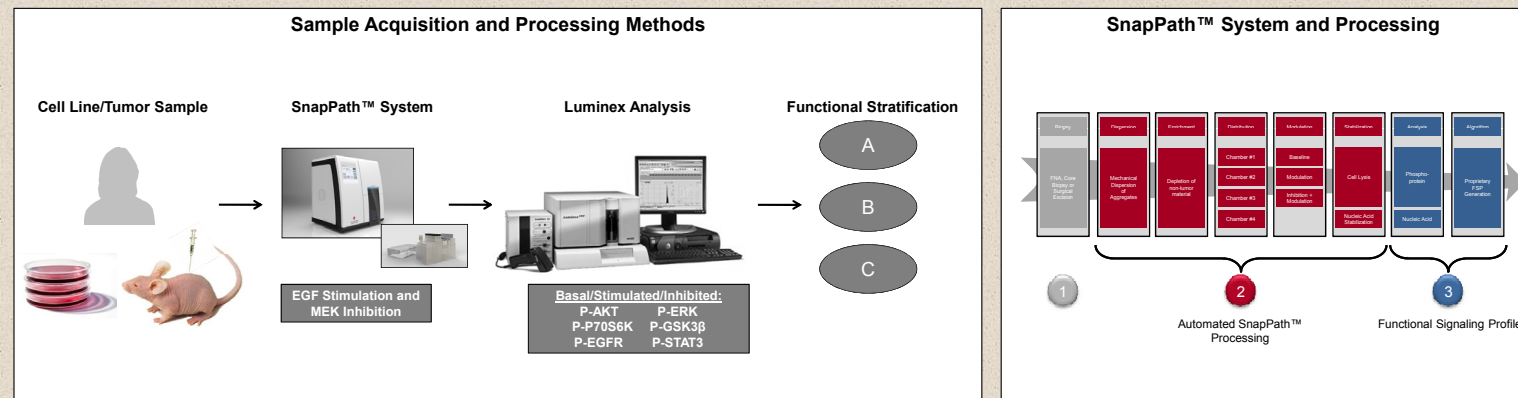
Abstract

Most targeted therapies still lack effective predictive biomarkers. A major limitation of the existing classes of biomarkers is the lack of functional information about the signal transduction networks targeted by molecularly targeted drugs. We have developed a functional assay based on ex vivo biomarkers produced by live tumor cells. The profile is elicited by short-term epidermal growth factor (EGF) stimulation in the presence or absence of a MEK inhibitor. The resultant changes in signal transduction phosphoprotein levels are used to create functional signaling profiles that stratify tumor cell lines into functional groups. This functional signaling profile is feasible by an automated platform that is amenable to tumor biopsy processing. **Methods:** Breast Cancer Cell lines (BT-474, MDA-MD-231, SKBR3, HCC-1937, BT-20, T47D, MCF-7, BT-549) were propagated and removed from the plate by gentle scraping to simulate a FNA biopsy sample. Following removal, the cells were placed on the SnapPath™ live-tumor-cell processing platform (BioMarker Strategies, LLC) to evoke ex vivo biomarkers. SnapPath™ disperses the sample, enriches for tumor cells, aliquots into test wells, and applies ex vivo stimulation by EGF (200ng/ml) in the presence or absence of the MEK inhibitor, U0126 (10uM). Cell lysates were then analyzed using the BioPlex platform for the following phosphoproteins: p-Erk 1/2, p-Akt, p-EGFR, p-Stat3 (BioRad). Functional profiles were generated for each cell line based on the levels of phosphoproteins. **Results:** Functional signaling profiles of breast cancer cell lines stimulated with EGF in the presence of U0126 revealed distinct functional groups that enabled the stratification. Two functional groups were identified based on AKT phosphorylation levels: one group displayed variable, but low levels of p-AKT inhibition, whereas another group showed unanticipated up-regulation of p-AKT. This second group may be resistant to MEK inhibition but sensitive to the combination of MEK/AKT inhibition. Two other functional groups were identified based on EGFR phosphorylation levels: one group displayed variable, but low p-EGFR inhibition, whereas the other group showed unanticipated up-regulation of p-EGFR. This second group may be resistant to MEK inhibition, but sensitive to combined MEK/EGFR inhibition. **Conclusion:** Functional signaling profiles of human cancers reveal unique details of signal transduction networks that permit stratification of tumors unavailable through traditional biomarkers. These profiles may correlate with targeted drug sensitivity or resistance and may yield successful companion diagnostics, including combination therapies of targeted agents. Such functional profiles can be reproducibly elicited from small numbers of tumor cells on an automated platform, suggesting that this approach to predictive tests is possible for human tumor biopsy samples.

