**An Ex-vivo Platform Predicts Anti-tumor Outcome of Metabolically-Targeted, Algorithm-Driven Combination Therapy in Triple-Negative Breast Cancer**

Aaron J. Goldman¹,²,³, Andrew Dhawan⁴, Biswanath Majumder⁵, Mohammad Kohande⁶, Pradip Majumder⁵ and Shiladiitya Sengupta¹,²,³

¹Harvard Medical School, Health Sciences and Technology; ²Harvard-MIT health sciences and technology; ³Division of biomedical Engineering, Brigham and Women’s Hospital; ⁴University of Waterloo department of applied mathematics; ⁵Mitra Biotechnology Bangalore India.

**Abstract:** Cancer cells undergo phenotypic cell state transitions in response to chemotherapy as a mechanism that can confer transient resistance. However, such cell state transitions can also unlock unique vulnerabilities that can be exploited using temporally-sequenced combination chemotherapy. Here, utilizing a primary breast cancer ex-vivo functional assay (CANScript™) that captures tumor heterogeneity, we report that in response to a chemotherapeutic agent, a subset of cancer cells can mount an acutely-induced phenotypic adaptive resistance to future cytotoxic pressure via the transient acquisition of a unique metabolic state defined by augmented glycolysis together with mitochondrial proficiency. These cells activate two complex, temporally-interdependent pathways that enable a glucose shunt towards the pentose phosphate pathway (PPP), which confers an adaptive cross-tolerance to different chemotherapeutic agents. Mathematically modeling translational and simulated drug schedules, we define a rationally-designed 3-drug combination therapy of metabolic inhibitors and cytotoxic agents, which results in improved cancer survival. Our findings highlight a new bioenergetics-based adaptive resistance mechanism through which cancer cells can survive combinations of chemotherapy. Administration of metabolic inhibitors in rational, temporal sequence with existing chemotherapy can emerge as a new paradigm in the treatment of cancer.

**Objectives:** The goal of our study was to identify novel therapeutic strategies that exploit the metabolic niche of cross-therapy resistant breast cancer cells in the context of relapse, refractory and metastatic disease. By implementing a novel ex-vivo platform that informs the temporal ordering of the molecular events leading to cross-resistance we reasoned that drug schedules could be designed to target the origins of resistance.

**Results**

**Fig. 1** Functional studies of human primary breast cancers reveal therapy-induced hybrid phenotype, glycolytic program, and cross-drug resistance

A) Schematic of human explant model to evaluate response of refractory human tissue to anti-cancer agents. B) Representative IHC from explant culture shows effect of drug treatments on the expression of a drug resistant phenotype (CD44B) and serial sections corresponding to cleaved Caspase 3, control tissue treated with platinum/gemcitabine regime shows response to therapy (activated caspase-3) (data not shown). C) Quantification of glucose consumption in patient tissue following re-treatment with chemotherapies

**Fig. 2** Mathematical modeling indicates a temporal order of signaling networks drive drug-induced glucose uptake

A) Systems biology was developed using available literature to loosely connect CD44 expression and glucose uptake. Evidence for an involvement of reactive oxygen species (ROS), hypoxia inducible factor 1 alpha (HIF) and GLUT-1 are all implicated in this network. B) Simulated temporal kinetics of molecular dynamics in the case where drug concentration in the system remains constant over time.

**Fig. 3** In vitro evaluation of a drug-induced hybrid epithelial mesenchymal phenotype correlates with cross therapy resistance and glucose uptake

A) Schematic describes generation of drug tolerant cells (DTC) Breast cancer cells were treated with a high dose (20x IC50) of docetaxel followed by drug washout and acute population outgrowth. B) DTC were analyzed by flow cytometry for epithelial and mesenchymal markers, CD24 and CD44, respectively. Right panel shows quantification of the distinct phenotypes, CD44HD24Lo (mesenchymal-like) or CD44HD24Hi (a hybrid phenotype). C) MDA-MB-231 breast cancer cells were treated with docetaxel (DTX) at a non-cytotoxic dose for indicated time points. Glucose uptake was measured by 2-NBDG uptake and CD44 expression was determined by antibody fluorescence using flow cytometry.

**Fig. 6** Glucose metabolism inhibition reverses acquired cross-resistance to anthracyclines

A) Cell viability curves show sensitivity of breast cancer cells to the anthracycline, doxorubicin. Cells were either derived from a patient cell line (non drug-treated) or cells that survive high dose treatment with docetaxel (docetaxel-tolerant). Note the acquisition of cross-therapy resistance in the drug tolerant breast cell population. B) Cell viability curves show sensitivity of breast cancer cells to doxorubicin in drug naive parent cells or docetaxel tolerant cells. Black lines indicate doxorubicin alone, red line indicates addition of the AKT kinase inhibitor P1103.

**Fig. 6** Glucose metabolism inhibition reverses acquired cross-resistance to anthracyclines

A) Western blot analysis of proteins that co-precipitate with AKT in breast cancer cells treated with low dose of doxorubicin or vincristine. siRNA targeting CD44 or scrambled control were transected prior to drug exposure. B) Glucose uptake determined by 2-NBDG fluorescence in parent cells or docetaxel tolerant breast cancer cells that were treated with AKT or mTOR inhibitors P1103 or Everolimus, respectively. C) Schematic depicts the mechanism of glucose uptake following exposure to docetaxel or cytotoxic chemotherapy-induced stress.

**Fig. 6** Glucose metabolism inhibition reverses acquired cross-resistance to anthracyclines

A) Ex-vivo CANScript platform was employed to test and predict the outcome of combination regimens that include taxane followed temporally by doxorubicin and an inhibitor of the hexokinase, Lonidamine. Lower panel shows histogram quantification of tumor cell viability (black bars) or glucose uptake in residual population of tumor cells (red bars). B) In vivo tumor volume curves of syngeneic mouse model (4T1 triple negative breast cancer cells) following treatment with doxetaxel and doxorubicin +/- P1103 in discrete sequence and temporal order. The temporally-constrained addition of Dox-P1103 overcomes tumor regrowth following cessation of treatment.

This research was supported by the American Cancer Society fellowship grant SPF-12-226-01