

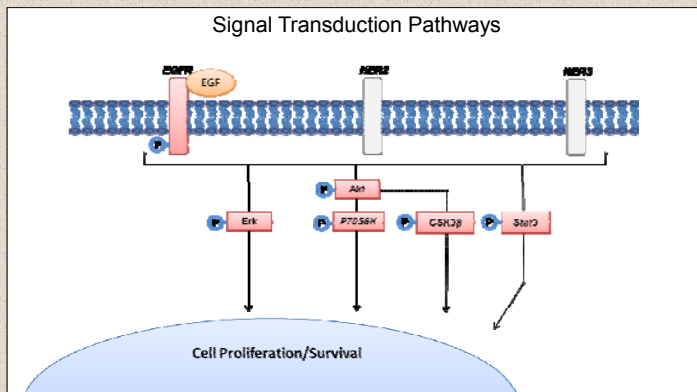
Stratification of breast cancers based on functional phosphoprotein signaling profiles elicited from live tumor cells

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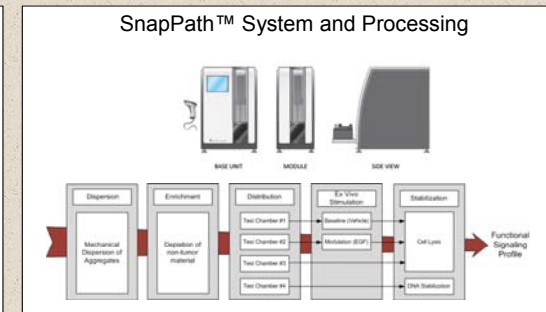
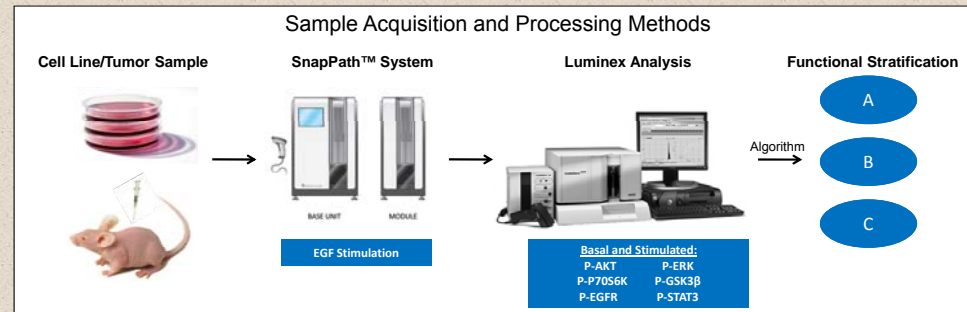
Abstract

Background: Abnormal signal transduction networks are frequent targets of existing and emerging molecularly targeted agents (MTAs). Unfortunately, most predictive biomarkers to guide therapeutic selection are based on indirect assessment of signal transduction through DNA mutations or transcriptional profiles rather than dynamic assessment of signal transduction proteins themselves. Classification of breast cancer based on functional signaling profiles derived from a set of signaling phosphoproteins induced upon growth factor stimulation of live breast cancer cells is likely to provide a more accurate system for MTA selection than indirect methods utilizing fixed or frozen tissue. **Objective:** This study aimed to demonstrate stratification of multiple breast cancer model systems based on functional signaling profiles elicited from live tumor cells in response to ex vivo stimuli. **Methods:** Breast cancer cell lines (MCF-7, HCC-1937, MDA-MB-231, BT474, and SKBR3) were exposed to either vehicle (control) or stimulated with 200 ng/ml epidermal growth factor (EGF) for 5 minutes then lysed and proteins extracted. Mean Fluorescence Intensity (MFI) levels of six phosphoproteins (pEGFR, pErk, pAkt, pP70S6K, pGSK3b, and pSTAT3) were determined in sextuplet using a multiplexed bead-immunoassay (BioPlex, BioRad) and a modulation score (MS), defined as the log₂ (MFI stimulated / MFI control), calculated for each. Scores were ranked by percentile relative to the median (0.66) and inter-quartile range (IQR) (1.54). Moderate responders were classified as those with MS between the 75th percentile (2.20) and the 75th percentile plus the IQR (3.74). High responders were those MS > 3.74. Low responders were those MS falling between the IQR and 75th percentile (1.54-2.20) whereas non responders were classified as MS < 1.54 **Results:** EGF stimulation resulted in high levels of EGFR-phosphorylation in all cells except BT474, which responded moderately (2.57). MS for pErk were high in MCF-7 cells (3.92), moderate in HCC-1937 (2.89) and none for the other lines tested. Moderate STAT-3 phosphorylation was observed in only MCF-7 cells (2.34) whereas low pAkt MS were observed in only SKBR3 (1.78). All other markers across the five cell lines tested were non responders (< 1.54), with pGSK3b and pP70S6K yielding MS < 1.0 for all five cell lines. Interestingly, the relative MS rank order of all six proteins differed across each cell line suggesting further opportunity for stratification. **Conclusions:** Our data demonstrate that different breast cancer cell lines display unique functional phosphoprotein signaling profiles, thereby providing a mechanism for stratifying tumors based on individual signal transduction pathway activation. Further studies are underway to correlate the functional signaling profiles identified here to sensitivity and resistance to specific MTA treatment.

