Tumor-fibroblast interactions that mediate HER2-therapy resistance in breast cancer: integrating proteomics, microfluidics and cyclic immunofluorescence

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Introduction: HER2 overexpressing (HER2+) breast cancer accounts for 15-20% of all breast cancer cases, and although HER2-targeted therapies offer improved patient outcomes, advanced disease is rarely cured. Although several studies have demonstrated the role of tumor cell growth factor pathways in HER2-therapy resistance, the roles of extrinsic signals stemming from the tumor microenvironment remain poorly understood. Here, we investigate the role of fibroblasts in HER2-therapy resistance by utilizing high-throughput screening, cyclic immunofluorescence, proteomic measurements and *in vivo* xenograft models.

Materials and Methods: We measured the dose-response of seven HER2+ breast cancer cell lines to the FDA-approved HER2-targeted agent lapatinib under monoculture and direct co-culture with breast fibroblasts (Fig 1A). We also performed viability assays using fibroblast conditioned medium and paracrine assays. To identify signaling pathways that are induced by fibroblasts in tumor cells we perform reverse phase protein arrays (Figure 1B) and *in situ* cyclic immunofluorescence under direct co-culture conditions. A high-throughput direct co-culture screen was also performed using a library of 144 selective inhibitors to identify novel combination therapies that restore drug sensitivity. The *in vivo* relevance of the fibroblast-induced drug resistance was investigated using tumor xenografts formed using mammary fat pad and intraductal injections to control for the extend of stroma infiltration.

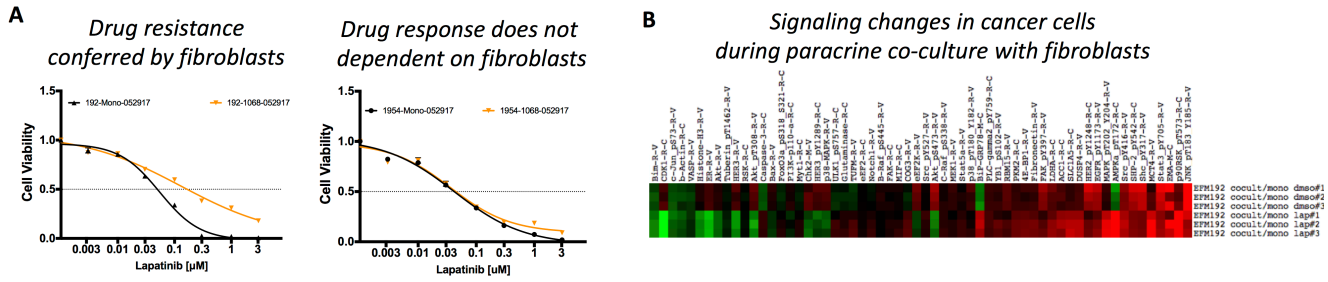

Figure 1. (A) Lapatinib dose response in a cell line (EFM192 - left) protected from lapatinib-induced growth inhibition and a cell line (HCC1954 – right) that does not dependent on fibroblasts (B) Fibroblast-induced protein expression alterations (red: upregulated, green: downregulated) in EFM192 cells under control and lapatinib treatment conditions.

Results and Discussion: *In vitro* drug sensitivity analysis to lapatinib treatment showed that direct co-culture with fibroblasts induced lapatinib resistance in 4/7 HER2+ breast cancer cell lines. Exposure to fibroblast conditioned medium in a subset of the HER2+ breast cancer cell lines could phenocopy the effects of direct fibroblast co-culture suggesting that paracrine factors are sufficient to induce HER2-therapy resistance.

To gain insight into the underlying response mechanisms we examined fibroblast-induced alterations in protein expression in the cell line (EFM192) that exhibited the strongest fibroblast-mediated protection from lapatinib. Factors secreted by fibroblasts upregulated pro-survival signaling in EFM192 cells noted by high RAS/MAPK activity, reduction in pro-apoptotic proteins (BIM and BAX) and increased anti-apoptotic protein activity (MCL-1). *In situ* immunofluorescence analysis confirmed that pro-survival signaling was also upregulated in direct co-culture with fibroblasts. Using a drug combination screening approach we identified that SRC inhibition reduced tumor cell viability under coculture with fibroblast and did not affect fibroblast viability or tumor cell viability under monoculture. Finally, our *in vivo* results of an enriched fraction of Ki67+ tumor cells in lapatinib-treated tumors at the tumor-fibroblast *in vivo* interface compared to untreated tumors suggest that stroma-rich areas mediate drug resistance *in vivo*.

Conclusion: Our studies highlight the critical role of fibroblasts in mediating drug resistance in a subset of HER2+ breast cancers that is mediated by activation of anti-apoptotic pathways. Furthermore, we identified a new combination therapy using SRC inhibitors to restore HER2-therapy sensitivity in breast cancer models that exhibit fibroblast-mediated resistance.