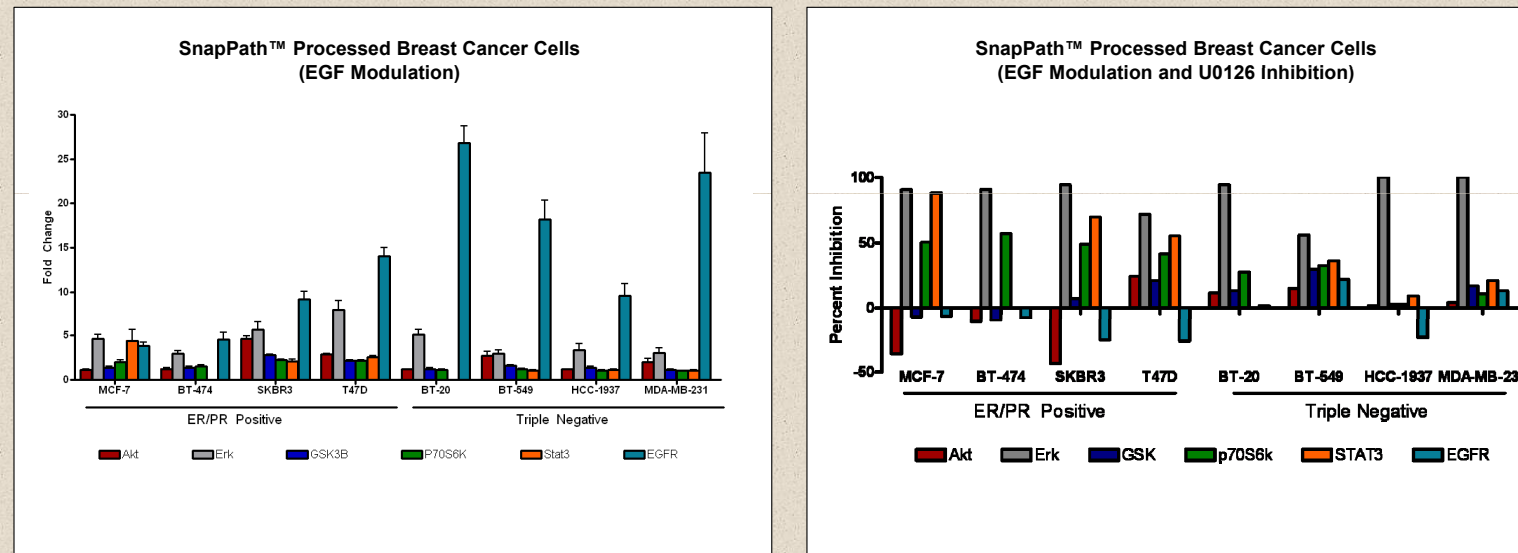
Background



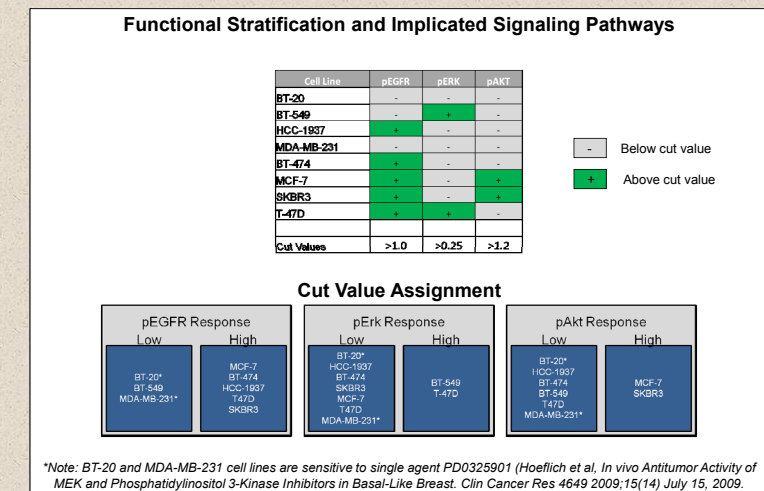
Methods



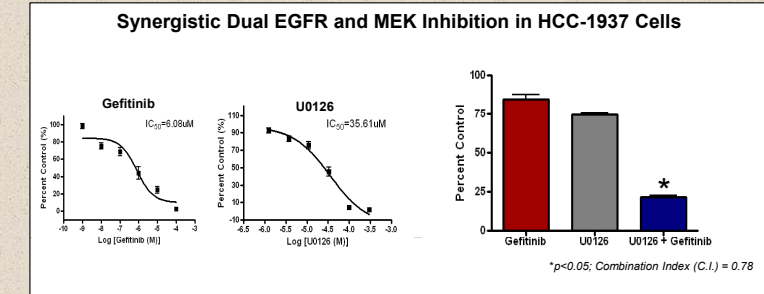
Results



Results



*Note: BT-20 and MDA-MB-231 cell lines are sensitive to single agent PD0325901 (Hoeflich et al. In vivo Antitumor Activity of MEK and Phosphatidylinositol 3-Kinase Inhibitors in Basal-Like Breast. Clin Cancer Res 4649 2009;15(14) July 15, 2009.



Conclusions

- Functional signaling profiles (FSPs) elicited from ex vivo biomarkers enable unique stratification of tumors.
- FSPs may correlate with targeted drug sensitivity or resistance, providing a basis for companion diagnostics and combination therapies.
- FSPs can be reproducibly elicited from small numbers of tumor cells on an automated platform, suggesting that this approach to predictive tests is possible for human tumor biopsy samples.