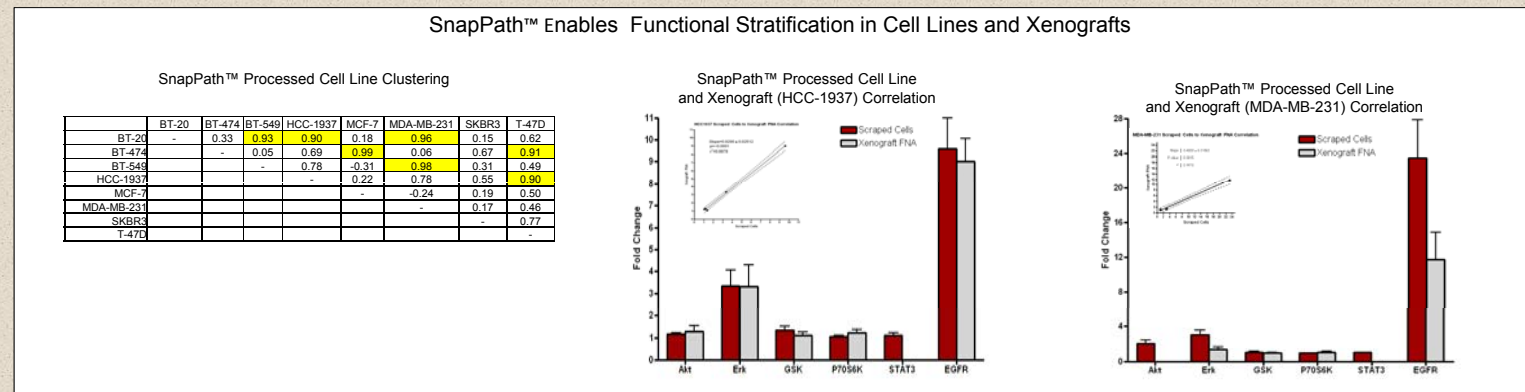
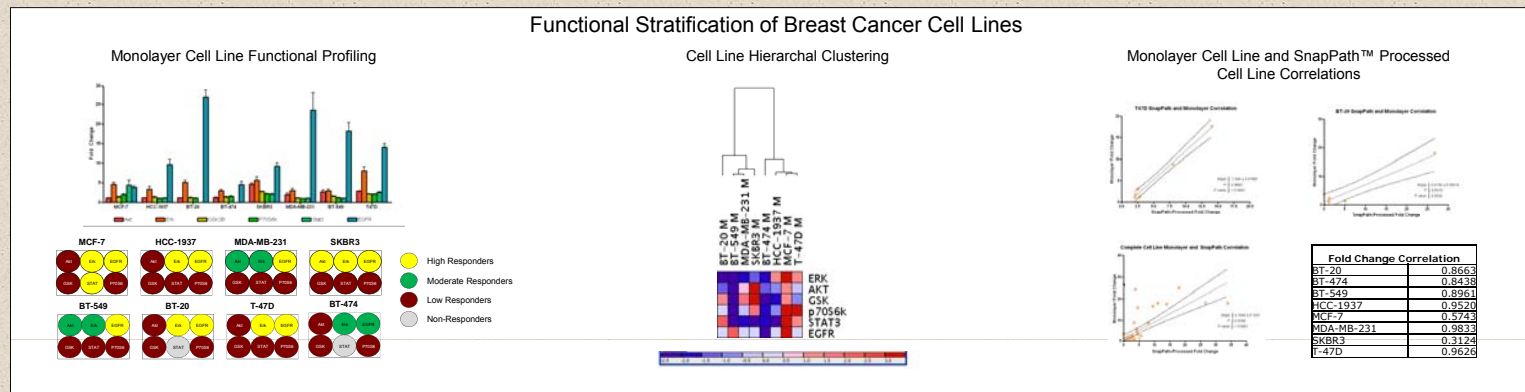
Background



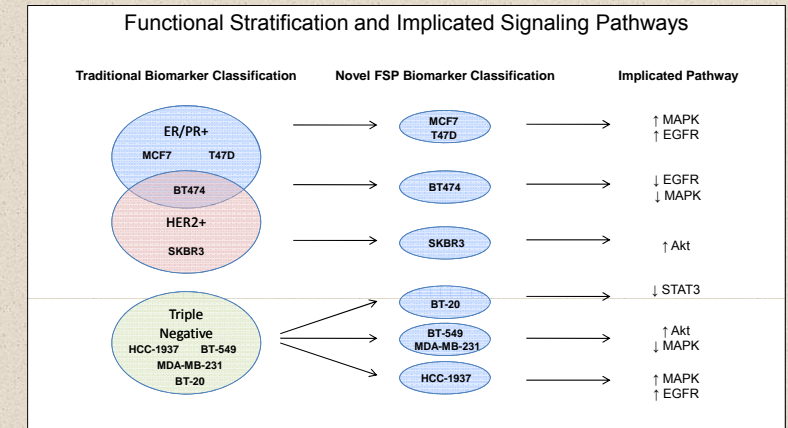
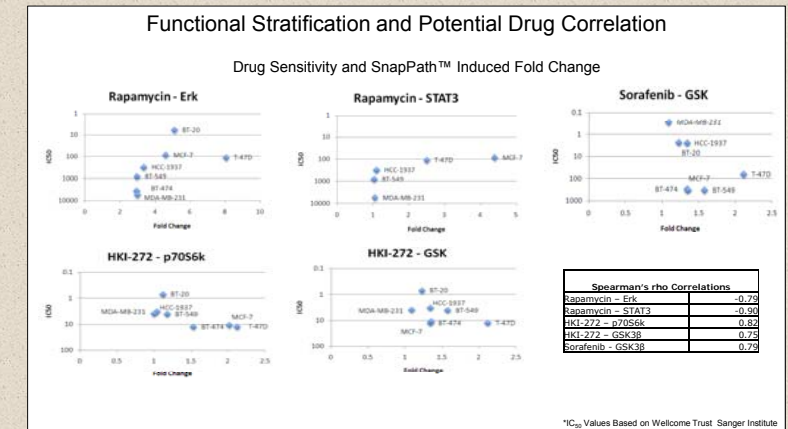
Methods



Results



Applications



Conclusions

- Different breast cancer cell lines display unique functional phosphoprotein signaling profiles, thereby providing a mechanism for stratifying tumors based on individual signal transduction pathway activation
- The SnapPath™ system is an automated platform capable of evoking function signaling profiles from cell lines and xenograft tumors
- Functional signaling profiles elicited from the SnapPath™ system can be correlated to drug sensitivity and resistance data providing the foundation for a predictive diagnostic platform